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Prediction of leaf:stem ratio in grasses using near infrared reflectance spectroscopy

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Abstract

Leaf:stem ratio of grass stands is an important factor affecting diet selection, quality, and forage intake. Estimates of leaf:stem ratios commonly are based on a labor intensive process of hand separating leaf and stem fractions. Near infrared reflectance spectroscopy (NIRS) has been used successfully to predict forage quality and botanical composition of vegetation samples. The objective of this study was to evaluate the use of NIRS to predict leaf:stem ratios in big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), and smooth bromegrass (*Bromus inermis* Leyss.). A total of 72 hand-clipped samples of each species was taken from seeded monocultures in eastern Nebraska throughout the 1992, 1993, and 1994 growing seasons. Leaf:stem ratio was determined first for each sample and then the entire sample was ground. Samples were scanned by a Perstorp model 6500 near infrared scanning monochromator. Three calibration equations were developed based on using 18, 36, and 54 (1/4, 1/2, and 3/4 of total samples, respectively) samples. These 3 calibration equations were used to determine the number of samples necessary to achieve an r^2 of 0.70 or higher for each data set. Big bluestem and switchgrass had coefficients of determination (r^2) of ≤ 0.69 for all calibration equations except for the equation using only 18 samples of big bluestem r^2 = 0.60). Smooth bromegrass had a r^2 ranging from only 0.06 to 0.14 for the calibration equations regardless of the number of samples used. Near infrared reflectance spectroscopy was a rapid means of estimating leaf:stem ratios in monocultures of big bluestem and switchgrass but it was not suitable for smooth bromegrass.

Key Words: NIRS, big bluestem, switchgrass, smooth bromegrass

Resumen

La relación hoja:tallo en poblaciones de plantas forrajeras es un factor importante que afecta la selección de la dieta calidad y consumo del forraje. La determinación de la relación hoja:tallo está basada comúnmente en un proceso que demanda mucho trabajo manual para separar las fracciones de hojas y tallos. La espectroscopía de reflectancia del infrarojo cercano (NIRS) ha sido usada con éxito para predecir la calidad de forraje y la composición botánica en especies vegetales. El objetivo de este estudio fue el evaluar el uso de NIRS para predecir la relación hoja:tallo en popotillo gigante (*Andropogon grardi* Vitman), zacate switchgrass (*Panicum virgatum* L.), y bromo (*Bromus inermis* Leyss.). Un total de 72 muestras de cada especie cosechadas manualmente , se colectaron de monocultivos sembrados en el Este de Nebraska, EE.UU durante largo los períodos de crecimiento de 1992, 1993, y 1994. Primero se determinó la relación hoja:tallo para cada muestra y luego la totalidad de la muestra fue molida. Un barredor electrónico Perstorp modelo 6500 infrarojo cercano moncromático fue usado para analizar las muestras. Se desarrollaron tres ecuaciones de calibración basándose en el uso de 18, 36, 54 del total de las muestras, y (1/4, 1/2, y 3/4), respectivamente. Las tres ecuaciones de calibración fueron usa das ron para determinar el número de muestras necesarias para alcanzar un r^2 de 0.70 o mayor para cada set de datos. Popotillo gigante y zacate switchgrass tuvieron un coeficiente de determinaci (r^2) ≥ 0.69 para todas las ecuaciones de calibración excepto para la ecuación que usó soló 18 muestras de popotillo gigante (r^2 = 0.60). Bromo tuvo un r^2 con un rango entre 0.06 a 0.14 para las ecuaciones de calibración independiente del número de muestras usadas. El uso de NIRS fue til para calcular la relación hoja:tallo en monocultivos de popotillo gigante y zacate switchgrass pero no así para bromo

Architecture of a grass canopy affects ingestive behavior of grazing livestock. Leaf:stem ratio is a component of canopy architecture that is an important factor determining diet selection and forage intake in tropical grasses (Chacon and Stobbs 1976, Chacon et al. 1978). Green leaf:stem ratio was the single most important component determining forage intake of old world bluestems (*Bothriochloa* spp.) (Forbes and Coleman 1993). Although leaf:stem ratio is an important measurement, grass stands are infrequently characterized in terms of leaf:stem ratios because separation by hand is time consuming and expensive.

