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Near infrared reflectance spectroscopy (NIRS) prediction of herbage quality from forage and browse legumes, and natural pasture grass grown in Zimbabwe

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Near infrared reflectance spectroscopy (NIRS) was used to predict the chemical composition of forage legumes *Vigna unguiculata* (cowpea), *Desmodium uncinatum* (Silverleaf desmodium), *Stylosanthes guianensis* (cv. Oxley fine stem stylo), natural pasture grass hay, *Stylosanthes scabra* (cv. Fitzroy) and an indigenous browse tree, *Brachystegia spiciformis* (Musasa). Crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analyzed. A software for scanning, mathematical processing and statistical analysis was supplied with the spectrophotometer and used multiple linear regression (MLR). A set of 35 samples that was analyzed for calibration was selected using the software SELECT on the basis of the NIR spectra of 82 samples. The samples were scanned and screened using a FOSS NIR systems model 5000 monochromator. Equations for predicting chemical composition of the legumes were derived using scores from partial least squares (PLS) as independent variables. Cross-validation procedures indicated good correlations between laboratory values and NIRS estimates. Prediction using independent samples validated the model developed. NIRS calibrations obtained from this study could be utilized in current and future programmes of evaluating quality of forage and browse legumes for animal production.

Key words: Tropical forage legumes, near infrared reflectance spectroscopy, chemical composition.

INTRODUCTION

The technology of near infrared reflectance spectroscopy (NIRS) has been used to determine many plant constituents related to forage quality (Shenk and Westerhaus, 1985; Undersander et al., 2005). The primary focus has been on the prediction of crude protein (CP) (Undersander et al., 2005), individual constituents of cell walls (Cosgrove et al., 1994) and more recently, intake and digestibility in ruminants (Decruyenaere et al., 2009). NIRS has also been used at the whole plant level to determine botanical (Coleman et al., 1990; Pitman et al., 1991) and morphological (Hill et al., 1988) composition. Abrams et al. (1988) showed promising calibrations for total, soluble and insoluble nitrogen in silages.

Waters and Givens (1992) and Todorov et al. (1992)

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tested NIRS for prediction of nitrogen degradation characteristics. More recently, ShuJing et al. (2009) established NIRS calibration models of *in vitro* dry matter digestion, acid detergent fibre (ADF), neutral detergent fibre (NDF) and water soluble carbohydrate (WSC) of maize stover.

Forage legumes like *Stylosanthes* and *Desmodium* species, *Macroptilium atropurpureum*, among others, were recommended for rangeland improvement under different soil, moisture and grazing management conditions in Zimbabwe (Mapiye et al., 2006). The major evaluation programmes have been aimed at evaluating and developing low-input legume-based forage production technologies for resource-poor farmers in different agro-ecological zones of Zimbabwe (Mapiye et al., 2006). However, these research efforts on forage legumes were disrupted due to civil strives in the early 2000. Quality estimates of forages are ultimately used to make inferences about potential animal performance. The use of NIRS for estimating the quality of the animal's diet has been limited, especially with tropical forages. The NIRS technology has been considered to be fast and accurate method for forage analysis, and reduces the need for conventional wet chemistry procedures (Dara et al., 1991). The objective of the present study was to determine the potential and precision of using NIRS to predict chemical composition [nitrogen, ash, organic matter, NDF, ADF and acid detergent lignin (ADL)] of tropical forage legumes.

