Broad NIRS calibrations to predict nutritional value of the southern feedbase

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Abstract
Near infrared reflectance spectroscopy (NIRS) is used to predict the nutritive characteristics of feeds consumed by livestock. Once calibration equations are developed between reflectance and measured nutritional traits, NIRS is rapid and inexpensive. Our aim was to develop broad NIRS calibrations for the southern feedbase of Australia. A total of 4385 samples from 154 accessions of 109 species of annual and perennial legumes, grasses and forbs were grown in common plots at two locations over 3 seasons. Plots were sampled across all growth stages. A quarter of these samples were subject to laboratory analysis of dry matter digestibility (DMD), total nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and organic matter (OM). Of the samples analysed, half were used to develop the calibration and half were set aside for validation. Development of broad calibrations across the sample range was very successful despite the large variation in the taxonomy and life history of the samples. When predicting samples from the collection that were not included in equation development, the statistics of prediction were; total N - r^2 0.96, RPD (relative percent difference) 5.3, *in vitro* DMD - r^2 0.93, RPD 3.7, ADF - r^2 0.93, RPD 3.9 and NDF - r^2 0.95, RPD 4.3. When the validation samples were separated into taxonomic groups, the prediction errors were considerably lower for annual species than perennial species. The data generated is being used to compare nutritional value of species over time and investigate opportunities to improve productivity and reduce methane emissions intensity from sheep in southern Australia.

Key words
Feed testing, multispecies, pasture, forage, plant improvement

Introduction

NIRS is used by industry to predict the nutritional characteristics of feeds consumed by livestock. The method relies on the development of mathematical relationships between measured traits and light absorption properties in the NIR region (wavelength range 700 – 3000 nanometres). Once calibration equations are developed, NIRS is rapid, inexpensive, non-destructive and can predict a large range of traits at the same time (Deaville and Flinn 2000). It is therefore a powerful tool within forage improvement/breeding programs for identifying plant species and genotypes within species with superior nutritional traits. NIRS also allows industry to conduct rapid assessment of the nutritional value of feeds and pastures, therefore informing feed purchasing and grazing management decisions. By providing a tool to improve quality of the feedbase and influence management, NIRS calibrations can be used to improve productivity, profitability and possibly reduce methane emissions intensity from ruminant industries.

There have been a number of studies exploring how much taxonomic and/or nutritional diversity is required to develop robust calibrations. Shenk and Westerhaus (1993) found that if enough samples are utilised, broad multi-forage species calibrations can be nearly as accurate as those for single species. In southern Chile, a calibration was successfully developed for mixed swards, comprising 8 perennial grass and legume species (Lobos et al 2013). The aim of this project was to investigate the feasibility of developing broad NIRS calibrations for predicting nutritional value of the southern feedbase of Australia. Using samples from 109 forage species we tested the hypothesis that it would be possible to develop a global calibration across a diverse range of forage species.

Materials and methods

We utilised 4385 samples representing 154 accessions from 109 species of temperate forages. The accessions were grown in common garden plots at 2 locations (Urrbrae in South Australia and Brookton in

Western Australia). The diversity of the sample base included commercialised and experimental material, with 60 accessions of annual legumes, 30 accessions of perennial legume, 18 accessions of annual grasses, 25 accessions of perennial grasses, 12 accessions of annual forbs and 9 accessions of perennial forbs. To capture the possible diversity in nutritional profiles, plants were sampled across all growth stages (approximately every 3-6 weeks).

Sample growth, collection and processing

Each accession at each site was located in randomised plots (1 x 8 m) within 3 replicated experimental blocks. Annual legumes, annual grasses and forbs, perennial legumes and perennial grasses and fobs, were in separate but adjacent plots within the same paddock. Sowing rates, herbicides, fertilisers and inoculants (for legumes) were according to recommended best practice. At sampling, biomass was harvested from a new area within the plot, so it was not regenerating growth from a previous cut. Samples were collected between June 2012 and December 2014 and were either frozen and later freeze dried or placed in a paper bag then oven dried at 60^{o}C. After drying, samples were ground to pass through a 1 mm screen using either a Cyclotech or Retsch Twister mill grinder. A preliminary study revealed that there was little bias associated with these grinders. Samples were then scanned by NIRS and throughout the project a subset was set aside for chemical analysis. Initially we selected a range of species and sampling times for analysis and then focussed on spectral gaps and outliers. Across the 3 year project a total of 1086 samples were subject to the full range of laboratory analyses.