Near infrared reflectance spectroscopy (NIRS) has been used successfully to predict forage quality components (Barton 1989, Barton and Windham 1988, Norris et al. 1976) with considerable savings in time and expense. In addition, NIRS has been used to estimate species composition of mixed pastures (Coleman et al. 1985, Moore et al. 1990, Peterson et al. 1987, Pitman et al.1991). Using NIRS to predict percentages of anatomical components of forages has not been fully investigated. Hill et al. (1988) predict-

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ed percentage leaf in artificial mixtures of alfalfa leaf and stem by NIRS, but research has not been conducted using NIRS to predict leaf:stem ratios in grasses. The objective of this study was to evaluate the use of NIRS to predict leaf:stem ratios in monocultures of big bluestem (*Andropogon gerardii* Vitman), switchgrass (*Panicum virgatum* L.), and smooth bromegrass (*Bromus inermis* Leyss.).

Materials and Methods

Hand-clipped samples of big bluestem, switchgrass, and smooth bromegrass were taken from seeded monocultures in 1992 and 1993 at the University of Nebraska Agriculture Research Development Center at Mead, Nebr. and in 1994 at a University of Nebraska research site in Lincoln, Nebr. The grasses were clipped at weekly intervals from the vegetative through reproductive stages in each of the 3 years. A total of 72 samples of each species was collected over the 3 years and 2 sites.

Grass plants were clipped at ground level within 2 randomly-placed quadrats (0.1 m^2) for each species on each collection date. The entire sample was separated into leaf blade and stem fractions. The stem fraction potentially included stems, leaf sheaths, and inflorescences. Each fraction was dried separately in a forced-air oven at 60° C, after which they were weighed to determine leaf:stem ratios. The 2 fractions were reconstituted and ground in a Wiley mill (Arthur Thomas Co., Philadelphia, Penn.) to pass a 1.0 mm screen and further ground through a cyclone mill (Udy Analyzer Company, Boulder Colo.) with a 1.0 mm screen. Samples were stored in plastic bags at room temperature prior to NIRS analysis.

Ground forage samples were scanned using a Perstorp model 6500 near infrared scanning monochromator (NIRSystems, Inc., A Perstorp Analytical Co., Silver Spring, Md). The NIRS software program SELECT normally is used to randomly select samples from the entire set to generate a calibration equation. We did not use SELECT because each sample was ground entirely after leaf:stem ratio was already determined. Three calibration equations were developed based on using 18, 36, and 54 (1/4, 1/2, and 3/4 of total samples, respectively) samples. Outliers were omitted as determined by the NIRS2 (ISI by NIRSystems, Inc., Silver Springs, Md) software using the partial least squares method to develop calibration equations. These 3 calibration equations were used to determine the number of samples necessary to acheive an r^2 of 0.70 or higher for this data set. Different curve fitting math treatments were screened to improve calibration by running an NIRS software option that determines the highest spectral correlation with the predicted variable (leaf:stem ratio). Calibration equations resulting in an r^2 of 0.70 and a level of accuracy of 81.7% were considered reasonable prediction equations. Stepwise multiple regression was also tried but did not predict leaf:stem ratio as well as partial least squares methods. Samples not in the calibration sets were used to validate the calibration equations for each species. Equations were validated by simple linear regression analysis comparing hand-separated values with NIRS-predicted values.

Table 1. Leaf:stem ratios, range, and standard deviation (SD) of big bluestem, switchgrass, and smooth bromegrass samples determined by hand separation.

Grass species	N	Mean	Range	SD
Big bluestem	72	1.76	0.27–3.97	0.77
Switchgrass	72	1.16	0.14–3.10	0.65
Smooth bromegrass	72	1.87	0.73–4.38	0.62

Results and Discussion

Leaf:stem ratios based on separation by hand for the 3 grass species are shown in Table 1. Vegetation characteristics of each sample set varied greatly and provided a broad range of leaf:stem ratios. Ratios were generally above 2:1 during the vegetative stage of growth. Leaf:stem ratios declined with maturity and were the lowest for switchgrass, especially in later stages of maturity. Within date of collection and species, leaf:stem ratios of the samples were consistent.

The equations for big bluestem and switchgrass had higher r^2 and lower standard error of cross validation than equations for smooth bromegrass (Table 2). Statistical parameters varied among equations within grasses. Equations developed using 18, 36, or 54 samples had higher r^2 and lower standard error of prediction for big bluestem and switchgrass than for smooth bromegrass (Table 3). Prediction of leaf:stem ratio for big bluestem improved moderately each time 18 more samples were added to the calibration equation, whereas use of more than 18 samples with switchgrass only slightly improved predictability.

Table 2. Calibration statistics† 3 equations for near infrared reflectance spectroscopy determination of leaf:stem ratio of big bluestem, switchgrass, and smooth bromegrass.

