Near infrared analysis of forage quality in tropical legumes from Zimbabwe

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Introduction

Tree legumes are widely utilised in agriculture and forestry, not only for fertility improvement, fuelwood production and soil conservation, but particularly as dietary supplements for ruminants in both intensive and extensive agroforestry systems in humid and semi-arid regions of the tropics.[1] For this last purpose, the nutritive value and the anti-nutritive factors of the forage shrub legumes have to be investigated, and their introduction and diffusion are now studied in more and more detail. With this objective, a research project was granted by the EU, involving different European Institutions, and the University of Zimbabwe in Harare. Our institute in Lodi was responsible for studying the presence of anti-nutrients, particularly saponins, in a wide range of different forage legumes, both herbaceous and browse trees, collected in several locations in Zimbabwe, during successive growing seasons over three years; on the same samples, a complete analysis of the chemical components of dry matter (DM) was undertaken, and specific near infrared (NIR) calibration equations have been derived, starting with pigeon pea; [2] a new series of wide calibrations is now presented, useful for a rapid assessment of the nutritive value of many different tropical forage legumes.

Materials and methods

One hundred and sixty-five plant samples, belonging to 32 tropical legume species, were included in this study and are listed in Table 1. The samples derived from plants grown in experimental trials in different locations of Zimbabwe, during the years 1993 to 1996, were collected and separated, when necessary, into leaves, stems and pods, dried at 65°C and ground to pass a 1 mm screen in a cyclone mill. All samples were analysed in duplicate for: protein content (N × 6.25) by the Dumas' method,[3] ash by ashing in a muffle furnace at 550°C for 5 h, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Goering and Van Soest.[4]

NIR scanning

About 2 g of sample was placed in a sample holder with a 3 cm diameter quartz window and scanned on an NIRSystems model 5000 monochromator (NIRSystems, Silver Springs, MD, USA). Reflectance spectra (log 1/*R*) from 1100 to 2500 nm were recorded at 2 nm intervals, giving 700 datapoints per sample. Using the SELECT algorithm described by Shenk and Westerhaus[5] and available in the ISI software (ISI, Port Matilda, PA, USA), a sub-population of samples was derived which represented the spectral characteristics of the whole population. This sub-population, comprising of 49 to 53 plant samples, was used for the development of all calibrations, while the remaining samples

Table 1. List of the tropical legumes grown in Zimbabwe, analysed in this study.

Species	Common name	Part of plant
Acacia angustissima	acacia	leaf–stem–petioles
Acacia boliviana		leaf–stem
Acacia karoo		leaf
Acacia nilotica		whole
Brachystegia spiciformis	musasa	whole
Cajanus cajan	pigeon pea	leaf–stem
Calliandra calothyrsus	calliandra	leaf–stem
Cassia rotundiflolia	cassia	leaf–stem–pod
Desmodium uncinatum	cv. Silverleaf	leaf
Desmodium intortum	cv. Greenleaf	leaf
Dichrostachys cinerea		whole
Flemingia congesta		leaf
Gliricidia sepium	gliricidia	whole
Glycine wightii	cv. Cooper	leaf
Lablab purpureus	lablab, dolichos	whole
Leucaena diversifolia	leucaena	leaf
Leucaena leucocephala	cv. Cunningham	leaf–petioles
Leucaena pallida		leaf–stem–pod
Lupinus albus	white lupin	seed
Lupinus angustifolius		leaf
Lupinus cosentinii		leaf
Lupinus luteus		seed–leaf
Lupinus pubescens		leaf
Macroptilium atropurpureum	siratro	leaf–whole
Macrotyloma axillare	cv. Archer	leaf
Securinega virosa		leaf
Sesbania macrantha		leaf
Sesbania sesban		leaf
Stylosanthes guianensis	stylo, cv. Oxley	whole
Stylosanthes scabra	stylo, cv. Fitzroy	leaf–whole
Vigna unguiculata	cowpea	whole
Ziziphus mucronata		leaf

Table 2. Chemical composition (% DM) of the set of samples of tropical legumes analysed.[a]

Variable	*n*	Mean	Range	*SD*
CP	160	13.6	4.4–28.6	6.7
NDF	162	54.4	27.1–76.2	12.0
ADF	163	38.6	13.4–59.5	11.3
ADL	165	13.5	5.3–26.0	4.1
Ash	163	5.8	2.2–14.2	2.2

[a]*n*, number of samples
CP, crude protein
NDF, neutral detergent fibre
ADF, acid detergent fibre
ADL, acid detergent lignin

were regarded as a validation set. Table 2 shows the distribution of the chemical components of dry matter in the whole population of samples analysed in this study. The sub-populations used for calibration and validation purposes had a similar and comparable composition.

