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Rapid evaluation of pasture quality for a critically endangered mammal, the northern hairy-nosed wombat (*Lasiorhinus krefftii*)

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Abstract. Near-infrared spectroscopy (NIRS) was used to predict the nutritive value of forage species available to the critically endangered northern hairy-nosed wombat (*Lasiorhinus krefftii*). Nutritive attributes of the forage successfully estimated included total nitrogen concentration, fibre (including neutral detergent fibre, acid detergent fibre and acid lignin), organic matter, water soluble carbohydrates and *in vitro* dry matter digestibility. The reported results demonstrate the seasonal variability of the forage resource available to *L. krefftii* in its tropical savanna habitat. Multivariate modelling of the spectra enabled the nutritive value of forage samples to be estimated with coefficients of determination (r^2) of 0.770–0.995 and standard errors of the cross-validation of 0.070–2.850 using a modified partial least-squares analysis technique. The standard error of the laboratory was 0.02–1.42. This study demonstrates that broad-based NIRS predictive equations can be used to predict the nutritive value of a number of plant types available to a herbivore over time. By using NIRS the analyst can rapidly analyse large numbers of samples with limited reduction of precision, thereby enabling large-scale ecological applications that may have previously been impeded by time and costs.

Introduction

The northern hairy-nosed wombat (*Lasiorhinus kreftii*) is one of the most critically endangered mammals in the world, with only about 65 individuals surviving in a small patch of tropical savanna at Epping Forest National Park in north-eastern Australia (Hoyle *et al.* 1995). Tropical savannas are strongly seasonal and, in Australia, at least, subject to frequent and severe droughts (Walker and Gillson 1982). In particular, at Epping Forest National Park, prolonged droughts in the last two decades associated with El Niño effects (Nicholls *et al.* 1996; Suppiah and Hennessy 1996) have reduced the ability of the population of *L. krefftii* to recover from its already diminished size as fecundity is most likely linked to the food resource (Johnson 1991; Woolnough 1998). Also, the native grasses that once dominated the habitat of *L. krefftii* are being replaced by an introduced pasture grass (*Cenchrus ciliaris*) (Woolnough 1998) but it is not known what effect this will have on the nutritive resources available to the animals. Therefore nutritional analysis of the pasture resource available to free-ranging *L. krefftii* is crucial to management of this large grazing mammal.

Traditional methods of measuring nutritive value of forage are expensive and time-consuming. However, near-infrared reflectance spectroscopy (NIRS) offers ecologists a way to overcome these constraints and measure forage quality with a high degree of precision and accuracy (Batten 1998; Foley *et al.*1998). NIRS works by analysing the interaction between light and the chemical bonds that make up plant tissues. However, the absorbance peaks in a near-infrared spectrum are not unique but the sum of absorbances of a number of chemical bonds. Therefore, calibration equations that describe the relationship between laboratory analyses of a group of representative samples and their spectra are used to interpret the spectra of unknown samples (Batten 1998; Foley *et al.*1998).

Many of the early applications of NIRS aimed to predict the composition of relatively homogenous products, such as the protein content of wheat grains in a single season (Norris *et al.* 1976). However, if the technique is to be useful to ecologists and wildlife habitats, it must be able to be used with different species of plants collected in several locations over several seasons and broad-based calibrations should be robust (Foley *et al.*1998). This study aims to demonstrate

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that calibrations developed with many plant species are just as robust as those developed for a single species.

In this study we aimed (1) to evaluate whether NIRS was suitable for the nutritional analysis of plants in *L. krefftii* habitat, and (2) to report some preliminary analyses of the nutritive value of forage available to *L. krefftii*.

Methods

Collection site

Epping Forest National Park (3300 ha) is a protected area of tropical savanna located in central Queensland, Australia (22°21′S, 146°41′E). The habitat is semi-arid with a mean annual rainfall of 671 ± 228 mm and is the last remaining refuge for *L. krefftii* (Horsup 1998). Woolnough (1998) describes the structure of the vegetation community, the soil characteristics and the diet of *L. krefftii* in this area.

Plant samples

Lasiorhinus krefftii occupies just 300 ha of Epping Forest National Park. It was from within this 300 ha that whole-plant samples were collected. Plant samples, including native and exotic grasses and native sedges and forbs, were collected bimonthly over a three-year period (1993–96) to account for temporal variability in nutritive value. All samples were cut and air-dried until return to the laboratory where they were oven-dried at 60°C for a minimum of 24 h. Samples were then ground in a cyclone mill (Udy Corporation, Fort Collins, CO, USA) to pass through a 1-mm screen.

In preparation for collection of NIR spectra, ground samples were placed in a constant-temperature (5°C) and relative-humidity (15%) environment for a minimum of two weeks so that residual moisture was standardised. Standardising for residual moisture increases the predictive ability and repeatability of NIRS models (Baker *et al.* 1994).

