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Research Note

Fecal Near-Infrared Reflectance Spectroscopy to Predict *Leymus chinensis* of Diets From Penned Sheep in North China

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Abstract

Selective foraging among free-ranging herbivores can make measuring botanical composition of diets challenging. Using near-infrared reflectance spectroscopy (NIRS) on feces for predicting botanical components of individual animal diets is a novel method for studying diet selection. This study was conducted to determine the ability of fecal NIRS to predict the percentage of consumption of *Leymus chinensis* (Trin.) Tzvel., a dominant species in north China, by sheep (*Ovis aries* L.). The calibration data set consisted of 47 diets of known *L. chinensis* composition, paired with corresponding fecal spectra. These pairs were generated in a trial using restricted feeding. Validation pairs ($n = 9$) were collected in a similar trial that used ad libitum feeding. Derived coefficients of determination (R^2) and standard error of calibration were 0.99% and 2.2% for partial least squares (PLS) regression and 0.89% and 7.3% for stepwise regression, respectively. Derived coefficients of determination (r^2) and standard error of prediction were 0.78% and 4.8% for PLS regression and 0.90% and 3.2% for stepwise regression, respectively. PLS regression resulted in better calibration than stepwise regression, but when the calibration data set was small, stepwise regression improved the precision and accuracy of predictions compared with the PLS regression. Results of the present study show that a fecal NIRS equation developed from a restricted feeding trial could be used to predict the percentage of *L. chinensis* in fecal materials collected from voluntary feeding trials.

Resumen

El forrajeo selectivo entre herbívoros de rango libre puede lograr medir la composición botánica de las dietas difíciles. El uso de la espectroscopía de reflectancia infrarroja cercana (NIRS) en heces para predecir los componentes botánicos de las dietas de un animal individual es una nueva aplicación de esta técnica. Este estudio se llevó a cabo para determinar la capacidad del NIRS fecal para predecir el porcentaje de consumo de *Leymus chinensis* (Trin.) Tzvel., una especie dominante en el norte de China, por la oveja (*Ovis aries* L.). El conjunto de datos de calibración consistió de 47 dietas de una composición conocida de *L. chinensis* junto con los espectros fecales correspondientes. Estos pares fueron generados en un ensayo utilizando alimentación restringida. Los pares de validación ($n = 9$) fueron recolectados en un ensayo similar que utilizó alimentación libre. Los coeficientes derivados de determinación (R^2) y el error estándar de calibración fueron: 0.99% y el 2.2% para la regresión de mínimos cuadrados parciales (PLS), y 0.89% y el 7.3% para la regresión gradual, respectivamente. Los coeficientes derivados de determinación (r^2) y el error estándar de predicción (SEP) fueron los siguientes: 0.78% y 4.8% para la regresión de PLS, y 0.90% y el 3.2% para la regresión gradual, respectivamente. La regresión de PLS resultó en una mejor calibración que la regresión gradual, pero cuando el conjunto de datos de calibración fue pequeño la regresión gradual mejoró la precisión y la exactitud de las predicciones en comparación con la regresión de PLS. Los resultados del presente estudio muestran que una ecuación fecal de NIRS desarrollada a partir de un ensayo de alimentación restringida puede ser utilizado para predecir los porcentaje de *L. chinensis* en materiales fecales recogidos de los ensayos de alimentación voluntaria.

Key Words: feces, foraging selectivity, *Leymus chinensis*, near-infrared reflectance spectroscopy (NIRS), steppe

INTRODUCTION

Diet composition provides important information for rangeland and animal nutritional management. Optical and chemical methods have been used to estimate the botanical components of herbivore diets. Near-infrared reflectance spectroscopy (NIRS) is a rapid, nondestructive, and nonpolluting optical technique, based on the use of chemometrics, to show the relationship between mathematically transformed spectra and the frequency of chemical bonds in an organic matrix, termed as reference values, quantified by classical laboratory procedures. Once a calibration equation is developed, the characteristic of interest can then be estimated on the basis of their spectra alone, provided the samples are from similar populations. The undigested residue of forage diets is likely to be closely correlated with the ingested herbage; thus, feces should contain information about the original diets (Coleman et al. 1995). Early research using fecal NIRS to predict nutritional parameters of diets of grazing livestock (Coleman et al. 1989) has been shown to be successful in predicting dietary crude protein, digestible organic matter (Lyons and Stuth 1992; Leite and Stuth 1995; Showers et al. 2006), and botanical components (Walker et al. 1998, 2002).

