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PREDICTION OF LEAF/STEM RATIO USING NEAR-INFRARED REFLECTANCE SPECTROSCOPY (NIRS): A TECHNICAL NOTE

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Abstract

Leaf/stem ratio of grass stands is an important factor affecting diet selection, quality, and forage intake. Determination of leaf/stem ratio involves a labor-intensive process of hand separating leaf and stem fractions. Our objectives in this study were to evaluate leaf/stem ratio prediction using near-infrared reflectance spectroscopy (NIRS) in monocultures of big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), intermediate wheatgrass [*Thinopyrum intermedian* (Host) Barkw. & D.R. Dewey], and smooth bromegrass (*Bromus inermis* Leyss.). Samples of each species were hand-clipped from monocultures at four locations throughout Nebraska in the 1997 and 1998 growing seasons. Ground samples were scanned by a Perstorp model 6500 near-infrared scanning monochromator. Calibration equations were developed using CENTER and SELECT procedures. Near-infrared reflectance spectroscopy estimated leaf/stem ratio in monocultures of big bluestem, switchgrass, intermediate wheatgrass, and smooth bromegrass with a coefficient of determination (r^2) of 0.73, 0.96, 0.75, and 0.84, respectively. Near-infrared reflectance spectroscopy was a rapid means of estimating leaf/stem ratio in these grasses.

Leaf/stem ratio of grass stands is an important factor affecting diet selection, quality, and intake of forages. An estimate of leaf/stem ratio commonly is based on a labor-intensive process of hand separating leaf and stem (including leaf sheath and inflorescence) fractions of a grass sample. A rapid technique to estimate leaf/stem ratio in grass would greatly reduce costs in evaluating pastures and grazing land management practices and increase efficiency in grass breeding programs (Smart et al., 1998).

Near-infrared reflectance spectroscopy has been used successfully to predict nutritive value components (Barton, 1989; Norris et al., 1976; Redfearn, 1998) with considerable time and expense savings. In addition, NIRS has been used to estimate species composition of mixed pastures (Coleman et al., 1985; Pitman et al., 1991; Wachendorf, 1999). Few studies have been conducted where NIRS was used to predict percentages of anatomical components of forages. Hill et al. (1988) predicted percentage leaf in artificial mixtures of alfalfa (*Medicago sativa* L.) leaf and stem by NIRS. Our preliminary research indicated that NIRS could be used to estimate leaf/stem ratio in the warm-season grasses big bluestem and switchgrass (Smart et al., 1998). Our objective in this study was to evaluate leaf/stem ratio prediction using NIRS in monocultures of big bluestem, switchgrass, intermediate wheatgrass, and smooth bromegrass.

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Materials and Methods

The grasses were sampled through parts of 1997 and 1998 growing seasons at four sites across Nebraska. At the Gudmundsen Sandhills Laboratory near Whitman in west-central Nebraska, naturalized plant communities of smooth bromegrass from a subirrigated meadow were sampled at random. Native switchgrass was collected at random from monocultures growing on upland range sites. At the West Central Research and Extension Center near North Platte in south-central Nebraska, all four grasses were collected from monocultures seeded in 1990 from a replicated randomized complete block design. At the campus of the University of Nebraska–Lincoln in southeastern Nebraska, all four grasses were collected from monocultures seeded in 1990 from a replicated randomized complete block design. At the Havelock Farm near Lincoln in southeastern Nebraska, big bluestem, switchgrass, and smooth bromegrass were collected from monocultures seeded in 1996 from a replicated randomized complete block design. No fertilizer had been added to the grasses 2 yr before sampling. The warm-season grasses, big bluestem and switchgrass, were sampled on a weekly basis from 5 June to 2 July in 1997 and from 19 May to 23 July in 1998. The cool-season grasses, intermediate wheatgrass and smooth bromegrass, were sampled on a weekly basis from 14 May to 26 June in 1997 and from 5 May to 23 June in 1998.

This sampling schedule provided the wide array of plant developmental stages needed to test the performance of NIRS. At each sampling date, tillers from each grass species were hand-clipped at ground level from four replications using one 0.25-m^2 circular plot frame. Samples were oven-dried at 60°C and hand-separated into leaf blade and stem (including leaf sheath and inflorescence) components. Components were weighed, and the leaf blade dry weight was divided by the stem dry weight to calculate leaf/stem ratio.

These components were ground separately in a Wiley mill (Arthur Thomas Co., Philadelphia, PA) to pass a 1.0-mm screen and further ground through a cyclone mill (Udy Analyzer Co., Boulder, CO) with a 1.0-mm screen. Ground forage samples were recombined according to component dry weight fraction before leaf/stem ratio determination, thoroughly mixed, and stored in plastic bags at room temperature. Samples were scanned using a Perstorp model 6500 near-infrared scanning monochromator (NIRSystems, Perstorp Analytical Co., Silver Spring, MD). The NIRS software program CENTER (WINISI2 ver. 1.02a, FOSS NIRSystems, Silver Springs, MD) was used to group samples based on spectra similarities. Calibration equations were developed using the NIRS software program SELECT (WINISI2 ver 1.02a, FOSS NIRSystems Inc., Silver Springs, MD) and using neighborhood H values of 0.6, 0.8, and 1.0 and math treatments 1, 4, 4, 1; 2, 4, 6, 1; and 3,

Abbreviations: NIRS, near-infrared reflectance spectroscopy; SEC, standard error of calibration.