Collection and storage of spectra

NIRS is commonly used in agriculture and standard methods have been developed (ASTM 1995). In our work we followed these standard procedures carefully and only give brief details here.

Ground samples (n = 154) were scanned with a near-infrared monochromator spectrophotometer (NIRSystems Inc. Model 6500, Silver Spring, MD, USA) with a spinning cup attachment. The instrument was operated under conditions of constant temperature (22°C) and relative humidity (55%). The instrument used ISI software (NIRS 3.11, Infrasoft International, Port Matilda, PA, USA), which was also used for subsequent data analysis.

Spectra were collected between 402 nm and 2498 nm at 2-nm intervals with the full spectra used in the prediction models. Repeatability of the NIR spectra was measured by deviations in optical data (log(1/R)) at each wavelength using an internal ceramic reference tile (Shenk and Westerhaus 1992). The log(1/R) function aims to linearise the relationship between the measured reflectance (R) and the absorbence of the substance (ASTM 1995). Sub-samples were scanned in duplicate, with the sample being re-packed between samples. Duplicate spectra were averaged and accepted as a spectral data point only if the difference between the two spectra did not exceed a root mean square of 50 (as suggested by Shenk and Westerhaus 1992).

Calibration set and calibration equation development

A calibration set was identified using the CENTER and SELECT algorithms of the ISI software. The CENTER algorithm was used to generate an algorithm to rank all spectra according to the Mahalanobis (H) distance (Mahalanobis 1936) from the mean spectrum using principal component scores. The principal component scores were calculated from the principal components (eigenvectors) of the spectra (Shenk and Westerhaus 1992, 1994). Mahalanobis distances were then standardised by dividing each value by the mean H to produce 'global H' values (Shenk and Westerhaus 1994). The ranking process of the CENTER-generated algorithm allowed identification of potential outliers or spectra belonging to a different population (Shenk and Westerhaus 1994).

The SELECT algorithm was used to identify spectra that were representative of all spectral variability. Selected spectra represented 'neighbourhoods' of common H distances (neighbourhood H), where spectra within the neighbourhood shared similar H distance with its neighbours (or similar spectral variability) (Shenk and Westerhaus 1992). The SELECT-generated algorithm operated on the assumption that one sample is representative of each neighbourhood (Shenk and Westerhaus 1994). In total, 86 samples were identified on the basis of spectral variability. An additional five samples were selected so that each species/genus was represented in the calibration set.

To develop the most appropriate predictive model for each measure of forage quality, a variety of models and mathematical transformations and smoothing functions were explored. Models examined included modified partial least-squares (MPLS), partial least-squares (PLS) and principal components regression (PCR), with each model cross-validated to prevent over-fitting (Shenk and Westerhaus 1994). The transformations and pre-treatment of the calibration models allowed comparisons of the linearisation process. Model variations included altering the derivatives of log(1/R) (e.g. no derivative, first derivative or second derivative), the number of data points over which the derivative is to be calculated, and/or the number of smoothing functions. For example, a mathematical transformation and smoothing function of 1,4,4,1 uses the first derivative of log(1/R) calculated every 4 data points (or 8 nm), with the first smoothing occurring across 4 data points and no second smoothing (Shenk and Westerhaus 1992). In addition, each calibration model used standard normal variate and detrend as a means for scatter correction. Scatter of data 'can distort the relationship between the NIR spectrum and the reference value' (Shenk and Westerhaus 1991) and is often caused by variation in particle size and moisture (Shenk and Westerhaus 1991). The standard normal variate and detrend method of correcting for scatter thereby improves the accuracy of the prediction (Shenk and Westerhaus 1991, 1992; Baker *et al.* 1994).

In total, 30 calibration models were derived for each nutritional variable; these were subsequently ranked on the basis of lowest standard error of the cross-validation (SECV) statistic and the highest coefficient of determination (r^2). The model that provided the best predictive capability for each nutritional variable was subsequently used to predict the variable value for each species. The relationship between observed and predicted values was assessed by least-squares linear regression for each quality attribute. Potential outliers, where the values predicted by the model were significantly different from the observed laboratory value, were identified using principal component scores and the Mahalanobis distance. In each case we repeated the laboratory analyses on these samples, and if necessary, eliminated the sample from the calibrations.

Chemical analysis

Variables of forage quality measured included organic matter (OM), total nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid lignin (AL), water soluble carbohydrates (WSC) and *in vitro* dry matter digestibility (IVDMD). Each attribute was measured in duplicate. An estimate of the standard error of the laboratory (SEL) procedures was calculated following the method of Smith and Flinn (1991).

