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# Mineral Analysis of Forages with Near Infrared Reflectance Spectroscopy<sup>1</sup>

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## ABSTRACT

Mineral concentration data could easily be generated by near infrared reflectance spectroscopy (NIRS) while determining quality parameters in forage samples. However, which minerals can be analyzed and why they can be determined has not been documented adequately. Therefore, NIRS spectra were collected on 200 samples of crested wheatgrass (*Agropyron* spp.), 203 tall fescue (*Festuca arundinacea* Schreb.), and 59 alfalfa (*Medicago sativa* L.) hays. Concentrations of Na, K, Ca, P, Mg, Fe, Mn, Cu, and Zn, as determined by atomic absorption, and calculated values of the Ca/P, K/Mg, and K/(Ca+Mg) ratios were regressed against reflectance values measured in 2-nm increments between 1100 and 2500 nm for each sample. Approximately one half of the samples in each forage set was used to develop the calibration equations, while the other half was used to validate the equations. The coefficients of variation [CV = (standard error of analysis ÷ the mean) × 100] generally ranged from 10 to 20% for K, Mg, Ca, and P concentrations in each forage type. The Ca/P ratio in alfalfa was determined with a CV of 18%. The CV values of other minerals and mineral ratios generally exceeded 20%. Chlorophyll and some inorganic salts and organic-acid salts of Ca, Mg, and K were scanned with NIRS for wavelength comparisons with those wavelengths used to determine mineral concentrations in forages. Some of the wavelengths used in the equations for Ca, K, and Mg were related to peaks and changes in slope observed in chlorophyll and organic-acid salts of Ca, K, and Mg, suggesting that NIRS is indirectly measuring these minerals by their association with organic molecules. Accurate use of NIRS to determine mineral cation composition in forages appears limited to certain major minerals (Ca, P, K, and Mg).

*Additional index words:* Crested wheatgrass, *Agropyron* spp., Tall fescue, *Festuca arundinacea* Schreb., Alfalfa, *Medicago sativa* L., Mineral ratio.

THE USE of near infrared reflectance spectroscopy (NIRS) for the analysis of quality measurements in feedstuffs has increased considerably in the last decade (3,4). Basically, scanning monochromators measure reflected light from samples in the near infrared region. Specific filters capable of scanning the appropriate wavelengths can be installed in less expensive, but accurate, filter instruments for rapid and accurate analysis. Apparent absorbance (log 1/Reflectance) at selected wavelengths can then be correlated with various quality components.

The ability of NIRS to determine various quality

components in agricultural type samples is due to the rotational or vibrational energies associated with hydrogen bonds. The vibrational motions of hydrogen bonded to other atoms (C, O<sub>2</sub>, N<sub>2</sub>, and S) can be measured indirectly through the amount of absorbed near infrared radiation. This bonding or stretching will vary depending on the presence of other elements and number of bonds that will affect the amounts of reflected light. Kaye (5) listed some of the functional groups (CH<sub>3</sub>, NH<sub>3</sub>, OH, etc.) and wavelengths that are detected by NIRS. Whetsel (10) reported the wavelengths found with NIRS for the determination of rare earth metals and inorganic metal complexes in simple solutions. The mineral complexes he reported are not found in living systems, and the solutions examined were pure.

The minerals in agricultural products probably exist in both inorganic and organic complexes. The possibility that NIRS could be used for determining mineral concentrations would therefore seem remote. Shenk et al. (8,9) reported the use of NIRS for determining K, Mg, Ca, and P in forages. However, the ability of NIRS to determine other minerals has not been documented. This study was initiated to examine the use of NIRS in determining mineral concentrations in three forages. A hypothesis is also offered to explain why NIRS can be used to estimate some mineral concentrations.

## MATERIALS AND METHODS

Mineral profiles and NIR spectra were determined on 200 crested wheatgrass (*Agropyron* spp.), 203 tall fescue (*Festuca arundinacea* Schreb.), and 59 alfalfa hay (*Medicago sativa* L.) samples. Crested wheatgrass samples were from plants grown at Lincoln and Alliance, NE. Tall fescue samples were from a broad genetic based population of three cultivars ('KenHy', 'Ky-31', and 'Mo-96' grown at Mount Vernon, MO) that was free of the endophyte. The grasses were sampled at the vegetative stage of growth. The alfalfa samples represented 59 hays of several maturities, cuttings, and locations in the Intermountain West (3).