MATERIALS AND METHODS

A total of 82 samples derived from *Vigna unguiculata* (cowpea), *Desmodium uncinatum* (Silverleaf desmodium), *Stylosanthes guianensis* (cv. Oxley fine stem stylo), *Stylosanthes scabra* (Scabra cv. Fitzroy) and the tree legume *Brachystegia spiciformis* (Musasa) were used for NIRS scanning. Cowpea samples (19) were harvested from Henderson Research Station (HRS), (18°15' S and 31°29'E) Mazowe, Zimbabwe, during the 1994/95 and 1995/96 rain seasons at three stages of growth, pre-anthesis, anthesis and post-anthesis and divided into leaf, stem and leaf/stem (whole) fractions. Silverleaf desmodium samples (9) were also harvested from HRS during the 1995/96 rainy season and treated in the same way as cowpea samples. Another sample of Silverleaf desmodium and one of Oxley fine stem stylo were harvested from the University of Zimbabwe Thornpark farm, (17°42' S and 31°01' E) Harare, Zimbabwe during the 1994/95 rain season. Eighteen (18) Scabra samples were harvested from HRS plots during the 1995/96 rain season and the other 12 samples of Scabra were derived from samples harvested at post-anthesis stage from Domboshawa (17°18' S and 31°15' E), Mhondoro (18°23' S and 30°38' E), Chikwaka (17°38' S and 30°57' E), and Chivu (19°02' S and 30°35' E) farm sites in April, 1996. *B. spiciformis* was harvested from Grasslands Research Station (18°11' S and 31°30' E) and HRS (18°15' S and 31°29'E) and Gwebi Agricultural College from September to December, 1996, and the other 10 were leaf and stem samples harvested from Henderson from September, 1995 to January, 1996. The veld hay was predominantly *Hyparrhenia* species cut at mature stage in the 1994/95 rain season. All the samples were milled through a 2 mm screen and pre-dried in an oven at 60°C overnight before scanning.

About 2 g of each sample were placed in a sample holder that had a 3 cm diameter quartz window and pressure pad. Spectral data were recorded in the wavelength range of 1100 to 2500 nm using a FOSS NIR system model 5000 scanning monochromator infrared spectrophotometer. The signals were recorded as log (1/R) by an IBM compatible computer. The software for scanning, mathematical processing and statistical analysis was supplied with the spectrophotometer by Infrasoft International (John Shenk and Associates, Port Matilda, PA, USA) using multiple linear regression (MLR). The alogorithm SELECT (Version 2.05, InfraSoft International, NIR Systems Inc.1995) was then used to choose samples for calibration based on the Mahalanobis H distance between neighbourhood groups of spectra. If the Mahalanobis distance was ≤ 0.6, the individual spectra in the group were considered similar and only one sample chosen for the calibration set. Use of 0.6 is considered to result in an adequate number of samples for accurate calibration (Shenk and Westerhaus, 1991). Mathematical transformations 1, 10 and 5 of the spectral data were carried out before derivation of MLR models. The first number "1" indicated the derivative used, the second "10" showed the length of the segment for data points, and the last "5" indicated the length of the smoothing segment. The calibration equations were done by MLR using log (I/R) data.

The samples for calibration were analyzed in duplicate for total nitrogen (N) by the Dumas' method (Kirsten, 1983), and values were multiplied by the constant of 6.25 to obtain CP. Ash content was determined by ashing in a muffle furnace at 500°C for 5 h (AOAC, 1990). NDF, ADF and ADL were determined as described by Goering and Van Soest (1970).

RESULTS AND DISCUSSION

From the 82 samples scanned, 35 (43%) were selected on the basis of spectral differences and were used for NIRS calibration against quality determinations. The mean and range of the CP, ash, NDF, ADF and ADL values are summarised in Table 1. Means and ranges reflect typical values for forages (Berardo et al., 1993, 1997; Ordoardi et al., 1999; Stuth et al., 2003). There was a wide variation in chemical composition because of the diversity of the legume species, stages of maturity at sampling and differences in plant fractions (leaves versus stems) used. The forage legumes were also harvested at sites with different agro-ecological conditions.