Organic matter was determined by burning each sample in a muffle furnace at 550°C. Total nitrogen concentration of plant samples was determined by a semi-micro-Kjeldahl technique with a selenium catalyst using a Gerhardt Vapodest nitrogen analyser. Recoveries of

ammonium were checked against ammonium sulphate standards and were in the range of 99.7–101.6% for all analyses. Cell wall constituents were measured as NDF and ADF following the methods of Van Soest *et al.* (1991) but with the filter bag modification described by Komarek *et al.* (1994). Acid lignin was measured on the ADF residue in the filter bags, using the 72% sulphuric acid method described by Van Soest *et al.* (1991). Water-soluble carbohydrates were extracted using the method of Radojevic *et al.* (1994) and the concentration of the extract was estimated by the anthrone method (Yemm and Willis 1954) and expressed as fructose equivalents. The IVDMD determination followed a modification of the Choo *et al.* (1981) method, where samples were placed in filter bags and incubated with acid pepsin solution and fungal cellulase solution at 37°C for 24 h and 48 h respectively. No *in vivo* measurements of forage digestibility have been made for *L. krefftii* so the value of the *in vitro* procedures used here are comparative and do not imply that comparable digestibilities would be achieved *in vivo.*

Results

Development of calibration equations

All spectra collected (n = 154) showed an even distribution across the first three principal components (Fig. 1), suggesting that all spectra belonged to the same population (global 'H') with the possible exception of three outlying spectra. Biologically, the three outliers were distinct from other forage species considered, being from two genera of dicotyledons. Table 1 shows the best predictive model for each attribute of forage quality out of the 30 models explored. The modified partial least-squares (MPLS) model explained the maximum variation for each attribute of forage quality. This accords with the findings of Shenk and Westerhaus (1994) who showed that MPLS is a more stable and accurate algorithm than PLS. Variation in the mathematical transformations and smoothing functions also improved the predictive model, with the second derivative of log(1/R) being most successful in predicting N, OM, IVDMD, NDF and ADF while the first derivative of log(1/R) is best for AL and WSC. The optimum gap and the smoothing function for each quality attribute was 5 points (or 10 nm), allowing calculation of second or first derivatives of log(1/R) over spectra segments 10 nm in length. The possible exception is for NDF (8 points or 16 nm); however, there is little difference between a gap and smooth of 16 nm and 10 nm respectively in the estimates of SEC and r^2. The highest-ranked model for each attribute of forage quality produces comparable values for the coefficient of determination and standard error of the calibration to those found for a range of crop and pasture species by other studies (Table 2).

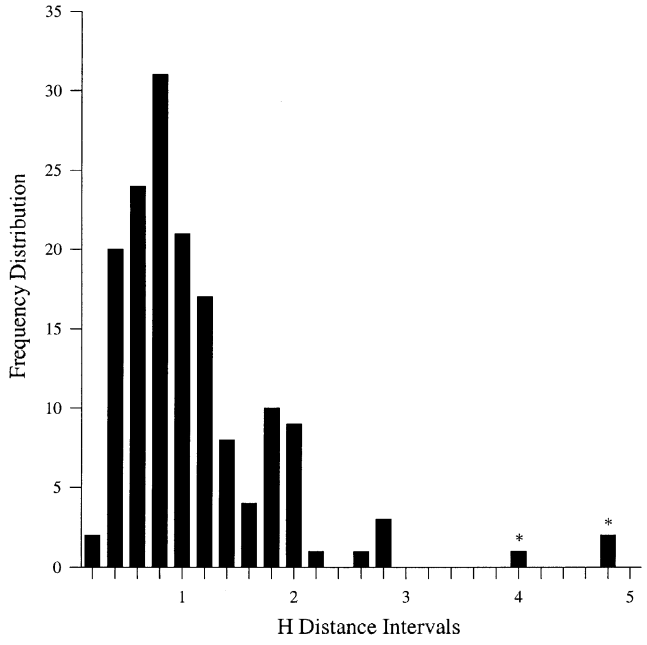

Fig. 1. Frequency distribution of global H values, demonstrating that all spectra, except three outliers (H > 3.0), belong to the same spectral population. Outliers, denoted by asterisks, are spectra from the dicotyledons *Salsola kalii* (n = 2) and *Carissa ovata.*

Agreement between predictive models and laboratory analyses

As expected, the laboratory analyses were most precise for constituents with well established and understood methods such as total nitrogen (measured by Kjeldahl), and water-soluble carbohydrate extractions with anthrone determinations proved the most accurate laboratory assays. The greatest source of error in the laboratory analysis was associated with the sequential fibre analysis.

There was excellent agreement between the NIRS method and the laboratory measurements. For every variable there was a significant relationship ($P \leq 0.001$) between values determined in the laboratory and those indicated by NIRS (Table 3, Fig. 2). The accuracy of the relationship was indicated by the SECV and the deviation of the regression line from the equation $y = x$ (where y is the NIRS-predicted value and x is the laboratory-measured value, with a slope of 1.00 and an intercept about the origin). The slopes of the relationship between values measured in the laboratory and the values predicted by NIRS did not differ significantly from 1.00 ($P \leq 0.001$) for all attributes of forage quality (Table 3).