Grass samples were hand clipped and dried at 60°C, and alfalfa samples were obtained by compositing 10 or more corings from each hay source. All samples were ground in a Wiley no. 4 mill to pass a 1-mm stainless steel sieve and again dried at 40°C for 24 h prior to chemical analyses. Nitric-perchloric acid (3:1) digestion of 0.5 g forage subsamples preceded analysis for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn. Phosphorus was measured colorimetrically as molybdovanadophosphoric acid. Potassium was determined by

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flame emission. All other elements were measured by atomic absorption spectrophotometry. Samples for K, Ca, and Mg analysis were prepared in 1 g L<sup>-1</sup> lanthanum (La). Mineral ratios of Ca/P, K/Mg, and K/(Ca+Mg) were calculated from the concentration data of the respective element. The Ca/P values were calculated on a mass concentration basis, whereas the K/(Ca+Mg) and K/Mg values were calculated on a chemical equivalence basis.

After subsampling for chemical analysis, the balance of the samples was ground using a UDY<sup>3</sup> cyclone mill (UDY Corp., Fort Collins, CO) (1-mm), and a portion (ca. 2 g) was packed into cups for NIRS scanning. Spectral response data were collected for all samples from 1100 to 2500 nm at 2-nm increments using a Pacific Scientific<sup>3</sup> Model 6350 scanning monochromator (Pacific Scientific Corp., Gardner/Neotec Instrument Div., Silver Spring, MD). Apparent absorption data for tall fescue, crested wheatgrass, and alfalfa hays were stored in separate files. Mineral data from chemical analysis of each sample were entered into the computer (DEC<sup>3</sup> PDP 11/23; Digital Equipment Corp., Nashua, NH). One-hundred-two (102) tall fescue samples, 100 crested wheatgrass samples, and 44 alfalfa samples were randomly selected for calibration. The absorption data and chemical analyses for these samples were used in developing multiple regression equations for each mineral and mineral ratio within each forage type.

Software used to collect reflectance spectra and to develop and test the equations are part of the USDA National NIRS Forage Network software (7). Equation development was performed using the 1985 version of the CAL program (7). The program first selected the wavelengths to be used in the equation, using all of the samples present in the development file. Mathematical treatments (log, first derivative, or second derivative of absorption values) were performed on selected wavelengths to obtain better fitting equations. Next, coefficients were calculated using half of the calibration samples, and validation of the equation was performed on the other half. This process was repeated twice so that every sample in the calibration set contributed to the validation process (Mark Westerhaus, 1985, personal communication). Equation selection, by the operator, was based on each wavelength in the equation having a partial  $F > 8.5$  and the equation having the lowest standard error of calibration (SEC) and highest  $R^2$ . The remaining samples (101 tall fescue, 100 crested wheatgrass, and 15 alfalfa hay) were used as the final validation of the developed equations. Near infrared reflectance determination on these samples consisted of making reflectance measurements and solving a regression equation of the form:

$$\text{Amount analyzed component} = B_0 + B_1X_1 + B_2X_2 + B_3X_3 \dots,$$

where  $X_1$ ,  $X_2$ , and  $X_3$  are reflectance (absorption) measurements or derivatives of these at wavelengths  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$ ;  $B_0$  is the regression constant; and  $B_1$ ,  $B_2$ , and  $B_3$  are partial regression coefficients. Standard error of analysis (SEA) and  $r^2$  for each mineral within each forage type were used to measure the amount of error associated with the determination of each mineral.