NIRS scanning and mathematical treatments

Spectra were collected using a Unity Spectrastar 2500X- rotating top window system (Unity Scientific). The spectrum file data from the Spectrastar was converted to a multifile for the chemometric software package Ucal (Unity Scientific) used to generate predictions. Partial least squares regression was used to develop the calibrations. We tested a range of pretreatment options including standard normal variate detrending and derivatization with different derivative gap and smoothing. From this the best performing equations were selected. No wave specification trims were utilised and the entire available spectra from 680 nm to 2500 nm was employed. Outlier limits were left at default settings; T limit = 2.5, GD limit = 3.0 and neighbourhood size = 0.20. A dataset of 910 spectra with chemistry that included samples across all taxonomic groupings from both sites across seasons was used to develop the calibration presented in this paper. Approximately half the dataset (n=460) was used to develop the calibration and the remaining half for independent validation (n=450).

Assessing predictive ability

The performance of calibration equations was assessed using a number of criteria. Initially the r^2 value, 1-vr, SECV and RPD value. RPD tests the strength of the relationship between a constituents values and the error of the NIR predicted results and was calculated by $RPD = 1 / (1 - r^2)^{0.5}$. The larger the RPD value the greater the predictive ability of the calibration. We have adopted the guide of Williams (2014) who suggested RPD values of 0.0–1.9 are very poor and not recommended for forage testing; RPD values of 2.0–2.4 are only of use for rough screening; RPD values of 2.5–2.9 offer a fair screening potential; RPD values of 3.0–3.4 are good; RPD values of 3.5–4.0 are very good and RPD values of 4.1+ are deemed excellent.

Wet chemistry

In vitro DMD, adjusted to predict in vivo DMD, was determined in duplicate using a modified pepsin-cellulase technique described by Clarke et al. (1982). Modifications include different sample weight (600 mg), the use of ANKOM Technology F57 filter bags, sealed plastic boxes as incubation vessels and use of an orbital mixer incubator (set at 48^{o}C and 2rpm). Duplicate samples of seven AFIA standards (AFIA 2007) with known in vivo DMD were included in each batch to allow raw laboratory values to be adjusted to predict *in vivo* DMD using linear regression (mean se across runs for standards was 0.261%). Concentrations of NDF and ADF of the material were measured sequentially, according to operating instructions, using an Ankom 200/220 Fibre Analyser (Ankom® Tech. Co., Fairport, NY, USA). Duplicate samples were analysed for each diet. An oaten hay QC sample was included in each of the 103 fibre analysis runs (NDF 30.19 ± 0.1137% DM and ADF 19.71 ± 0.0665% DM). Total ash was measured on duplicate samples according to the methods of Faichney and White (1983). Total N and C were determined by combustion using a Leco CN628 N Analyser (Sweeney and Rexroad 1987).

Results

Table 1 presents the performance statistics for the mature calibration. Total N was predicted with an RPD of 5.3, falling into the excellent category of Williams (2014). The mean error of prediction was 0.17% (equating to about 1% CP). Predictions of NDF were also excellent with an RPD of 4.3 and an error of 3.5% units. The ADF predictions were very good with an RPD of 3.9 and error of 2.1% units. DMD also fell into the very good category of Williams (2014) with an RPD of 3.7 and an error of 2.6% units. Predictions of OM were not as accurate with an RPD of 2.2 and an error of 0.85% units. We could not predict total C.

Table 1 Performance statistics of the mixed species NIRS calibrations

Trait	r^2	1-VR	SECV	RPD	r^2 (validation)	RPD (validation)
NDF	0.941	0.918	3.50	4.1	0.945	4.3
ADF	0.957	0.935	2.10	4.8	0.933	3.9
DMD	0.937	0.916	2.60	4.0	0.926	3.7
OM	0.905	0.851	0.02	3.2	0.794	2.2
N	0.977	0.967	0.17	6.6	0.964	5.3
C	0.713	0.634	0.71	1.9	0.495	1.4

Using the validation set, we investigated errors of prediction for each of the following groups; annual grasses, annual legumes, perennial grasses, perennial legumes and forbs. Table 2 presents the r^2 and RPD values derived from a regression of laboratory against predicted values. Across all groups, prediction of total N (thus crude protein) was excellent. The NIRS predictions were most accurate for the forbs, annual grasses and annual legumes. With the exception of OM, the RPD values indicate that we generated excellent predictions for these groups (RPD > 4.1). The calibration resulted in very good predictions of ADF and DMD for the perennial grasses but for NDF only yielded prediction that would be of value as a rough screening tool. For perennial legumes, ADF, DMD and OM predictions were very good but NDF was poor.