Temporal changes in forage quality

Lasiorhinus krefftii mainly eats the native grasses *Aristida* spp. and *Enneapogon* spp., the exotic grass *Cenchrus ciliaris*, the sedge *Fimbristylis dichotoma*, and a wide variety (but small quantity) of forbs (Woolnough and Johnson 2000). Our NIRS-derived analyses indicated marked seasonal differences in the nutritive value of these plants. Importantly, the introduced species (*Cenchrus ciliaris*) was consistently richer in protein and showed higher *in vitro* dry-matter digestibility than many of the other major food items (Table 4). Overall, the nutritive value of the forage was

Table 1. Best calibration equations for each attribute of forage quality, ranked according to lowest SECV followed by highest coefficient of determination

All equations were derived using log(1/R) data subjected to standard normal variate and detrend methods of correcting for scatter (Baker *et al.* 1994). The tabulated results represent the best equations using permutations of various mathematical transformation and smoothing functions (0,4,4; 1,4,4; 2,4,4; 0,5,5; 1,5,5; 2,5,5; 0,8,8; 1,8,8; 2,8,8; 1,10,10) and the statistical methods MPLS, PLS and PCR. See text for explanation of variables. MPLS, modified partial least-squares; SECV, standard error of cross-validation; SEC, standard error of calibration; r^2, coefficient of determination

Quality variable assessed	Equation type	Derivative	Gap	Smooth	SECV	SEC	r^2
Total N	MPLS	2	5	5	0.070	0.043	0.995
NDF	MPLS	2	8	8	2.378	1.485	0.979
ADF	MPLS	2	5	5	2.850	1.918	0.891
AL	MPLS	1	5	5	2.284	1.816	0.770
OM	MPLS	2	5	5	1.705	0.808	0.967
WSC	MPLS	1	5	5	0.798	0.588	0.906
IVDMD	MPLS	2	5	5	3.386	2.184	0.964

greater during the wet season than during the dry season but was still low compared with other habitats.

Discussion

Near-infrared spectroscopy – a valuable tool for ecology

The benefits of NIRS have long been known among agricultural scientists (Shenk *et al.* 1992; Shenk and Westerhaus 1994) but there is little use of the method by ecologists (Foley *et al.* 1998). There are two major benefits of the application of NIRS. The first is the reduction of analytical cost and time without compromising precision or accuracy. Secondly, once the predictive equations have been developed, large numbers of samples can be processed. For example, once we had established calibration equations, we were able to analyse 120–140 ground plant samples in duplicate per day for eight nutritional attributes. In

Table 2. Review of validation statistics for studies using NIRS to predict variables of terrestrial forage quality

Validation statistics include coefficient of determination (r^2) and a measurement of standard error[A]. See text for explanation of variables

Forage description and country of study	N		NDF		ADF		AL		OM		IVDMD		Source
	r^2	SE[A]	r^2	SE[A]	r^2	SE[A]	r^2	SE[A]	r^2	SE[A]	r^2	SE[A]	
Cultivated crops, USA	0.99	0.74	0.98	2.39	0.96	1.56	0.96	0.80	–	–	–	–	Norris *et al.* (1976)
Cultivated grain crops, USA	0.96	0.95	0.95	2.64	0.86	2.31	–	–	–	–	0.89	2.73	Shenk *et al.* (1979)
Semi-arid rangeland grasses, forbs and browse, USA	0.98	0.37	–	–	0.90	1.26	0.67	0.67	–	–	–	–	Ward *et al.* (1982)
Temperate grasses and legumes, USA	1.0	0.03	1.0	0.09	1.0	0.36	0.99	0.23	0.72	1.52	1.0	0.73	Brooks *et al.* (1984)
Semi-natural grasses and legumes, Spain	0.95	0.56	0.91	1.97	0.87	1.24	0.91	0.47	–	–	–	–	García-Ciudad *et al.* (1993)
Mature annual legumes, Australia	–	–	0.93	0.31	0.91	0.44	0.79	1.09	–	–	–	–	Kellaway and Stimson (1993)
Trees and shrub foliage, France	0.98	0.11	0.99	1.36	0.97	1.85	0.97	1.04	0.97	0.54	0.99	1.51	Meuret *et al.* (1993)
Grass silage, UK	–	–	–	–	–	–	–	–	–	–	0.82	2.35	Baker *et al.* (1994)
Semi-arid rangeland grasses, Argentina	0.90	0.31	0.94	0.43	–	–	0.93	0.21	–	–	0.97	1.14	Rabotnikof *et al.* (1995)
Semi-arid rangeland grasses, Spain	–	–	–	–	–	–	–	–	0.88	4.6	–	–	Vázquez de Aldana *et al.* (1996)
Semi-arid tropical savanna grasses, sedges and forbs, Australia	0.99	0.04	0.98	1.49	0.89	1.92	0.77	1.82	0.97	0.81	0.96	2.18	This study