Coefficients of variation were calculated [ $CV = (\text{SEA}/\text{mean}) \times 100$ ] for each mineral and mineral ratio for comparing different minerals within and across the three forage types. Standard error of calibration and analysis were calculated as follows:

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

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

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

As a means of trying to explain why NIRS responds to Ca, K, Mg, and P concentrations in forage samples, spectra of inorganic and organic acid salts of Ca, P, K, and Mg were scanned with NIRS. Chlorophyll extracted with an azeotropic mixture of ethanol and absorbed to Alundum was also scanned since some of the Mg in forages is associated with chlorophyll. These spectra were compared with the wavelengths chosen during the calibration process as a means of explaining the response of NIRS to mineral concentrations.

## RESULTS AND DISCUSSION

### Tall Fescue

Table 1 lists the statistics associated with regression and NIRS wavelengths selected to best explain the variation in mineral concentrations determined by wet chemical analysis of tall fescue forage samples. Our SECs for Ca, P, and K were 1.0, 0.3, and 2.6 g kg<sup>-1</sup>, respectively. Shenk et al. (8) reported SECs of 1.3, 0.4, and 3.7 g kg<sup>-1</sup> for the same minerals, respectively, in a diverse group of forages with a different model of scanning monochromator than the one used in this study. Large errors occurred in our study when the spectral data were regressed on the Na data, preventing development of a Na equation. This may be due to the extreme range in Na values (see Table 1).

Equations were also developed for mineral ratios [Ca/P, K/(Ca+Mg), and K/Mg], and the associated calibration errors were calculated. The SECs were 0.2, 0.3, and 0.4 for K/(Ca+Mg), K/Mg, and Ca/P, respectively. Mean values and ranges are presented to illustrate the variability present in the samples used for equation development. Wavelengths and transformations (i.e., first or second derivative of reflectance data) used in the equations are listed for each mineral and ratio.

Equations were then tested on the remaining 101 tall fescue samples, with the results shown in Table 2. Shenk et al. (9) reported SEAs (g kg<sup>-1</sup>) and corresponding  $r^2$  values for forage samples from around Pennsylvania of 1.4 and 0.90, 0.4 and 0.70, and 4.1 and 0.60 for Ca, P, and K, respectively. We observed SEAs (g kg<sup>-1</sup>) and  $r^2$  values of 1.1 and 0.80, 0.3 and 0.83, and 3.4 and 0.74 for Ca, P, and K, respectively. Bias values, which are the mean of the samples analyzed with NIRS, minus the mean from chemical determinations, were quite variable.

Trace elements had lower  $r^2$  values and higher SEAs than the other minerals. Copper and Mn had  $r^2$  values of 0.30 and 0.44 in comparison with  $r^2$  values of 0.31 and 0.31 as reported by Shenk et al. (9) for the same elements. The Ca/P ratio was determined because of its impact on phosphorus nutrition, while the other two ratios were computed because of their implication in the grass tetany syndrome (6). Values for the two K ratios could be determined with a CV of 20%, whereas Ca/P had a CV of 27%. The SEA of 0.6 for the Ca/P ratio is too large for practical use. The two K ratios could be calculated if one is interested in the potential tetany hazard of this forage. As a rule, minerals with a wide range in concentration values also had high CVs.

<sup>3</sup> Mention of a tradename does not imply an endorsement or recommendation by the U.S. Department of Agriculture or Utah State University over similar companies or products not mentioned.

Table 1. Statistical data for mineral concentrations determined by wet chemistry for 102 tall fescue samples used in NIRS calibration and equation development.

Variable†	Wet chemistry			R <sup>2</sup>	Wavelengths‡					
	Mean	Range	SEC‡							
Ca	5.8	3.0-9.9	1.0	0.82	2390#	1514¶				
P	2.7	1.9-4.7	0.3	0.79	1788¶	1688¶	1768#			
Mg	4.4	2.2-7.0	0.6	0.73	1850¶	1768¶	2350¶			
K	29	19-39	2.6	0.88	2224#	2418#	1942¶	1488¶	2372#	2318¶
Cu	5.5	3.5-8.5	0.8	0.46	1454#	1268¶	1838#	2246#	1588#	1822#
Zn	20	13-35	2.7	0.51	1790#	1992#	1914¶			
Mn	84	42-280	19	0.46	1200#	1704#				
Na	1200	70-4700								
Fe	760	200-3900	200	0.69	1358#	1868#	2046¶	1416#	1912¶	
Ca/P	2.2	1.1-4.3	0.4	0.50	2342#	1502¶	1748¶	2372#	2316#	2288#
K/(Ca + Mg)	1.2	0.6-2.0	0.2	0.71	2386¶	1950¶	2228#	2248#	1746¶	1888#
K/Mg	2.1	1.0-3.8	0.3	0.72	2362¶	1748¶	1480¶	1558#	2256#	2434#

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, Na, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis; K/(Ca + Mg) and K/Mg were calculated on a chemical equivalence basis.

