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Near-infrared reflectance spectroscopy calibrations for sorghum silage

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NEAR-INFRARED REFLECTANCE SPECTROSCOPY CALIBRATIONS FOR SORGHUM SILAGE

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Summary

Calibrations for near-infrared reflectance spectroscopy (NIRS) analysis of sorghum silage and sheep feces samples were developed, with mixed success. For sorghum silage, the standard errors of calibration (SEC) and correlation coefficients of calibration (R^2) for crude protein (CP) were .405% and .927; for acid detergent fiber (ADF), 1.667% and .943; and for neutral detergent fiber (NDF), 1.589% and .964, respectively. The statistics for crude protein were not as good as similar work reported in the literature, but the data for the fiber components was as good as or better than similar reported work.

For sheep feces, the SEC and R^2 for CP were .300% and .949; for ADF, 1.438% and .875; and for NDF, 2.016% and .846, respectively. These statistics are similar to other reports. Calibration should improve as we add more calibration samples.

Introduction

Research at Kansas State University generates thousands of varied samples annually. The feasibility of incorporating NIRS analysis into research on sorghum silages and sheep feces generated by sorghum silage digestion trials is being investigated. Successfully implementing NIRS analysis has the potential of saving large amounts of time and money.

An NIRS unit was recently acquired, along with calibration software. The calibrations purchased include those for grass hay, mixed hay, legume hay, small grain silage, corn silage, and mixed feed. Since those calibrations were developed using calibration sets with laboratory data acquired elsewhere, they needed to be verified under our conditions. In NIRS terminology, the equations needed to be re-biased.

The corn silage and hay equations were all successfully biased to our laboratory reference methods, and they satisfactorily predict protein and fiber. However, the small grain silage calibration, which was developed using a wide variety of silages, including wheatlage, oatlage, and rylage, failed to work satisfactorily with sorghum silages (Table 26.1), even after biasing. Thus, we wanted to develop a calibration specifically for sorghum silages. In addition, a calibration was developed for sheep feces from sorghum silage digestion trials. Successful application of such a calibration would allow considerable time and cost savings.

Experimental Procedures

Two hundred seventy forage and grain sorghum silages were collected from K-State research plots from four different growing seasons, encompassing a wide range of growing conditions and cultivars. One hundred six sheep feces samples were collected from three separate forage and grain sorghum digestion trials. These samples had previously been ground with a Wiley mill through a 1 mm screen. To minimize particle size effects, all the samples were re-ground with a Udy cyclone mill through a 1 mm screen.

The samples were then scanned with a Pacific Scientific 4250 NIR scanner, equipped with three tilting filters that provide a continuous scan (291 data points) from 1900 to 2320 nm. The spectral data were stored as $\log 1/R$, where R is percent reflectance. The data were analyzed using software purchased with the instrument, and samples with similar spectral data were grouped, allowing selection of calibration sets with maximum spectral variability. Fifty-one sorghum silage samples and 31 fecal samples were selected from the two groups for use as calibration samples. In addition, 37 silage, and 14 fecal samples were randomly selected for validation.

The selected samples were then analyzed for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) according to Association of Official Agricultural Chemists procedures, with very tight restrictions on replications to ensure precise laboratory data. Despite this, one or two laboratory values were omitted for some of the constituents because of poor replication, even after re-runs. A summary of the laboratory data for the calibration and validation sets of sorghum silage and feces is shown in Table 26.2.

The laboratory values were compared (via the software) with the spectral data for each of the samples in both calibration and validation sets. Multiple step-wise linear regression was then used to select the wavelengths and coefficients in the equations that provided the best statistics: highest R^2 and lowest standard error of calibration (R^2 and SEC) and highest R^2 and standard error of prediction (R^2 and SEP). The "best" equations for each of the constituents for both silage and feces were then stored into system equation files, for use in subsequent routine analysis.