Table 2 Validation of global calibration with validation samples split into groups

Trait	Annual grasses		Annual legumes		Perennial grasses		Perennial legumes		Forbs	
	r^2	RPD	r^2	RPD	r^2	RPD	r^2	RPD	r^2	RPD
NDF	0.951	4.5	0.955	4.7	0.818	2.3	0.767	2.1	0.980	7.1
ADF	0.956	4.8	0.973	6.1	0.927	3.7	0.880	2.9	0.983	7.7
DMD	0.964	5.3	0.959	4.9	0.927	3.7	0.887	3.0	0.981	7.3
OM	0.920	3.5	0.907	3.3	0.790	2.2	0.930	3.8	0.880	2.9
N	0.986	8.5	0.980	7.1	0.987	8.8	0.966	5.4	0.984	7.9

Discussion

The data presented in this paper suggest that a large, robust NIRS calibration for estimating nutritional value of a broad range of samples from the southern feedbase of Australia is feasible. It is likely that these calibrations could be further improved by using a greater number of samples for calibration and less for validation. While the literature would suggest that increasing diversity leads to stronger calibrations (Shenk and Westerhaus, 1993), the diversity between species in this data set was much larger than others reported in the literature with inclusion of 109 species across a number of plant families. During the three year project, the accuracy of predictions from the calibrations declined after the first year as we increased the spatial and temporal diversity of the sample range with samples from the site in Western Australia and a second season in South Australia (data not presented). This highlights the need to include spatial and temporal diversity within the dataset if calibrations are to be used beyond the reference sample collection sites. If this calibration is to be developed further, we would seek to build spatial diversity by inclusion of the same species from other sites in southern Australia. We would also investigate ability to predict nutritional value of mixed swards.

These calibrations represent a useful tool for livestock industries in southern Australia as they are likely to encompass nearly all of the species that could appear in monocultures or mixed swards across all of their lifecycles. Inexpensive and rapid prediction of the nutritional value of pastures assists producers to optimise feed purchasing decisions, grazing management and growth rates of animals. This may lead to increased

profitability and reduced methane emissions intensity if animals reach slaughter weight faster with less feed inputs. For a subset of these samples, we have developed very good predictions of methane produced during batch culture fermentation.

Development of accurate calibrations can be very useful in plant development programs where large numbers of plants require assessment of their nutritional value. A recent study, where NIRS was used to predict the nutritional value of lucerne accessions within a breeding program, demonstrates this point. The team found significant variation between lucerne accessions for all traits and estimated M/D values of material at the same vegetative stage to range from 9.34 to 10.75 MJ ME/kg DM. Using the ruminant feeding model GrazFeed (Freer et al 1997), it was predicted that a pregnant Merino ewe (day 100 of gestation) offered the accession with the highest DMD would eat 1.2 kg of DM per day and grow at a rate of 210 g/week. In contrast the same ewe eating the accession with the lowest DMD would eat 0.97 kg of DM per day and lose 112 g/week. For mature, dry sheep, the difference in predicted weight gain was 3-fold (Norman et al 2013).

A critical factor leading to success of this work has been the quality of the laboratory data behind the calibration (Deaville and Flinn 2000). Not all differences between NIRS predictions and reference values can be ascribed to NIRS prediction error (Coates 2002) as the error sources of the reference method are incorporated into the model. By using a single, highly trained laboratory operator and adoption of a range of quality control samples, we managed to keep lab errors to a minimum (*in vitro* DMD 0.23%), NDF (0.11%) and ADF (0.7%). Our inability to develop good predictions for NDF in perennial legumes is an example of the importance of the quality of the reference data. Our variances between replicate samples during NDF measurement are larger for perennial legumes than for either annual legumes or annual grasses, suggesting a problem with the method we are using.

The current data set with over 1000 samples with matching scans and chemistry provides an excellent platform for future refinement, adoption of sample-specific PLS models or generation of calibrations for new nutritive traits. All reference samples from this study have been vacuum sealed and stored in dry conditions at 4^{o}C to allow measurement of other nutritional traits of interest in the future. Ongoing research is investigating tradeoffs in the use of fresh and unground material with a view to using hand held ASD for near real-time estimation of nutritional traits and to reduce costs.

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