‡ SEC = standard error of calibration in units of measure appropriate to each mineral.

§ Wavelengths (nm) used in the equation in order of decreasing contribution.

¶ Math treatment, first derivative.

# Math treatment, second derivative.

Table 2. Statistical data for mineral concentrations determined by wet chemistry and NIRS for 101 tall fescue samples.

Variable†	Wet chemistry			SEA‡	r <sup>2</sup>	Bias	Standard deviation	
	Mean	Range	CV‡				Wet chemistry	NIRS
			— % —					
Ca	5.8	3.0-8.8	19	1.1	0.80	-1.8	1.9	1.6
P	2.6	1.8-4.5	12	0.3	0.83	-0.7	0.4	0.3
Mg	4.6	2.6-6.0	15	0.7	0.56	1.4	1.0	0.9
K	28	17-40	12	3.4	0.74	-0.6	3.4	3.3
Cu	5.6	3.6-8.6	20	1.1	0.30	2.7	1.1	1.2
Zn	21	13-36	16	3.3	0.41	-2.7	4.0	3.7
Mn	85	38-280	29	25	0.44	-15	32	17
Fe	760	240-3600	31	235	0.58	-101	360	270
Ca/P	2.2	1.1-4.3	27	0.6	0.31	2.3	0.6	0.6
K/(Ca + Mg)	1.1	0.6-2.0	20	0.2	0.49	-0.9	0.3	0.3
K/Mg	2.1	0.1-3.7	20	0.4	0.63	-0.4	0.6	0.6

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, Na, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis; K/(Ca + Mg) and K/Mg were calculated on a chemical equivalence basis.

‡ CV = [(SEA/mean) × 100].

§ SEA = standard error of analysis in units of measure appropriate to each mineral.

Table 3. Statistical data for mineral concentrations determined by wet chemistry for 100 crested wheatgrass samples used in NIRS calibration and equation development.

Variable†	Wet chemistry			R <sup>2</sup>	Wavelengths‡					
	Mean	Range	SEC‡							
Ca	2.7	1.4-5.0	0.6	0.86	1834¶	1342#				
P	1.6	1.1-2.6	0.2	0.77	2156#	2332¶				
Mg	1.0	0.6-1.7	0.2	0.77	2076¶	2190#	1124¶			
K	14	5.7-22	1.4	0.83	1470¶	1938¶	1656#	2326¶	2410#	
Cu	3.5	0.9-6.2	0.8	0.41	2172#	1674#	1502#	1734¶	1520#	1404#
Zn	18	6.9-33	4.3	0.44	1336#	2104#	1204#	1718¶		
Mn	38	14-100	12	0.50	1898#	1754¶	1710#	2186#		
Na	38	20-180	6	0.27	1368¶	1612#	1718¶			
FE	280	130-930	69	0.72	1188¶	1974#	1804#	1114¶	2138#	
Ca/P	1.8	0.7-2.9	0.3	0.52	1188¶	1868¶	1768¶	1208¶	1568¶	
K/(Ca + Mg)	1.7	0.7-5.2	0.4	0.46	2092¶	1652#	1824#			
K/Mg	4.5	1.4-13	1.1	0.53	1764¶	1216¶	1568¶	1872#		

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, Na, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis; K/(Ca + Mg) and K/Mg were calculated on a chemical equivalence basis.

‡ SEC = standard error of calibration in units of measure corresponding to each mineral.

§ Wavelengths (nm) used in the equation in order of decreasing contribution.

¶ Math treatment, first derivative.