The calibration equations were derived for each parameter using MPLS (modified partial least squares) regression and then tested for predictive performance by applying them to the remaining samples and calculating the standard error of prediction (*SEP*) and the variance accounted for (R^2 validation), in line with the procedures described by Windham *et al.*[6]

Results and discussion

From the 165 samples scanned, up to 53 were selected, on the basis of spectral differences, and were used for calibration against chemical determinations. The mean and range values of the chemical composition (% DM) of the whole set of samples are summarised in Table 2. There was a wide and statistically significant variation in chemical composition because of the diversity of legume species, stages of maturity at sampling, differences in plant fractions (leaves, stems, pods, whole plant), effects of the agro-ecological sites and growing seasons. In fact, the mean crude protein (CP) concentration ranged from 4.4 to 28.6% DM, while the percentage of the cell wall and structural components, expressed as NDF, ADF and ADL, ranged from 27.1 to 76.2, from 13.4 to 59.5 and from 5.3 to 26.0% DM, respectively.

Table 3. Statistics of calibration and cross-validation of the selected samples, including standard error of calibration (*SEC*), coefficient of determination (R^2), coefficient of determination of cross-validation (R^2CV) and standard error of cross-validation (*SECV*).

Variable	*n*	Mean	*SEC*	R^2	*SECV*	R^2CV
CP	52	13. 7	0.98	0.98	1.56	0.94
NDF	49	52.0	1.84	0.97	2.70	0.95
ADF	49	35.1	1.79	0.97	3.42	0.91
ADL	43	11.3	0.57	0.97	1.39	0.81
Ash	53	6.6	0.75	0.94	1.21	0.85

Table 4. Statistical results of validation of the remaining samples, including mean predicted v. laboratory values, standard error of prediction (*SEP*) and squared coefficient of correlation (r^2).

		Mean			
Variable	*n*	Predicted	Laboratory	r^2	*SEP*
CP	105	13. 1	12.7	0.94	1.63
NDF	110	55.9	55.8	0.96	2.59
ADF	112	40.2	39.8	0.94	3.01
ADL	104	13.9	13.5	0.76	1.90
Ash	110	5.6	5.7	0.75	0.96

The statistics of calibration and cross-validation for the equations obtained for each of the chemical constituents are reported in Table 3, including standard errors of calibration (*SEC*) and R^2 values, the R^2CV values for the cross-validation and standard errors of cross-validation (*SECV*). The standard errors of calibration were of high quality ($R^2 \geq 0.97$) for all the variables examined, except for Ash, in which a lower coefficient of determination was found (0.94). The trends of response were different and decisively lower for the variables in the cross-validation procedure, where R^2CV values ranged from 0.81 for ADL to 0.95 for NDF; for all variables the standard errors of cross-validation were substantially low, indicating good accuracy.

In Table 4, the statistics of validation are reported, which confirmed the high level of reliability of the NIR prediction of chemical parameters, compared to the laboratory values. The relationships between laboratory and NIR predicted parameters (r^2) were quite high for almost all variables (r^2 0.94), with the exception of minor components such as Ash (0.75) and ADL (0.76); this is also confirmed by the low values of the standard error of prediction (*SEP*), ranging from 0.96 to 3.01 for Ash and ADF, respectively.

In conclusion, the results presented here could be utilised widely for determing the chemical composition of forages derived from the same, or comparable, leguminous species. In fact, NIR reflectance spectroscopy offers new possibilities of applications, due to the well-known advantages of this analytical method (particularly minimal sample preparation, short time of analysis, cost-effectiveness), in research programmes aimed to increase forage productivity in developing countries through the utilisation of novel species, such as shrubs and tree legumes.

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