Grass species	N	C	Mean	Range	SEC	R^2	SECV	OR	Math
Big bluestem	18	18	2.03	0.62–2.95	0.16	0.94	0.37	0	1,4,4,1
	36	34	1.70	0.27–2.74	0.23	0.88	0.32	2	1,4,4,1
	54	54	1.87	0.27–3.97	0.34	0.78	0.46	0	2,4,4,1
Switchgrass	18	17	1.10	0.31–2.14	0.25	0.81	0.29	1	3,4,4,1
	36	34	1.00	0.14–2.80	0.23	0.86	0.27	2	4,4,4,1
	54	52	1.09	0.14–3.10	0.25	0.96	0.25	2	2,4,4,1
Smooth bromegrass	18	18	1.88	1.09–2.51	0.18	0.75	0.37	0	3,4,4,1
	36	33	1.78	0.87–2.77	0.18	0.83	0.76	3	1,4,4,1
	54	52	1.76	0.73–2.77	0.30	0.52	0.65	2	1,4,4,1

†Screening values: T = 2.5, H = 10, maximum number of outliers removed per pass = 2.
N = the number of samples used for calibration, C = the number of samples used for calibration after outliers omitted, SEC = the standard error of calibration, R^2 = multivariate coefficient of determination, SECV = standard error of cross validation, OR= number of outliers removed, and Math= derivative, gap, smooth, and smooth 2.

Table 3. Validation statistics of 3 equations for near infrared reflectance spectroscopy determination of leaf:stem ratio of big bluestem, switchgrass, and smooth bromegrass.

Grass species	N	C	r^2	Slope	SEP	Bias
Big bluestem	18	18	0.60	0.86	0.53	–0.17
	36	34	0.69	1.03	0.43	0.01
	54	54	0.75	0.94	0.39	–0.08
Switchgrass	18	17	0.69	1.20	0.37	0.00
	36	34	0.73	0.95	0.34	0.01
	54	52	0.75	0.87	0.34	0.05
Smooth bromegrass	18	18	0.06	0.46	0.63	–0.01
	36	33	0.14	0.53	0.61	0.03
	54	52	0.13	0.59	0.77	0.10

N = the number of samples used for calibration, C = number of samples used for calibration after outliers removed, r^2 = coefficient of determination, SEP = the standard error of prediction

Smooth bromegrass had a poor fit between NIRS-predicted and hand-separated leaf:stem ratios for all equations. Thirty-six samples were adequate for big bluestem and 18 samples were adequate for switchgrass to develop calibration equations to achieve an r^2 >0.69 between NIRS-predicted and hand-separated leaf:stem ratios.

The relationship between NIRS-predicted and hand-separated leaf:stem ratios varied by species. In principle, NIRS can be used to estimate leaf:stem ratio because chemical properties (e.g., neutral detergent fiber or N) and resulting spectral characteristics of leaf blades and stem tissue differ. Leaf sheaths, however, are included as part of the stem fraction in most studies in which leaf:stem ratios are estimated. Inclusion of the leaf sheath may obscure the spectral differences that exist between stems and leaf blades in some plant species because chemical properties of the leaf sheaths differ from that of stem tissue. Concentrations of neutral detergent fiber (NDF) in leaf sheaths and stem tissue are different in smooth bromegrass (Sanderson and Wedin 1989) indicating the NIR spectra of the stem fraction may be affected by the inclusion of the leaf sheath. In switchgrass, NDF and N concentrations of leaf sheaths are similar to that of stem tissue but different than leaf blades (Twidell et al. 1988) suggesting that the spectral properties of the stem fraction may not be significantly affected by inclusion of leaf sheath. The spectral correlations of big bluestem and switchgrass showed many peaks with high correlations throughout the entire visible and infrared bands, which corresponded to the major bond categories of C-H, O-H, and N-H. Smooth bromegrass did not show high correlations at any wavelength. We hypothesize that the use of NIRS to predict leaf:stem ratios may be limited to those grass species which have leaf sheaths and stems of similar spectral characteristics. Although the NIRS method was an effective means of estimating leaf:stem ratios in the 2 C_4 grasses, there is not sufficient evidence to conclude that the NIRS method is better suited to C_4 than C_3 grasses (e.g.,smooth bromegrass).

Conclusions

In conclusion, NIRS could be used effectively to predict leaf:stem ratios of such species as big bluestem and switchgrass in studies involving canopy characterization. The NIRS method provided rapid, inexpensive prediction equations (r^2 >0.69) for leaf:stem ratios in big bluestem and switchgrass when using a closed population and double-sampling technique. Use of NIRS could substantially reduce the number of samples requiring separation by hand. Application of NIRS for predicting leaf:stem ratios may be limited to monocultures or simple mixes in which the spectral characteristics of the plant fractions (e.g., leaf blade and stem) are consistent and distinctly different.

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