# Math treatment, second derivative.

### Crested Wheatgrass

Equation data for crested wheatgrass samples are shown in Table 3. The SEC values for P and Cu were similar in the tall fescue and crested wheatgrass equations (0.3 vs. 0.2 g kg<sup>-1</sup> and 0.8 and 0.8 mg kg<sup>-1</sup>, respectively). A Na equation was developed; however,

the R<sup>2</sup> was low (R<sup>2</sup>=0.27). Standard errors of calibration were lower for Ca and Mg than reported by Shenk et al. (8) and for the tall fescue samples in this study (Table 1). Three of the five wavelengths used for K analysis in the crested wheatgrass equation (1938, 2326, and 2410 nm) were similar to three wavelengths for

Table 4. Statistical data for mineral concentrations determined by wet chemistry and NIRS for 100 crested wheatgrass samples.

Variable†	Wet chemistry				$r^2$	Bias	Standard deviation	
	Mean	Range	CV‡	SEA§			Wet chemistry	NIRS
			— % —					
Ca	2.8	1.4-4.8	25	0.7	.88	-1.7	0.6	0.4
P	1.5	1.3-2.4	13	0.2	.84	0.1	0.3	0.1
Mg	1.0	0.7-1.7	20	0.2	.88	0.5	0.2	0.2
K	14	7.4-21	11	1.5	.81	0	3.5	3.1
Cu	3.2	1.1-6.2	31	1.0	.20	0.7	0.9	0.6
Zn	18	6.9-32	44	8.1	.22	-26	7.6	4.0
Mn	37	14-110	43	16	.14	136	14	14
Na	41	18-180	44	18	.01	-4.2	18	5
Fe	290	130-930	32	93	.64	426	140	150
Ca/P	1.8	1.0-3.6	22	0.4	.14	2.0	0.4	0.3
K/(Ca + Mg)	1.6	0.8-3.3	25	0.4	.30	-0.7	0.5	0.4
K/Mg	4.2	2.1-9.1	29	1.2	.23	-3.9	1.3	1.0

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, Na, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis; K/(Ca + Mg) and K/Mg were calculated on a chemical equivalence basis.

‡ CV = [(SEA/mean) × 100].

§ SEA = standard error of analysis in units of measure corresponding to each mineral.

Table 5. Statistical data for mineral concentrations determined by wet chemistry for 44 alfalfa hay used in NIRS calibration and equation development.

Variable†	Wet chemistry				$R^2$	Wavelengths§				
	Mean	Range	SEC‡							
Ca	14	9.8-20.0	1.1	0.75	1954#	1462#	1394#	2406#		
P	2.2	1.2-3.4	0.3	0.62	1540#	1504#	1910#	2208#		
Mg	2.8	1.7-4.1	0.3	0.80	1592#	1768#	2304#	2036#	2404#	1818#
K	22	12-31	1.8	0.88	2366#	2010#	1734#	1256#	1834#	1538#
Cu	7.6	3.0-16	1.2	0.84	1534#	1856#	2352#	1714#	1492#	
Zn	17	11-27	2.3	0.61	2376#	2056#	1160#			
Mn	31	14-69	6	0.24	2384#	2026#	1342#	2426#		
Na	490	200-960	160	0.24	1296#					
FE	260	90-730	110	0.45	1322#					
Ca/P	6.8	4.2-15	1.2	0.66	2344#	1948#	1484#	2246#	1762#	

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, Na, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis.

‡ SEC = standard error of calibration in units of measure corresponding to each mineral.

§ Wavelengths (nm) used in the equation in order of decreasing contribution.

# Math treatment, first derivative.

# Math treatment, second derivative.

Table 6. Statistical data for mineral concentrations determined by wet chemistry and NIRS for 15 alfalfa hay samples.