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Table 1. Botanical properties of the forage diets (dry matter basis) fed to sheep as determined by conventional techniques.

Constituent	Calibration data set ($n = 47$)				Validation data set ($n = 9$)			
	Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
Leymus chinensis	44.7	21.9	16.3	90.0	28.4	9.7	15.7	42.9
Elymus sibiricus	20.3	18.4	0	82.7	5.6	4.5	2.5	16.6
Puccinellia distans	17.6	17.0	0	60.4	36.3	8.0	29.4	51.3
Chenopodium album	6.8	7.2	0	31.9	14.9	5.3	9.0	24.4

Fecal NIRS has not been used for predicting botanical components in sheep (*Ovis aries* L.) diets in north China. *Leymus chinensis* (Trin.) Tzvel, *Elymus sibiricus* L., *Puccinellia distans* (Jacq.) Parl., and *Chenopodium album* L., based on taxonomic classification according to Chen and Jia (2001), comprise the major species in the majority of diets among free-ranging sheep, with *L. chinensis* being the most dominant species in the vegetation consumed by sheep (Lin 2006). A satisfactory fecal NIRS equation for predicting the contribution of *L. chinensis* to sheep diets would be useful for developing proper stocking strategies.

Although fecal NIRS is a promising tool for measuring the botanical composition of diets, it is difficult to obtain fecal samples representing a variety of known diets from free-ranging herbivores. Therefore, to produce a variety of fecal materials for developing NIRS equations, restricted feeding trials, with diets containing known components, are needed. Because diets in feeding trials are restricted to a few forages, NIRS equations developed from penned animals would not be expected to accurately predict botanical composition of diets of free-ranging herbivores. The first objective of this study was to determine whether a fecal NIRS equation developed from a restricted feeding trial could be used to predict the components of consumed herbage via fecal materials collected from voluntary feeding trials. In addition, alternative statistical techniques can be used to predict botanical composition from the NIRS data. Shenk and Westerhaus (1991) and Walker et al. (2002) suggested the partial least squares (PLS) regression resulted in better calibration than stepwise regression. A second objective of this study was to compare stepwise and PLS calibrations for predicting the proportion of *L. chinensis* in sheep diets.

METHODS

Feeding trials were conducted at the National Field Station of Grassland Ecosystems (lat 41°42′N to lat 41°57′N, long 115°32′E to long 115°59′E) located in northern Hebei province, China. Feeding trials, conducted in 2006, were used to develop fecal NIRS equations with known levels of *L. chinensis* in designed diets. Validation of the fecal NIRS equation to predict the percentage of *L. chinensis* in the diets was conducted with a data set obtained from a feeding trial conducted in 2007.