[A]Standard error estimates in the different studies are standard error for calibration (SEC) (Meuret *et al.* 1993; Rabotnikof *et al.* 1995; Vásquez de Aldana *et al.* 1996; this study), standard error of prediction (SEP) (Shenk *et al.* 1979; Kellaway and Stimson *et al.* 1993; Baker *et al*. 1994; García-Cuidad *et al.* 1995; Rabotnikof *et al.* 1995) or standard error of the regression (SE) (Norris *et al.* 1976; Ward *et al.* 1982; Brooks *et al.* 1984). SEC is the error due to differences between the laboratory values and the NIR predicted values *within* the calibration set, while SEP is the same error but *outside* the calibration set (Kellaway and Stimson 1993).

Table 3. Performance of both laboratory methods and NIRS to measure variables of forage quality

For assessment of laboratory accuracy, '*n*' represents the number of samples in the calibration set, with the mean and standard deviation (SD) of laboratory values for each variable expressed as a percentage. The SD of duplicates and the standard error of the laboratory (SEL) both provide estimates of laboratory precision [SEL = $\sqrt{(\Sigma(y_1 - y_2)^2/n)}$ with y_1 and y_2 being duplicates of analyses (Smith and Flinn 1991)]. For assessment of the performance of the statistical model, least-squares linear regression statistics for predicted values for forage-quality variables using NIR spectra, with the appropriate statistical model, were compared with actual measurements made by laboratory analyses. The error between laboratory values and the values predicted by NIRS is measured by SECV and 1–VR. The SECV provides an estimate of accuracy and 1–VR explains variation in the reference method values explained by NIRS (Shenk and Westerhaus 1992). Validity of the statistical model is assessed by the difference of the slope from 1.0; this slope was not different from 1.0 for all variables. See text for explanation of variables

Forage-quality variable	Laboratory accuracy assessment					Statistical model performance assessment					
	n	Mean	SD	SD of duplicates	SEL	Regression slope	Regression intercept	r^2	SECV	1–VR	*P*
N	80	1.03	0.57	0.01	0.02	1.00	0.01	0.99	0.07	0.99	<0.001
NDF	87	68.11	10.70	0.86	1.42	0.98	1.15	0.98	2.59	0.95	<0.001
ADF	82	39.67	6.36	0.71	1.16	0.91	3.77	0.89	2.85	0.76	<0.001
AL	79	8.80	5.08	0.69	1.29	0.78	1.95	0.77	2.28	0.63	<0.001
OM	75	89.57	6.92	0.83	0.44	0.98	2.21	0.97	1.71	0.86	<0.001
WSC	85	3.16	2.20	0.004	0.08	0.92	0.25	0.91	0.80	0.92	<0.001
IVDMD	82	42.31	11.34	0.75	1.34	0.97	1.26	0.96	3.39	0.83	<0.001

comparison, a technique such as NDF analysis, even with improved methods, may be able to process 48 samples in duplicate per day at best (not including weighing and drying time). The reduction in time spent in the laboratory and reduced analysis cost then allows ecological experimentation or ecological monitoring of a single population on a broader scale than by conventional methods.

Another major benefit of NIRS is the measurement itself. Since data for each sample are recorded as spectra, the measurement is non-destructive. Therefore there are no expensive chemicals or hazardous by-products in measurements. In addition, spectra can be measured on relatively small samples (e.g. seagrass samples weighing less than 0.5 g dry matter: Aragones 1996), allowing many attributes to be measured on samples for which only one or two attributes could be measured by conventional methods.

The collection of spectra is subject to two major influences: the environment during collection (temperature and humidity) and the particle size of the samples (Shenk and Westerhaus 1991, 1992; Baker *et al.* 1994). To obtain repeatable and comparable spectra, standardising the collecting environment is critical. By standardising samples prior to measurement and the collecting environment, potential problems associated with sample moisture are reduced (Baker *et al.* 1994). Likewise, by applying standard normal variate and detrend to correct for scatter, the effect of variation in particle size is reduced and statistical models become more robust (Shenk and Westerhaus 1991, 1992; Baker *et al.* 1994).

The multivariate techniques of PCR, PLS and MPLS are the recommended standards for quantitative analysis techniques for NIR spectra (ASTM 1995). Each of these multivariate techniques aim to reduce the spectra to linear functions of the sample absorptions (ASTM 1995). Modified partial least-squares produced the best predictive models for each attribute of forage quality (Table 1), supporting the findings of Baker *et al.* (1994) and the recommendations of Shenk and Westerhaus (1991, 1994). The MPLS technique appears to be advantageous as most of the spectral data are reduced into relatively few linear combinations (Baker *et al.* 1994). The MPLS model for each forage-quality attribute was validated by cross-validation, producing a measurement of the SECV, standard error of the calibration (SEC) and a measurement of precision (either the correlation coefficient and/or 1–VR (one minus the variance ratio; where the variance ratio is the explained variance divided by the total variance)). Estimates of SECV, SEC, r^2 and 1–VR compare favourably with other studies (Table 2). The values of SECV and SEC increase as the model's ability to predict the forage-quality attribute decreases and the slope of the observed laboratory data *versus* values predicted by the NIRS moves away from 1.0.