Variable†	Wet chemistry				$r^2$	Bias	Standard deviation	
	Mean	Range	CV‡	SEA§			Wet chemistry	NIRS
			— % —					
Ca	14	5.8-19	14	2.0	0.68	0.6	3.7	2.3
P	2.2	1.4-3.0	14	.3	0.74	0	0.5	0.4
Mg	2.7	1.6-4.8	19	.5	0.71	0.1	0.9	0.7
K	22	13-33	12	2.6	0.80	1.1	5.4	4.0
Cu	7.6	4.5-13	29	2.2	0.50	0.3	3.1	2.6
Zn	17	11-20	18	3.1	0.35	1.3	2.7	3.8
Mn	33	15-55	24	8.1	0.51	1.0	11	9.8
Na	490	270-850	32	160	0.12	-7.0	160	86
Fe	220	120-340	42	91	0.33	-22	65	110
Ca/P	7.1	2.1-14	18	1.3	0.80	0.4	2.9	2.7

† Ca, P, Mg, and K are expressed as g kg<sup>-1</sup>; Cu, Zn, Mn, and Fe are expressed as mg kg<sup>-1</sup>; Ca/P values were calculated on a mass basis.

‡ CV = [(SEA/mean) × 100].

§ SEA = standard error of analysis in units of measure corresponding to each mineral.

the same mineral in the tall fescue equation (1942, 2318, and 2418 nm).

Results of testing the equations on the remaining 100 crested wheatgrass samples are in Table 4. The SEA values were lower for Ca, P, Mg, K, Cu, Mn, and Fe in crested wheatgrass than in tall fescue. Although an equation for Na was developed, validation with 100 crested wheatgrass samples accounted for none of the variability present ( $r^2=0.01$ ). Iron had similar  $r^2$  val-

ues in the tall fescue and crested wheatgrass samples (0.58 and 0.64, respectively); however, the SEA for Fe was lower in the crested wheatgrass samples (93 vs. 240 mg kg<sup>-1</sup>, respectively). The CVs exhibited a greater range when compared with the tall fescue samples. Potassium had the lowest CV, with Zn and Na having the highest CV in the crested wheatgrass samples. Bias values exhibited more variation compared to values obtained for the tall fescue samples.

### Alfalfa Hays

The same minerals were determined in alfalfa hay samples as in tall fescue and crested wheatgrass samples. The Ca/P ratio was the only ratio calculated (Table 5). The SEC values were similar for both Ca and P in alfalfa and tall fescue, while SEC values for P, Mg, and K were similar in alfalfa and crested wheatgrass samples. Sodium and Fe exhibited high SECs, which may be due to the large range in concentrations.

The alfalfa equations were verified on 15 additional alfalfa hay samples (Table 6). The SEA values were higher for Ca and Cu compared with the tall fescue and crested wheatgrass equation. The CV values for Ca were greatest for the crested wheatgrass samples, followed by the tall fescue and alfalfa samples (25, 19, and 14%, respectively). The mean and range in Ca values and in the Ca/P ratio were also larger in the alfalfa samples than tall fescue and crested wheatgrass. Sodium concentrations were considerably higher than crested wheatgrass, and the  $r^2$  for Na in alfalfa and crested wheatgrass reaffirmed the amount of error associated with equation development, although the equations could be developed. The CVs were variable, with Fe being the largest and K the smallest. The CV for Ca/P was lower than the CVs for Ca/P in crested wheatgrass or tall fescue samples. Bias values for alfalfa were less variable than tall fescue or crested wheatgrass; however, a smaller sample set was used to test the equation.

### Mineral Ratios

Data shown in Table 7 utilized the NIRS generated values for Ca, P, K, and Mg to calculate ratios [Ca/P, K/(Ca+Mg), and K/Mg]. These ratios were then correlated with ratios calculated from wet chemical determinations for the same minerals. The strongest correlation was for the Ca/P ratio ( $r^2=0.88$ ) for alfalfa samples. Using NIRS values of Ca and P to calculate Ca/P ratios in tall fescue and crested wheatgrass samples accounted for approximately 50% of the variability ( $r^2=0.44$  and  $0.52$ , respectively).

It appears that using NIRS determined mineral values to calculate ratios [Ca/P, K/(Ca+Mg), or K/Mg] does not have the same degree of reliability as the same ratios calculated from wet chemistry. This may be due to the forage populations used for this study or to the

Table 7. Correlations between mineral ratios calculated from NIRS and wet chemistry values.

