Using near infrared reflectance spectroscopy (NIRS) to determine nutritive value of tropical perennial grasses

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Abstract: *Near infrared reflectance spectroscopy was used to develop calibrations for nitrogen (N), dry organic matter digestibility (DOMD), acid detergent fibre (ADF) and neutral detergent fibre (NDF) for digit grass (Digitaria eriantha ssp. eriantha) cv. Premier and Rhodes grass (Chloris gayana) cv. Katambora. The coefficient of determination of calibrations developed for N and ADF were excellent, while the calibrations developed for DOMD and NDF were lower quality, but suitable for most applications. With additional sampling and calibration development these calibrations can be used to analyse plant samples from similar environments and could be broadened to other species and environments.*

Key words: NIR

Introduction

Near infrared reflectance spectroscopy (NIRS) is an accurate, rapid and cost effective analytical technique that has been commonly used to determine many organic compounds in a wide range of products. Since NIRS was first identified as having potential to determine nutritive value constituents of forage in the 1970s (Norris *et al*. 1976), there have been many advances in the technology and calibration methods, and the technique is now an accepted method for determining nutritive value of forages and is extensively used throughout the world (e.g. Alomar *et al*. 2003; Shenk and Westerhaus 1994).

This paper describes the development of calibrations on an NIRS for nitrogen (N), dry organic matter digestibility (DOMD), acid detergent fibre (ADF) and neutral detergent fibre (NDF) for two tropical perennial grasses. The calibrations will be used to determine these four nutritive value constituents on tropical perennial grass samples collected from an experiment near Tamworth, New South Wales (NSW).

Materials and methods

Field experiment

A study was conducted on a red chromosol soil (Isbell 1996) near Tamworth, NSW (31°16'S, 150°52'E, 490 m). The experiment was a split-plot design with three replicates. Main-plots were defoliation frequency; defoliated every 2 and 6 weeks (to a height of 50 mm using a rotary mower fitted with a catcher), with three forage species and five N rates randomised within each defoliation treatment. Forage species consisted of two perennial grasses; digit grass (*Digitaria eriantha* ssp. *eriantha*) cv. Premier and Rhodes grass (*Chloris gayana*) cv. Katambora sown in December 2005, and forage sorghum (*Sorghum bicolor* ssp. *bicolor* x *S. bicolor* ssp. *drummondii* hybrid) cv. Sweet Jumbo, which was sown in spring each year. Nitrogen treatments were applied at rates of 0, 50, 100, 150 and 300 kg N/ha as Easy N® (425 g N/L). Nitrogen was applied as 50 kg N/ha every 6 weeks (after defoliation), except for the 300 kg N/ha rate which was applied in three applications of 100 kg/ha. Defoliation treatments were from spring–autumn when the tropical grasses were actively growing for two seasons; 2006–07 and 2007–08. Nitrogen applications commenced with the first defoliation in spring of each season. Over the experimental period herbage mass and plant frequency were assessed and samples collected for analyses of soil N and forage nutritive value.

Sample collection for nutritive value analyses

Immediately prior to the application of each 6-week defoliation treatment, forage samples (0.4 x 0.4 m quadrat) were taken from digit grass and Rhodes grass plots fertilised with 0, 100 and 300 kg N/ha in both defoliation treatments from replicates 1 and 2 (i.e. a total of 24 plots sampled at each assessment). Samples were cut to a height of ~50 mm above the soil surface and stored in paper bags. In the laboratory, each

sample was separated into four components; green and dead leaf, and green and dead stem (when present). These components were dried at 65°C for 48 h and ground to pass through a 1-mm sieve (Shenk and Westerhaus 1994). Plots were sampled five times in each growing season, giving a total of 146 and 175 samples in 2006–07 and 2007–08, respectively.

Additional tropical grass samples were collected from an adjacent experiment to give a broader range in nutritive value for NIRS calibration development. These samples were collected and processed using the method described above.

Chemical analyses of reference samples

All samples collected in the 2006–07 season, additional tropical grass samples from an adjacent experiment and 60 samples from the 2007–08 season were analysed (Anon. 2009) for N (Australian Fodder Industry Association (AFIA) method 1.5R), DOMD (AFIA method 1.7R), ADF [AFIA method 1.8A(a)] and NDF [AFIA method 1.9A(a)].

NIRS calibration development

Spectra for all samples were measured using a NIRSystems Model 6500 spectrophotometer (Foss NIRSystems Inc., Laurel, MD, USA) in reflectance mode using a quarter-full small sample cell. All spectra were recorded for the 408–2492 nm range and saved as the average of 32 scans per sample, however the colour range (408–807 nm) was not used in calibration development. Data analyses were performed using WinISI software (Foss NIRSystems Inc., Laurel, MD, USA).

Results and discussion

Identification of appropriate mathematical treatments

All spectra from samples collected in the 2006–07 season and the additional samples were inspected and outliers removed from the data set. A subset of 55 samples, representing the range in each constituent was selected as a preliminary validation set, leaving the remaining 92 samples for preliminary calibration development. For each of the four nutritive value constituents, the NIR spectra in the preliminary calibration sample set were initially transformed using the mathematical treatment 2,6,4,1 with each of five scatter transformation options; standard normal variate (SNV) and detrend, SNV only, detrend only, standard multiplicative scatter correction (MSC) and weighed MSC. In the mathematical treatment, the first value is the order of the derivative, the second the segment gap in data points over which the derivative is calculated, and the third and fourth values are the number of data points used for smoothing (Williams 1987). Several regression methods were also tested and modified partial least squares (PLS) was found to be superior to PLS and principal components regression. The calibration developed using each mathematical treatment and scatter option adjustment was validated using the preliminary validation sample set to determine the optimum mathematical treatment. The treatment 2,6,4,1 with scatter option SNV and detrend provided the best predictions for N and ADF. Adjustment of the derivative and smoothing resulted in good predictions for NDF and DOMD, and DOMD also performed better with the detrend only scatter transformation option (Table 1).