2006 Feeding Trial

Twenty wethers, 6–8 mo of age, were individually housed and fed fresh forage mixtures. Body weight (BW) of the wethers was 30 kg ± 2 standard deviation (SD). Fresh herbage was harvested to a 5-cm stubble height from a typical steppe pasture every morning from July to August. Forage was immediately chopped into approximately 20-mm lengths using a small garden shredder before mixing. Samples were divided into three approximately equal portions by fresh weight and offered at 0900 hours, 1200 hours, and 1600 hours. It was assumed that the animals were unable to select between leaves and stems in the 20-mm section; thus, botanical composition of refusals was not determined (Hameleers and Mayes 1998). Approximately $50\ g \cdot kg^{-1} \cdot BW^{-0.75}$dry matter (DM) was initially fed to each sheep, and amounts were adjusted during the familiarization period to ensure less than 5% refusal. Refusals were weighed daily at 1800 hours to estimate the actual DM intake. Diets contained 15%, 30%, 45%, 60%, or 75% *L. chinensis* by fresh mass, with a base diet of *E. sibiricus*, *P. distans*, and *C. album* in the following proportions by fresh mass: 100:0:0, 0:100:0, 0:0:100, 50:50:0, 0:50:50, 50:0:50, and 1:1:1, which resulted in a total of 35 different diets (5×7). Each animal was fed one diet for 10 d (6-d adaptation period and 4-d collection period), and fecal samples from the last 4 d of the trial were composited by individual animals for scanning. The feeding trial consisted of two 10-d tests. Twenty wethers were fed in the first test, and 15 wethers were fed in the second test, resulting in 35 fecal collections, one for each diet. Additional diets were included to aid in predicting diets of grazing animals. The added diets consisted of two forage mixtures, one containing *L. chinensis*, *E. sibiricus*, *P. distans*, *C. album*, *Leymus secalinus* (Georgi) Tzvel., and *Medicago sativa* L., in a 6:4:2:2:4:1 proportion by fresh mass; and the other diet contained *L. chinensis*, *E. sibiricus*, *P. distans*, *C. album*, *L. secalinus*, *M. sativa*, *Saussurea japonica* (Thunb.) DC., and *Bromus inermis* Leyss, in a 9:6:3:3:6:1:0.75:0.75 proportion by fresh mass. Each of these two diets was fed to six animals, resulting in 12 diets:fecal pairs. The feeding trial consisted of a 6-d adaptation period and 4-d collection period. The fecal samples from the last 4 d of the trial were composited by individual animal as one fecal sample for scanning. The purpose of additional diets was to simply increase the calibration sample size. Summary distributional statistics for the calibration dataset are shown in Table 1.

2007 Feeding Trial

This feeding trial was conducted to evaluate the potential of the equation developed from the 2006 feeding trial to predict the percentage of *L. chinensis* in simulated diets for free-foraging sheep. Nine individually housed wethers (30 kg ± 2 SE body weight) were offered ad libitum *L. chinensis*, *E. sibiricus*, *P. distans*, and *C. album* placed in separate containers. Fresh herbage was harvested to 5-cm stubble height from a typical steppe pasture every morning from July to September and

Table 2. Comparison of stepwise to partial least squares regression equations on calibration and validation statistics for the percentage of *L. chinensis* (% dry matter).[1]

	Calibration				Validation					
Equation	N_c	RSQ	SEC	SD:SEC	N_v	rSQ	SEP	SD:SEP	Slope	Average H
Stepwise	47	0.89	7.3	3.2	9	0.90	3.2	3.1	0.80	0.77
PLS	46	0.99	2.2	10	9	0.78	4.8	2.0	0.64	0.90
	Calibration (combined)				Validation (combined)					
Stepwise	42	0.92	6.0	3.5	13	0.87	8.7	2.6	0.99	0.87
PLS	43	0.93	5.6	3.7	13	0.84	10.2	2.2	1.03	0.93

[1] N_c indicates the number of observations with outliers excluded for calibration; RSQ, coefficient of determination for calibration; SEC, standard error of calibration; SD, standard deviation; N_v number of observations with outliers excluded for validation; rSQ, coefficient of determination for validation; SEP, standard error of prediction; slope, slope of the line between reference and predicted values; average H, the Mahalanobis distance values; and PLS, partial least squares.

chopped into about 20-mm lengths using a small garden shredder. Forages were offered twice daily at 0700 hours and 1500 hours to make sure animals had ad libitum access to forage. Refusals were weighed, and fecal samples were collected daily at 0700 hours. The feeding trial consisted of a 6-d adaptation period and a 4-d collection period. The fecal samples from the last 4 d of the trial were composited by individual animal as one fecal sample for scanning. Summary distributional statistics for the validation data set are shown in Table 1.

Measurements

The weight of forage offered and forage refusals were measured daily. Dry matter was determined by drying 100 g of fresh samples at 105°C for 48 h in an oven. All calibrations for diet species composition were based on DM.