Mineral ratio	$r^{\dagger}$
<u>Tall fescue</u>	
K/Mg	0.29
K/(Ca + Mg)	0.55
Ca/P	0.44
<u>Crested wheathgrass</u>	
M/Mg	0.19
K/(Ca + Mg)	0.30
Ca/P	0.52
<u>Alfalfa</u>	
Ca/P	0.88

$\dagger$  NIRS determined values of Ca, Mg, K, and P were used to calculate ratios, which were correlated with ratios calculated from wet chemistry.

large variation noted earlier in determining mineral values (see CVs for Ca, P, K, and Mg in Tables 2, 4, and 6).

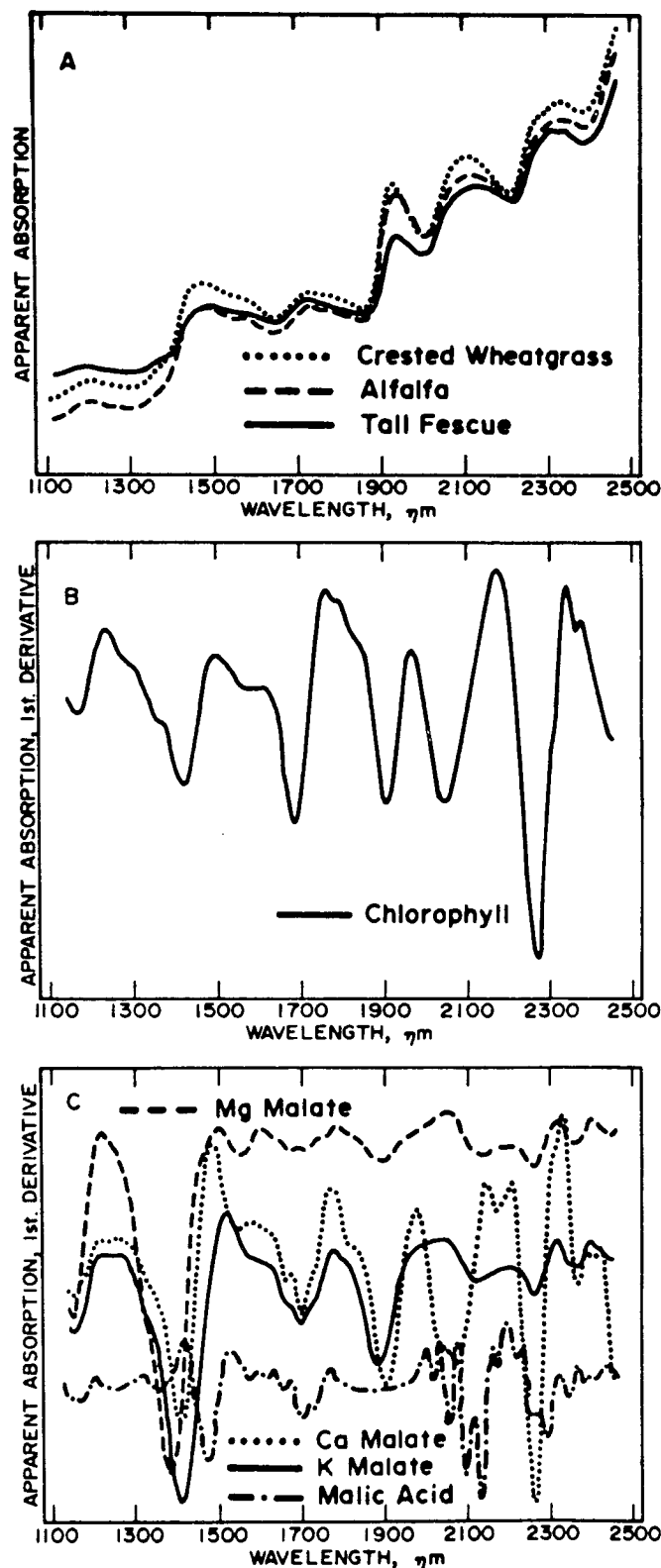


Fig. 1. First derivative spectra of forages (A), chlorophyll (B), and malic acid and Mg, Ca, and K malate (C).