Table 2 summarises the statistics, including the standard error of calibration (SEC), squared coefficient of multi-determination (R^2) for the predictions obtained for each of the chemical constituents. R^2 values ranging from 0.74 in ADL to 1.00 in ADF were obtained. CP R^2 was 0.97 and is comparable to values of 0.92 reported in other forages (Castro, 2002; Stuth et al., 2003). Performance of the cross-validation set, expressed as the squared coefficient of correlation (1-VR) and standard error of cross validation (SECV) are also shown in Table 2. Highest SEC and SECV of 1.70 and 1.91, respectively were obtained for ADL. The squared coefficient of correlation (1-VR) for the cross validation was very poor for ADL (0.67). Precision in estimating lignin has been reported to be generally lower than protein or ADF (Stuth et al., 2003). This could be related to the sequential method of ADL determination used for this work, as a

Table 1. Mean and range of chemical composition (g/kg DM), and standard deviations (SD) of the calibration set for NIRS of samples for cowpea, Silverleaf desmodium, Fine stem stylo, Scabra, *B. spiciformis* and veld hay from Zimbabwe.

Chemical component	N	Mean	Minimum	Maximum	SD
CP	31	127	47.1	264	5.66
Ash	34	83.3	25.7	152	3.60
NDF	31	494	284	738	11.3
ADF	30	344	148	560	11.4
ADL	32	109	53	197	3.36

N = number of samples used.

Table 2. Statistics of NIRS calibration equation for best fit and cross validation, including SEC, coefficient of determination (R^2) and standard error of cross-validation (SECV), coefficient of correlation (1-VR) and wavelength for cowpea, Silverleaf desmodium, Fine stem stylo, Scabra, *B. spiciformis* and veld hay from Zimbabwe.

Chemical component	N	SEC	R^2	SECV	1-VR	Wavelengths (No.)
CP	31	0.90	0.97	1.21	0.95	168
Ash	34	1.09	0.91	1.22	0.89	168
NDF	31	1.31	0.99	1.65	0.98	172
ADF	30	0.80	1.00	1.67	0.98	172
ADL	32	1.70	0.74	1.91	0.67	168

N = number of samples used.

Table 3. Statistics of a NIRS prediction set (N = 47), including means and standard error of laboratory analysis (SEL) and SEP for cowpea, Silverleaf desmodium, Fine stem stylo, Scabra, *B. spiciformis* and veld hay from Zimbabwe.

Chemical component	Laboratory mean (g/kg DM)	SEL	Predicted mean (g/kg DM)	SEP
CP	12.7	5.66	12.7	5.52
Ash	83.3	3.66	83.6	3.49
NDF	499	11.8	492	11.0
ADF	339	11.4	344	11.9
ADL	10.9	3.36	10.9	2.96

greater 1-VR value for ADL of 0.81 has been obtained for *Cajanus cajan* (pigeon pea) (Berardo et al., 1997) by determining ADL without first going through the NDF and ADF determination. ShuJing et al. (2009) reported determination coefficients of calibration (R_{cal}^2) were 0.9870 and 0.9931 and those of cross validation (R_{cv}^2) were 0.9413 and 0.9678 for ADF and NDF, respectively.

The precision with which the NIRS predicted laboratory analysis using results from the remaining 47 samples of forage legumes (Table 3) confirmed that the validation was as precise as those obtained by other authors using grasses (Berardo, 1992; Berardo et al., 1993; Cosgrove et al., 1994; Baker et al., 1994). The standard error of prediction (SEP) was used to judge the predictive ability of the calibration equation (Stuth et al., 2003). Generally, SEP reflects the accuracy of the laboratory chemical analysis and in this case the SEP values obtained ranged from 2.96 in ADL to 11.9 in ADF. These values obtained were slightly smaller in almost all chemical components predicted.

Conclusion

It appears that NIRS can also be used to predict the chemical composition of tropical forage legumes with high accuracy. Therefore, this method could be more widely used in the evaluation of tropical forage legumes for the assessment of their chemical composition and, consequently, their nutritional value. However, caution needs to be employed when applying the developed calibration for new materials, as their spectral variability may not yet be covered.

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