All fecal samples were dried at 60°C for 48 h in a forced draft oven and ground to pass through a 1-mm sieve. Samples were redried at 60°C for 1 h and equilibrated in a desiccator at ambient air temperature for 1 h before scanning (Abrams 1985). Dried and ground fecal samples were packed into circular quartz cups and scanned at 2-nm intervals over the 1 000-nm to 2 500-nm wavelength range using a Fourier transform near-infrared spectrometer (Thermo Scientific, Waltham, MA). Spectral data were recorded as log 1/*R*, where *R* is the reflectance value, by averaging 32 scans, and spectral data was mathematically analyzed using TQ Analyst, Version 6.2.1.509 (Thermo Scientific). Outliers were identified using the spectrum outlier diagnostic with TQ Analyst, and only one sample was found to be an outlier in the calibration using standard PLS regression. Scatter correction (multiplicative signal correction) was applied to spectra before PLS and stepwise regression, using Savitzky–Golay filter smooth with TQ Analyst.

Equation Calibration and Validation

Mathematical treatment was original spectra in stepwise regression and second derivative in PLS regression. The 47 fecal samples (35 + 12) from the 2006 feeding trials were used to calibrate the fecal NIRS equation. Calibration models were developed for predicting the percentage of *L. chinensis* in sheep diets using PLS and stepwise regression (Shenk and Westerhaus 1991; Walker et al. 2002). Only one outlier was found in calibration with PLS. The nine fecal samples from the 2007 feeding trials were used to validate the fecal NIRS equation. However, for the further testing, the 2006 calibration and 2007 validation data sets were combined; then, 13 randomly selected values from the combined data set (47 + 9), termed the *combined validation set*, were analyzed again. Only one outlier was found in combined calibration with stepwise regression.

In most studies, robustness of NIRS calibration is evaluated in terms of the coefficient of determination for calibration (R^2) and standard error of calibration (SEC). The SEC represents the variability in the difference between predicted values and reference values when the equation is developed from the calibration data set. Validation statistics used in this study included standard error of prediction (SEP) and the coefficient of determination for validation (r^2). SEP represents the variability in the difference between predicted and reference values when the equation is applied to an external validation data set.

The SD:SEC or SD:SEP ratios were considered estimators for evaluating the effectiveness of the calibration equations (Williams and Soberig 1992). We also calculated the ratio between the SD of the reference values for calibration and the SEC or SEP. An SD:SEC or SD:SEP ratio > 3 indicated a good calibration or validation, respectively (Williams and Soberig 1992). Therefore, the minimum criteria to evaluate how well the calibration or validation data were fit includes 1) a coefficient of determination > 0.80, and 2) an SD:SEC or SD:SEP ratio > 3 (Westerhaus 1989). The Mahalanobis distance (H) values provide information about how the spectra of the standards are distributed from the mean spectrum.

RESULTS AND DISCUSSION

Calibration

The percentage of *L. chinensis* in the diet was accurately predicted using both stepwise and PLS regression (Table 2). Coefficients of determination (R^2) for calibration developed by the two regression approaches were both high, > 0.80, but the R^2 for calibration using PLS was clearly higher than stepwise regression. The SD:SEC ratios for calibration developed by the two regressions were > 3, but the SD:SEC ratio for calibration for PLS was higher than for stepwise regression. Calibration statistics for the percentage of *L. chinensis* in the combined data set using stepwise and PLS regression equations are presented in Table 2. Coefficients of determination (R^2) for combined calibration developed by stepwise regression were similar to the R^2 using PLS, and both were > 0.80. The SD:SEC ratio for calibration developed by stepwise regression was similar to the SD:SEC ratio using PLS, and both were > 3.0. These values compared favorably with those from previous reports (Walker et al. 1998, 2002; Landau et al. 2004). The calibration statistics also indicated that PLS regression might be superior to stepwise regression for calibration.