Table 1. Leaf/stem ratios, range, and standard deviation (SD) of big bluestem, switchgrass, intermediate wheatgrass, and smooth bromegrass determined by hand separation from samples collected in 1997 and 1998 at four locations throughout Nebraska.

		Leaf/stem ratio		
Grass species	N	Mean	Range	SD
Big bluestem	169	2.96	0.44–7.07	1.07
Switchgrass	222	1.76	0.40–8.00	1.05
Intermediate wheatgrass	142	1.94	0.23–6.24	1.09
Smooth bromegrass	268	1.93	0.34–9.84	1.42

5, 5, 1. Outliers were omitted as determined by the software using the partial least-squares method. Ten samples not in the calibration set were used to validate the calibration equations for each species. The equation with the lowest standard error of prediction was chosen as the calibration equation.

Results and Discussion

Leaf/stem ratios based on hand separation for the four grass species are shown in Table 1. Vegetation characteristics of each sample set varied greatly and provided a broad range of leaf/stem ratios due to the different sampling locations, dates, and years. Leaf/stem ratios declined logarithmically over time for switchgrass, intermediate wheatgrass, and smooth bromegrass while leaf/stem ratio for big bluestem remained relatively constant during early summer followed by a rapid decline in midsummer (data not shown).

NIRS Prediction

Near-infrared reflectance spectroscopy was effective in predicting leaf/stem ratio. Calibration equations for all grasses were acceptable based on high r^2 and low standard error of calibration (SEC) (Table 2). Equations predicted leaf/stem ratio accurately for all grasses (Table 3). The suggested minimum number of samples to develop an adequate calibration equation for any nutritive value parameter is 50 in a narrow-based population and 150 in a broad-based population (Windham et al., 1989). In addition, selected samples from both population types should be representative of major factors influencing sample physical and chemical characteristics (Windham et al., 1989). In our study, each species was representative of a fairly broad-based population because sampling occurred from multiple harvest dates, locations, and years. Our results suggest that a range of 50 to 100 samples was needed for calibration equations to give accurate predictions. Adding samples beyond this range may not significantly improve leaf/stem ratio prediction; however, the addition of samples with special features that represent values outside the normal population (e.g., extremely low or high leaf/stem ratio values) may improve prediction more than just adding more samples (Shenk and Westerhaus, 1994). Another possibility, which was not explored in this study, would be to evaluate the performance of leaf/stem ratio prediction by using coarser grinding. The variation in the mean particle size and its distribution would be larger in this case. This might be useful because it would be an indirect measurement of forage chemistry due to the smaller particles sizes that would occur for leaves and larger sizes for stems with a larger sieve diameter (Shenk and Westerhaus, 1994).

Table 2. Calibration statistics† of near-infrared reflectance spectroscopy equations developed for leaf/stem ratio in big bluestem, switchgrass, intermediate wheatgrass, and smooth bromegrass.

Grass species	Math	H	N	Mean	SD	SEC	R^2	SECV
Big bluestem	1,4,4,1	0.8	60	2.79	1.22	0.376	0.90	0.509
Switchgrass	2,6,4,1	0.6	99	1.62	0.91	0.230	0.94	0.306
Intermediate wheatgrass	2,6,4,1	0.6	49	1.83	0.83	0.225	0.93	0.434
Smooth bromegrass	1,4,4,1	1.0	61	1.71	1.18	0.486	0.83	0.577

† Math = math treatment refers to derivative, gap, smooth, and smooth 2; H = neighborhood H; N = number of samples used for calibration; SEC = standard error of calibration; SECV = standard error of cross validation.

Table 3. Validation statistics† on 10 samples from near-infrared reflectance spectroscopy equations of leaf/stem ratio of big bluestem, switchgrass, intermediate wheatgrass, and smooth bromegrass.

Grass species	Math	H	N	Slope	Bias	SEP	r^2
Big bluestem	1,4,4,1	0.8	10	0.96	−0.45	0.543	0.73
Switchgrass	2,6,4,1	0.6	10	1.09	0.13	0.230	0.96
Intermediate wheatgrass	2,6,4,1	0.6	10	1.19	0.02	0.504	0.75
Smooth bromegrass	1,4,4,1	1.0	10	1.03	−0.07	0.378	0.84

† Math = math treatment refers to derivative, gap, smooth, and smooth 2; H = neighborhood H; N = number of samples used for validation; SEP = standard error of prediction.

Although validation statistics for big bluestem were similar to those reported earlier (Smart et al., 1998), switchgrass and smooth bromegrass predictions were higher in this study compared with Smart et al. (1998). The low r^2 and high SEC for smooth bromegrass in the previous study (Smart et al., 1998) was probably related to the method of sample selection for calibration development. In the previous study, calibration equations were developed by a stratified sampling method, selecting the *i*th sample to adequately represent the population. The NIRS software SELECT and CENTER, which use the scanned spectra, group similar samples together into neighborhoods and select samples to use in calibration development based on the distance the samples are apart. This is probably a more reliable method than stratified sampling because samples that look different in color or come from different environments may actually have very similar spectra.

Leaf/stem ratio of big bluestem, switchgrass, smooth bromegrass, and intermediate wheatgrass was adequately predicted using NIRS. Based on the grasses used in this study, NIRS likely would be an acceptable predictor of leaf/stem ratio of most grasses.

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