In general, the precision of the NIRS measurements (comparing the SECV and the SEL) was at least twice as low as the precision of the lab measurements. This reflects the quality of the laboratory work, the agreement between duplicates and the relatively small size of the calibration set. Variability in the precision of predictive models between each attribute was a direct result of the methodology used in the conventional analysis. The predictive model for total nitrogen was very precise because of the high occurrence of nitrogen–carbon bonds in plant material. Other attributes of forage quality such as AL were comparatively low in precision but can still predict more than 77% of the variation. The decrease in precision for an attribute such as AL is, in part, due to the relatively complex structure of cellulose compared

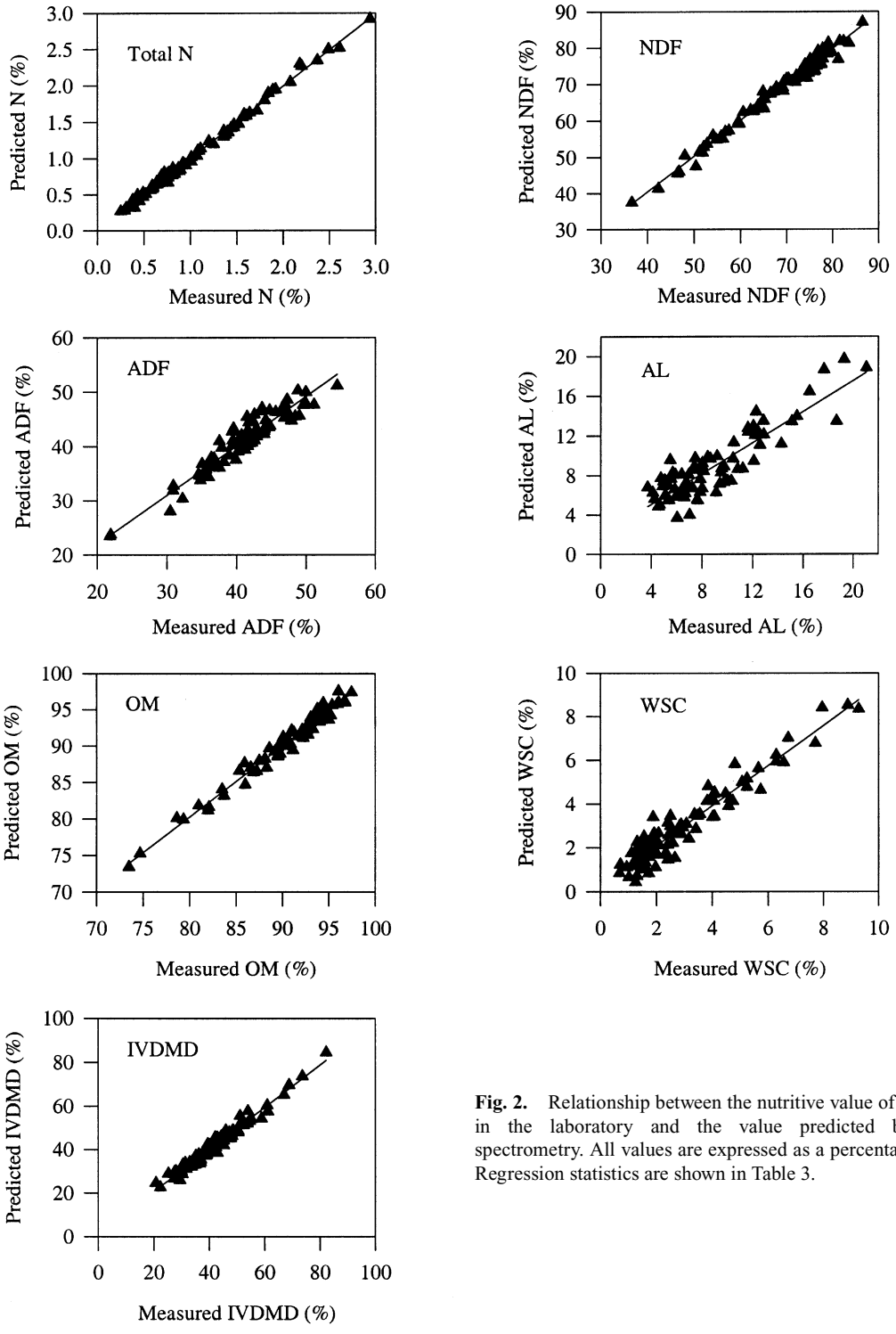

Fig. 2. Relationship between the nutritive value of forage measured in the laboratory and the value predicted by near-infrared spectrometry. All values are expressed as a percentage of dry matter. Regression statistics are shown in Table 3.

with the simple X–H bonds (where X represents carbon, nitrogen or oxygen and H represents hydrogen) as described by Shenk *et al.* (1992). Further contributing to the reduced precision of AL is the sequential fibre analysis used as the reference method. Potential errors in the sequential method will be magnified in AL compared with NDF. However, the reduction of precision is not significant and the predictive ability is still very useful in broad-scale ecological studies.