### Basis for Detecting Minerals

Theoretically, NIRS responds to rotational and vibrational bonding energies of hydrogen. Inorganic salts would therefore be transparent to the energy of NIRS. That NIRS can determine some cation concentrations may occur because of their association with organic or hydrated inorganic molecules. Spectra of the three forages show that they are different (Fig. 1A). However, the main differences are due to protein, moisture, and fiber content. Examination of peaks found in chlorophyll (Fig. 1B) and wavelengths used for the determinations of Mg in the forages (Tables 1, 3, and 5) showed some similarities (1768, 1818, 1850, 2076, 2304, 2350 nm). Calcium, Mg, and K malate spectra were collected and examined. Malic acid was used since it has been found in high concentrations (percent of total organic acids) in crested wheatgrass (2), tall fescue (1), and alfalfa (6). Few similarities are evident between malic acid and the three mineral acid-salts due to the presence of the minerals (Fig. 1C). Shifts in the area of 1400 nm (water region) are evident and are due to the presence of the minerals on the malate molecule. Some of the peaks (or slope changes) for Mg malate and chlorophyll corresponded to wavelengths used in the forages (1768, 1850, 2304, 2350 nm). Peaks or slope changes for Ca malate corresponded to wavelengths used in the forage equations (1394, 1834, 2390, 2406 nm), while other similarities were noted for K malate (1538, 1659, 2318, 2326, 2366, 2410, 2418 nm). No similarities were found in wavelengths chosen for P and those highlighted in phytate or phosphate spectra.

### CONCLUSIONS

Accurate use of NIRS to determine mineral cation composition in forages appears limited to certain major minerals (Ca, P, K, and Mg). From the statistical

data and Fig. 1B and Fig. 1C, it appears that NIRS is sensing Ca, K, and Mg associated with organic acids. The wavelengths chosen for P were not similar to those at which NIR responses were noted when scanning phytate, potassium phosphate, or calcium phosphate. Because of the high degree of variability, there was no consistent indication as to how well NIRS will perform when determining mineral ratios. Therefore, caution should be observed in using NIRS-determined mineral values in balancing mineral levels or ratios in feed rations.

### REFERENCES

1. Boland, R.L., G.B. Garner, K.H. Asay, and C.J. Nelson. 1976. Organic acid composition of tall fescue leaves from diverse genotypes. *Crop Sci.* 16:677-679.
2. Burns, J.C., C.H. Noller, C.L. Rhykerd, and T.S. Rumsey. 1968. Influence of fertilization on some organic acids in alfalfa, *Medicago sativa* L. *Crop Sci.* 8:1-2.
3. Clark, D.H. 1985. Predicting digestibilities of alfalfa hays with near infrared reflectance spectroscopy. Ph.D. diss. Utah State Univ., Logan (Diss. Abstr. 85-23676).
4. ———. 1985. History of near infrared reflectance spectroscopy analysis of agricultural products. p. 7-11. In G.C. Marten et al. (ed.) Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. ARS Agric. Handb. 643. U.S. Government Printing Office, Washington, DC.
5. Kaye, W. 1954. Near infrared spectroscopy-I. Spectral identification and analytical application. *Spectrochim. Acta* 6:257-287.
6. Mayland, H.F., and D.L. Grunes. 1979. Soil-climate-plant relationships in the etiology of grass tetany. p. 123-175. In V.V. Rendig and D.L. Grunes (ed.) Grass tetany. Spec. Pub. 35. American Society of Agronomy, Madison, WI.
7. Shenk, J.S. 1985. Public software. p. 18-21. In G.C. Marten et al. (ed.) Near infrared reflectance spectroscopy (NIRS): Analysis of forage quality. ARS Agric. Handb. 643. U.S. Government Printing Office, Washington, DC.
8. ———, 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.
9. ———, M.O. Westerhaus, and M.R. Hoover. 1979. Analysis of forages by infrared reflectance. *J. Dairy Sci.* 62:807-812.
10. Whetsel, K.B. 1968. Near infrared spectrophotometry. *Appl. Spectros. Rev.* 2:1-67.