Calibration development

Samples from the preliminary calibration and validation sample sets were recombined and used to develop a calibration (n = 147) for each of the 4 nutritive value constituents using the optimum mathematical treatments (Table 1). Calibrations were then used to predict the four constituents from a validation sample set represented by 60 samples collected in the 2007-08 season. These samples were identified by the WinISI software based on their spectral diversity and covered the range in the calibration of each constituent. The statistics of the validation samples and regressions are also shown in Table 1. The coefficient of determination was high (r^2 >0.85, Table 1) for each constituent, in particular N and ADF, indicating the suitability of the calibrations for many applications (Osborne *et al*. 2002). The calibration for DOMD was the poorest and should not be used to replace chemical analysis, however it is suitable for screening purposes.

Our calibrations were similar to those reported by others. For example, Smith *et al*. (1998)

reported better prediction statistics for dry matter digestibility of annual ryegrass (*Lolium rigidum*) (r^2 = 0.93, standard error of prediction (SEP) = 3.4) than for N (r^2 = 0.88, SEP = 1.3).

Prediction of nutritive value constituents for all samples

To predict the four nutritive value constituents for all of the samples in the experiment described above, all samples from both the calibration and validation sample sets were combined and new calibrations developed using the optimum mathematical treatments (Table 1). These calibrations (n = 207) had smaller standard deviations and standard errors of calibration to those developed using only samples from the 2006-07 season (n = 147) and would be best used to predict these nutritive value constituents on any future samples representative of the range included in the calibration sample set. Such calibrations should be validated by selecting ~1 in every 10 samples for chemical analysis that cover (or extend) the range of each constituent in the calibration. Inclusion of these samples and

Table 1. NIRS mathematical treatment, calibration and validation sample and regression statistics, and statistics for combined sample calibrations for nitrogen (N, %), dry organic dry mater digestibility (DOMD, %), acid detergent fibre (ADF, %) and neutral detergent fibre (NDF, %)

SNV, standard normal variate; SD, standard deviation; SEC, standard error of calibration; r^2, coefficient of determination between NIRS and chemical values; SEP, standard error of prediction; Slope, slope of the regression between chemical and NIRS values; Bias, mean difference between chemical and NIRS values; SEP(C), standard error of prediction corrected for bias.

Statistics	N (%)	DOMD (%)	ADF (%)	NDF (%)
	Mathematical treatment and scatter transformation			
	2,6,4,1, SNV and detrend	3,6,1,1 and detrend	2,6,4,1, SNV and detrend	3,6,1,1, SNV and detrend
	Calibration sample and regression statistics			
n	147	147	147	147
Mean	2.3	56.09	30.67	63.73
Minimum	0.2	43.0	16.0	27
Maximum	4.6	71.0	45.0	84
SD	1.02	5.15	4.67	6.58
SEC	0.10	1.85	0.92	1.61
r^2	0.99	0.87	0.96	0.94
	Validation sample and regression statistics			
n	60	60	60	60
Mean	2.4	56.5	29.7	62.8
Minimum	0.8	44.0	22.0	52.0
Maximum	3.4	63.0	45.0	78.0
r^2	0.95	0.80	0.94	0.83
SEP	0.12	2.09	1.09	2.01
Slope	0.98	1.02	1.07	0.99
Bias	-0.02	-0.24	0.10	-0.15
SEP(C)	0.12	2.09	1.09	2.02
	Combined regression statistics			
n	207	207	207	207
SD	0.91	5.00	4.61	6.16
SEC	0.09	1.66	0.92	1.44
r^2	0.99	0.89	0.96	0.95

any outside the current range would improve its robustness. The calibrations described in this paper were for only two species and so should be considered 'species-specific'. Calibrations based on a range of species from different environments (i.e. 'broad-based' calibrations) have been found to give values with a similar degree of accuracy as species-specific calibrations, but to be effective broad-based calibrations need to include samples representing all possible sources of variation (Brown *et al*. 1990).

NIRS is an effective method to predict nutritive value of forages, including tropical perennial grasses. However, error is associate with all methods and in NIRS measurement it may result from a range of sources (Hruschka 1987), principally sampling error (e.g. homogeneity of the sample), reference error (i.e. variation between duplicate samples used for chemical analysis) and NIR method error (e.g. spectral measure error and poor choice of mathematical treatment). The development of robust calibrations relies on the inclusion of appropriate samples for calibration, using the best mathematical procedures to obtain the most accurate calibration and including samples that are representative of all possible sources of variation (Hruschka 1987).

Conclusions

Optimum mathematical treatments were identified for calibration of an NIRS for four nutritive value constituents (N, DOMD, ADF and NDF) for two tropical perennial grasses and used to develop a calibration to predict each constituent. The coefficient of determination of calibrations developed for N and ADF were excellent, while the calibrations developed for DOMD and NDF were lower but suitable for most applications. These calibrations will be used to predict the nutritive value of leaf and stem samples collected over two growing seasons in the current experiment.

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