Validation

The predictive performance of the NIRS calibration equation was evaluated using samples from animals allowed to choose among forages. The analysis using validation data ($n = 9$) indicated that the equations for predicting percentages of *L. chinensis* developed from PLS regression were not satisfactory, with a low r^2 (< 0.8) and relatively high SEP (Table 2). Stepwise regression improved the accuracy of *L. chinensis* prediction for the external data set, with a high r^2 (0.9) and a relatively lower SEP. The SD:SEP ratios (3.2 vs. 2.0) also indicated that the prediction equation developed using PLS regression was inferior to that of stepwise regression. The validation results showed the fecal NIRS equation using stepwise regression on data from the restricted feeding trial could be used to predict percentages of *L. chinensis* via fecal materials collected from the voluntary feeding trials. The average *H* value was higher for the PLS predictions compared with the stepwise prediction using the validation data set (Table 2), which is consistent with those data reported by Walker et al. (2002), because all wavelengths were used in the PLS regression and only five wavelengths were used in stepwise regression.

The combined validation analyses indicated the equations for predicting the percentage of *L. chinensis* developed using PLS and stepwise regression had the same predictive performance (Table 2). However, the quality of the equation using stepwise regression was better than that using PLS, with a relatively higher r^2 and a relatively lower SEP.

The relationship of the proportion of *L. chinensis* predicted by NIRS to the proportion measured in the laboratory (Fig. 1) also suggests that the accuracy of prediction was lower with PLS than with stepwise regression. The slope was lower for PLS (0.6381) than for stepwise regression (0.7973) and the *Y* intercept was higher for PLS (38.68% vs. 21.55%).

The three grasses and one forb offered as forage in the 2006 and 2007 feeding trials were collected at the same stage of development and treated the same before being fed to the animals. Thus, it was appropriate to use the equation developed from the 2006 feeding trial to extrapolate and predict the percentage of *L. chinensis* in the diets used in the 2007 feeding trial. In the calibration trial the PLS regression resulted in better predictions than the stepwise regression, with a higher R^2 and SD:SEC ratio. However, in the validation trial, the fecal NIRS equation developed using stepwise regression did predict the percentage of *L. chinensis* of free-foraging animals' diet with acceptable precision. One reason for the inaccurate prediction with PLS regression may be the use of too many factors that led to overfitting of the model, where a better fit occurs in the calibration set but a worse fit occurs in validation. Prediction with such a model is often poor for external validation samples (Shenk and Westerhaus 1991; Walker et al. 2002). Walker et al. (2002) used 137 samples in a calibration data set, whereas we used only 46 samples for calibration using PLS regression. Considering the small calibration data set ($N = 46$), the five PLS factors used in the calibration seemed high and may have increased the R^2 for calibration but reduced the robustness of the equation. Another reason may be that using all the wavelengths in the PLS regression apparently increases the spectral distance between validation and the mean calibration spectra, which would reduce the robustness of the equation using PLS regression. All the spectral variation that may be encountered in a population was likely not included in the small calibration data set. Consequently, stepwise regression appeared to be a better method for developing equations.