The precision of the predictive models can be constantly refined and improved. Increasing the sample size of the

Table 4. Seasonal variation in the nutritive value of forage species at Epping Forest National Park, central Queensland, Australia
Sample means (±1 s.d.) represent three years of data with the dry season from April to October and the wet season from November to March. The categories of plants analysed included native grasses (from 12 genera), an exotic grass, a native sedge and native forbs (from 6 genera). All nutritive variables are expressed as a percentage of dry matter, with the exception of WSC, which is expressed as fructose equivalents of dry matter. Table 1 provides abbreviations for nutritive variables

Nutritive variable	Plant type	Dry season				Wet season			
		n	Mean	SD	Range	*n*	Mean	SD	Range
Total N	Native grasses	56	0.70	0.26	0.28–1.66	27	1.01	0.60	0.27–2.5
	Exotic grass	18	0.89	0.49	0.38–1.94	15	1.10	0.52	0.56–2.15
	Sedge	8	1.47	0.43	0.56–2.15	5	1.72	0.52	1.24–2.52
	Forbs	12	1.47	0.58	0.69–2.92	13	2.03	0.41	1.38–2.71
NDF	Native grasses	56	76.60	4.20	62.85–86.84	27	75.37	5.66	54.91–84.66
	Exotic grass	18	75.06	4.09	66.7–81.99	15	74.51	3.37	65.78–80.19
	Sedge	8	67.45	3.28	63.26–73.27	5	67.79	7.04	57.3–76.47
	Forbs	12	56.95	9.17	45.63–75.89	13	51.38	8.82	37.38–64.33
ADF	Native grasses	56	44.24	3.56	35.27–56.09	27	43.21	3.22	36.94–48.29
	Exotic grass	18	41.14	3.20	35.52–47.26	15	40.81	2.912	34.8–45.94
	Sedge	8	40.84	4.05	34.16–46.42	5	38.04	5.96	30.44–44.29
	Forbs	12	39.30	6.66	29.17–51.28	13	36.96	6.50	24.1–47.22
AL	Native grasses	56	7.87	1.62	3.71–13.21	27	7.57	2.07	5.47–12.09
	Exotic grass	18	6.84	1.53	3.94–9.94	15	6.48	0.99	4.71–8.34
	Sedge	8	12.11	2.27	8.44–15	5	9.88	2.75	7.2–13.45
	Forbs	12	11.23	3.09	5.51–16.44	13	12.26	5.30	4.46–19.71
OM	Native grasses	56	92.46	3.58	81.16–99.27	27	91.83	5.22	75.24–96.64
	Exotic grass	18	88.31	4.49	78.17–94.96	15	88.98	4.20	82.86–96.31
	Sedge	8	86.56	3.54	80.1–90.01	5	87.40	3.87	81.59–91.53
	Forbs	12	88.45	7.55	73.4–95.75	13	87.43	6.17	76.5–94.81
WSC	Native grasses	56	2.25	1.01	0.43–5.00	27	2.54	1.57	0.16–5.94
	Exotic grass	18	2.57	1.56	0.33–5.26	15	2.69	1.15	1.06–4.96
	Sedge	8	2.08	1.11	0.72–4.53	5	2.67	2.45	1.11–6.79
	Forbs	12	4.23	2.69	0.66–8.52	13	4.28	2.63	1.57–9.68
IVDMD	Native grasses	56	33.72	6.47	22.64–57.66	27	35.31	7.75	25.89–65.13
	Exotic grass	18	41.58	6.68	28.17–50.75	15	43.12	6.53	28.33–52.06
	Sedges	8	47.12	4.57	37.11–53.08	5	48.50	5.37	42.52–54.28
	Forbs	12	51.98	14.52	31.18–84.53	13	55.24	12.35	41.91–78.93

calibration set will improve each model by reducing the effect of outliers. Further, increasing the sample size will improve the ability of the model to predict a diverse range of species and temporal variation. Consequently, NIRS models can be continuously improved to suit the function and purpose of the model.