Results and Discussion

Calibration and validation results for sorghum silage and fecal matter are listed in Table 26.3. The sorghum silage calibration we generated contains five wavelengths for both CP and ADF and six wavelengths for NDF. This high number indicates the complexity of the problem and is consistent with research conducted elsewhere with similar forages. The calibration equation worked well with the group of unknowns used for validation. The correlations and standard errors of prediction were very close to those of calibration, indicating that the equations adequately predict composition of the validation samples. However, a SEP of $\pm .417\%$ for protein is higher than expected and needs more work. The correlation coefficients and standard error terms for both ADF and NDF are acceptable, considering the variability and nature of these two measures.

For the fecal calibration, the relatively small sample set had a negative effect on the resulting calibration. Generally, a minimum of 40 to 50 samples is needed for a calibration set. Eight to 10 samples are needed per term in the equation. With only 31 samples in our calibration set, this limited the maximum number of terms to three to four and may have hindered our ability to calibrate for a substance as complex as fiber. With the exception of the NDF numbers, however, the statistics are similar to those in the literature. The low R^2 and high SEP for NDF in the validation set (.697 and 2.459%, respectively) could be partially explained by the size of the validation set. A small validation set ($n=14$) is susceptible to outliers, and the bias value of 1.14 (Table 26.3) indicates that it might contain one or two fairly large outliers. Although the calibration is generally sound, we expect improvement as more calibration samples are added.

Table 26.1. Analysis of Sorghum Silage Validation Set Using Pacific Scientific Small Grain Silage Calibration^a

Item ^b	n	SEP, %	R^2	Bias, %
CP	37	.850	.725	-.35
ADF	35	3.943	.858	1.33
NDF	37	4.438	.764	.50

^aCalibration previously biased for DM, CP, ADF, and NDF using 40 sorghum silages and 10 rye silages.

^bCP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber.

Table 26.2. Composition^a of Sorghum Silage and Sheep Fecal Matter as Determined by Standard Laboratory Methods

Item ^b	Calibration Set				Validation Set			
	n	Mean	SD, %	Range	n	Mean	SD, %	Range
Sorghum Silage								
CP	51	6.86	1.57	3.62-12.10	37	6.66	1.49	3.68- 9.28
ADF	49	32.71	7.00	19.78-50.50	35	33.89	6.58	21.47-48.50
NDF	50	51.94	8.41	35.25-73.25	37	51.96	8.12	39.94-71.95
Fecal Matter								
CP	31	12.19	1.33	9.50-14.68	14	12.38	0.80	10.80-13.89
ADF	30	39.02	4.07	30.08-45.83	13	40.26	3.40	35.04-43.84
NDF	30	64.90	5.13	52.59-73.09	14	67.06	2.19	64.30-71.08

^aAll data reported on 100% dry matter basis.

^bCP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber.

Table 26.3. Sorghum Silage and Sheep Fecal Matter Calibration and Validation Data for NIRS^a Analysis

Item ^b	Calibration Set				Validation Set				Bias (%)
	n	WL ^c	Math ^d	SEC (%) ^e	R ^{2f}	n	SEP (%) ^g	R ^{2h}	
Sorghum Silage									
CP	51	5	1	.405	.927	37	.417	.934	.03
ADF	49	5	2	1.667	.943	35	1.752	.928	-.16
NDF	50	6	1	1.589	.964	37	1.838	.960	-.26
Fecal Matter									
CP	31	4	1	.300	.949	14	.298	.874	.07
ADF	31	4	2	1.438	.875	13	1.532	.797	-.21
NDF	30	4	1	2.016	.846	14	2.459	.697	1.14

^aNIRS = near infrared reflectance spectroscopy.

^bCP = crude protein, ADF = acid detergent fiber, NDF = neutral detergent fiber.

^cNumber of wavelengths (terms) in calibration equation.

^dMathematical treatment of spectral data: 1 = first derivative, 2 = second derivative.

^eStandard error of the calibration group (SEC).

^fCorrelation coefficient of calibration.

^gStandard error of prediction (corrected for bias) (SEP).

^hCorrelation coefficient of NIR predicted vs. lab values.