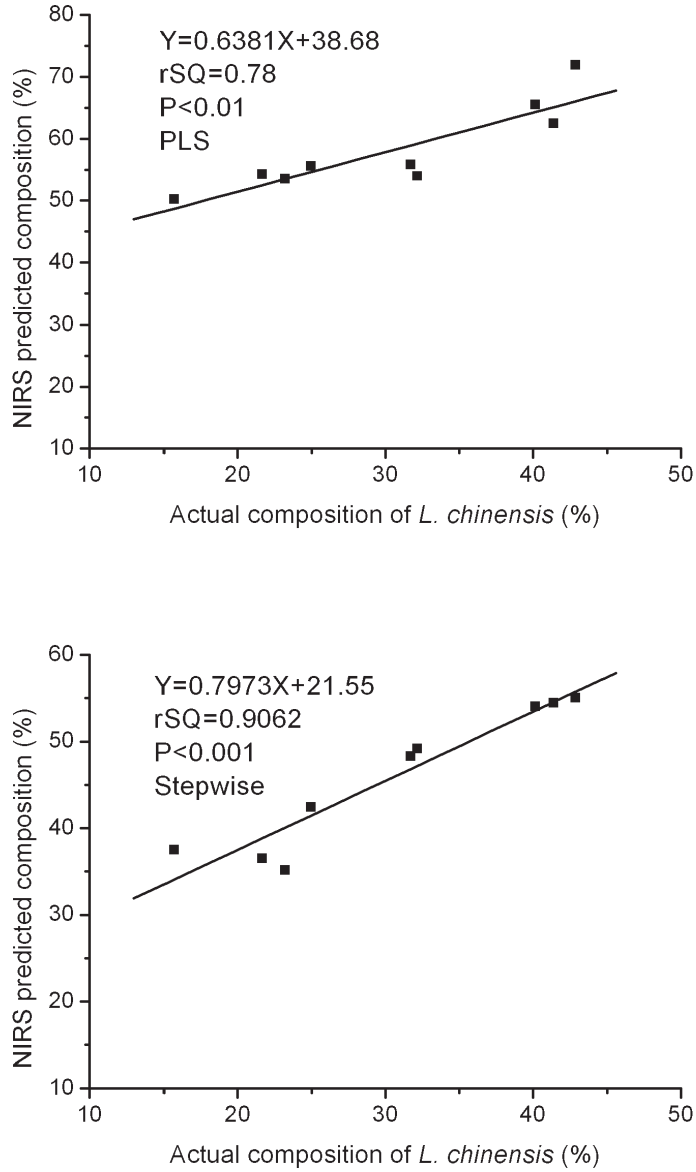

Figure 1. Comparison of partial least squares (PLS) and stepwise regression to predict the percentage of *L. chinensis* (percentage of dry matter) in the diet of sheep from near-infrared reflectance spectroscopy (NIRS) using the validation data set.

Stepwise regression improved the accuracy of prediction, but the quality of the prediction (slope and intercept) was less linear or accurate (Fig. 1) compared with previous reports (Walker et al. 1998, 2002; Landau et al. 2004). The bias between the reference values and the NIRS predicted values may result from the small sample number used for calibration ($n = 47$) compared with previous reports of Walker et al. (2002) ($n = 137$) and Landau et al. (2004; $n = 143$). Windham et al. (1989) reported that at least 150 samples were necessary for calibration with a more complex background diet. Another reason for the observed performance of the calibration in this study could be that the background diet was more complex than that used in previous studies.

Compared with previous reports on fecal NIRS calibrations for chemical components (Coleman et al. 1989; Lyons and Stuth 1992; Leite and Stuth 1995), the fecal NIRS spectra were less accurate in predicting botanical composition. Nevertheless, in view of the potential of fecal NIRS to accurately predict the botanical components and the rapid and nondestructive analysis of samples, this technique indeed can provide a promising tool for range management. The predictive performance of equations requires assessment of free-ranging sheep in the field, which is essential to evaluate the accuracy of the calibration for any component. The samples covered a limited diversity, which could limit the usefulness of the applications in the prediction equation; therefore, diversity of the calibration data set should be expanded to develop robust equations. It is generally recommended that calibration samples represent the full range of factors that give rise to spectral diversity, such as plant species, geographical location, soil type, management regime, season, and year (Boval et al. 2004). Therefore, considerable effort should be given to continued development of fecal NIRS as an animal nutritional and rangeland monitoring tool.

MANAGEMENT IMPLICATIONS

Fecal NIRS is a promising technique for predicting the diet components of sheep in north China. The equation developed from restricted feeding trials accurately predicted diets of animals given a free choice of food. When the size of the calibration data set was small, the equation developed using stepwise regression was a better predictor than the PLS regression. The calibrations based on small numbers of samples resulted in lower accuracy in predicting components of an unknown sample. Therefore, additional work in this area should be conducted to develop usable fecal NIRS equations encompassing a variety of temporal and spatial conditions.

The development of usable and satisfactory fecal NIRS equations would be laborious; however, once equations are successfully calibrated, the NIRS models could be used to estimate the diets of individual grazing animal as well as the whole herd. Monitoring individual animals would allow the grazier to obtain information about the preference of individual animals. Understanding diet preferences of sheep and developing grazing management strategies based on these preferences could help maintain the plant diversity on rangelands in China.

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