The potential application of NIRS to aspects of ecological research seems great, particularly in plant–herbivore systems. As well as in studies of forage quality such as this one and others (Table 2), NIRS has been applied to other aspects of grassland studies, including measurement of water-use efficiency estimated from $\delta^{13}C$ discrimination (e.g. Clark *et al.* 1995) and measurement of botanical composition of mixed-species hay harvests (e.g. Coleman *et al.* 1985; García-Ciudad *et al.* 1991, 1993). NIRS has also been applied to animal-production studies to predict rates of intake (e.g. Flinn *et al.* 1992; Poppi 1996) and estimate botanical composition of diet from oesophageal extrusa (e.g. Volesky and Coleman 1996) or faeces (e.g. Lyon *et al.* 1995). Future broad-scale applications may include measuring botanical composition of free-ranging herbivore diets, estimating rates of intake of free-ranging herbivores, with excellent potential for application in the field of herbivore-antifeedants and quantification of secondary metabolites produced by plants (e.g. Windham *et al.* 1988).

The products of this study can be specifically applied to the ecological management of the forage environment of *L. krefftii*. With robust NIRS equations developed for the forage at Epping Forest National Park, ongoing studies of pasture dynamics can examine real-time changes in forage quality with minimal interference to the forage and with minimal cost. Active evolution of the NIRS equations will improve the predictive ability of equations developed in this study and other long-term ecological monitoring.

Application of NIRS to the northern hairy-nosed wombat

This study has shown that NIRS is a suitably robust tool for analysing the composition of plants available to, and fed on by, *L. krefftii*. Importantly, the calibration equations developed incorporated many different plant species. Most previous applications have been made in cultivated or semi-natural habitats and these often developed separate calibrations for a single plant part in a single season (e.g. Barker *et al.* 1994). Our results show that the technique can be applied to whole plants in a natural habitat with high species diversity just as successfully. The application of predictive equations developed in this study has allowed successful measurement of seven attributes of forage quality (N, OM, NDF, ADF, AL, IVDMD and WSC) for 25 plant species, representing perennial grasses, annual grasses, sedges and forbs encompassing a three-year sampling regime across wet and dry seasons (Table 4).

Plants available to, and consumed by, *L. krefftii* vary considerably in nutritional value. The standard indicators of plant nutritive value (N, NDF, ADF and IVDMD) exhibit identical trends in relation to plant groups. These are that forbs have higher total nitrogen, lower NDF and higher IVDMD compared with the sedge *Fimbristylis dichotoma*, the introduced grass *Cenchrus ciliaris* and the native grasses in that order. Only indicators such as AL, OM and WSC do not show the same pattern. Moreover, these general trends found for N, NDF, ADF and IVDMD are also repeated between seasons (Table 4).

From a nutritional viewpoint, *L. krefftii* should consume plants with the highest total nitrogen concentration, lowest fibre concentration and highest IVDMD. In both the wet and dry seasons, this would suggest that the wombat should consume forbs and sedges more readily than grasses. This is not the case. Freeland and Janzen (1974), among others, have long pointed out the role of secondary compounds and plant defenses. The forbs available to *L. krefftii* are potentially unpalatable because of such defenses, both physical (stellate trichomes) and chemical (Horsup and Marsh 1992). The second best choice, the sedge *Fimbristylis dichotoma*, is also not highly selected by the wombat. Rather than defensive mechanisms or a problem with abundance, *F. dichotoma* has plant morphology such that the cost–benefit analysis, in terms of handling time by the wombat, is not in favour of its consumption. The fine leaves, small size and sparse distribution are not conducive to high intakes necessary for a large-hindgut fermenter like a wombat.

A potential problem for the conservation and management of *L. krefftii* has been identified by this study. Protein has long been seen as a limiting factor for large-bodied grazers in tropical savannas (Owen-Smith 1982). Indeed, if the crude protein concentration of tropical grasses falls below a threshold level of 5% (0.83% N), then the loss of body condition of large grazers is likely to occur through a negative nitrogen balance (Owen-Smith 1982). Clearly, *L. krefftii* is likely to be consuming a diet below this threshold level, particularly during the dry season. For wombats, the actual crude protein threshold for maintenance of nitrogen balance is likely to be lower than 5% (Barboza *et al.* 1993) but, even so, a prolonged exposure to a food resource low in total protein is likely to have a deleterious effect on the fecundity of the population (Freeland and Choquenot 1990). Indeed conservation research and management efforts should be directed at establishing a relationship between protein and ovulation at conception and then increasing the nitrogen concentration of the food available to NHNWs.

The role of the introduced grass *Cenchrus ciliaris* on the nutritional ecology of *L. krefftii* may be crucial for the maintenance of the population during drought. *Cenchrus ciliaris* clearly has higher nutritional value than native grasses (Table 4) and this is why it has become an economically important component of the herbage to the grazing industry in the Australian savanna. Woolnough and Johnson (2000) found that *C. ciliaris* is one of three principal grass species consumed by *L. krefftii* regardless of season. The consumption of *C. ciliaris* must benefit *L. krefftii*, particularly in relation to maintaining a positive nitrogen balance during the dry season. Indeed, its high nutritional value compared with native grasses may be of considerable value to the future viability of *L. krefftii*.

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