CSIRO PUBLISHING
www.publish.csiro.au/journals/ajz *Australian Journal of Zoology*, 2003, **51**, 95–98

Validation of near-infrared reflectance spectroscopy to estimate the potential intake of *Eucalyptus* foliage by folivorous marsupials

I. R. Wallis[A] *and W. J. Foley*[A,B]

[A]School of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia.
[B]To whom correspondence should be addressed. Email: william.foley@anu.edu.au

Abstract

We validated an existing model of food intake by captive common ringtail possums (*Pseudocheirus peregrinus*), a folivorous marsupial, by feeding foliage from 18 individual *Eucalyptus polyanthemos* trees and measuring dry matter intake. Near-infrared reflectance (NIR) spectra of a sample of each foliage were recorded and compared against a previously derived model relating food intake in common ringtails and NIR spectra. We found excellent agreement between the predicted and measured food intake, with the standard error of prediction being 3.6 g kg(body mass)$^{-0.75}$ day^{-1}. NIR spectroscopy is a suitable tool for modelling complex attributes such as potential feeding rates of mammals. This makes it theoretically possible to remotely sense critical nutritional determinants of animal habitat on a landscape scale.

Introduction

Browsers encounter great variability in the nutritional quality of their food plants. This variation, which depends on complex interactions between nutrients, such as nitrogen, and refractory components such as fibre, tannins and other plant secondary metabolites, results in differences in feeding rates and digestibility on different plants. Several recent studies have shown that there may be as much variability between different plants of the same species as there is between different species (Snyder 1992; Ernest 1994; Lawler *et al.* 1998, 2000). This variability challenges ecologists because often it is unclear which factors determine an animal's response to a plant and sometimes there are not appropriate analyses for quantifying particular chemical entities, e.g. tannins.

McIlwee *et al.* (2001) argued that the best way to integrate all of these factors was to model the relationship between feeding rates in standardised experiments (termed the 'potential food intake') and the NIR spectra of the plant samples. NIR spectra capture the chemical composition of the plant in a holistic manner (Foley *et al.* 1998) and this approach has been used by Lawler *et al.* (2000) to study feeding by a small marsupial (the common ringtail possum, *Pseudocheirus peregrinus*) on foliage from individual *Eucalyptus polyanthemos* trees. They concluded, from NIR-based estimates of potential intake of foliage, that common ringtail possums inhabit a highly patchy landscape that dictates selective foraging.

Lawler *et al.* (2000) optimised their model by a procedure called 'cross-validation' (Davies 1998), in which groups of samples are removed and the model parameters adjusted until all samples have been both included and omitted from the model. In spite of the name, 'cross-validation' is best described as a method of optimising a model and guarding against overfitting, rather than validation *per se*. Although cross-validation is a standard procedure in chemometric manipulation of NIR spectra (Anon. 1995) some see it as a circular

 10.1071/ZO02027 0004-959X/03/010095

argument because the samples that are included in the model are also used to optimise it. In other words, the samples used for the validation are not truly independent (Davies 1998). Cross-validation is usually used when there are insufficient samples to form a separate calibration data set, as typically occurs with feeding studies. In such instances, cross-validation is a reasonable alternative to arbitrarily dividing the sample set into calibration and validation subsets. In the cases of both McIlwee *et al.* (2001) and Lawler *et al.* (2000), a minimum number of samples were used for the calibration but this still left no 'spare' samples for independent validation.

Cross-validation is particularly useful when prediction equations from NIR spectra involve the use of modified partial least-squares regression (Shenk and Westerhaus 1991). With this approach the researcher needs to avoid overfitting the model by adding too many terms and cross-validation achieves this (Davies 1998). Both these procedures are unfamiliar to many ecologists, who would undoubtedly prefer to see models tested and validated with data collected independently. Convincing the ecological community that spectral measurements are a valid approach to predicting how animals will feed on different plants requires an unequivocally independent validation.

In this note, we validate the approach of McIlwee *et al.* (2001) for estimating the potential rate of food intake of herbivores by validating a model described by Lawler *et al.* (2000) with a new, independent data set. The model of Lawler *et al.* (2000) predicts potential food intake of *Eucalyptus polyanthemos* foliage by common ringtail possums. We used common ringtail possums caught in a different area and fed different samples of *E. polyanthemos* foliage by a different experimenter (IRW). Of course, there was also a time factor with this research coming several years after that of Lawler *et al.* (2000).

Materials and Methods

Six common ringtail possums were caught by hand in woodland near Canberra, Australian Capital Territory, and maintained in individual metabolism cages as described by Lawler *et al.* (2000). They were fed a mixture of *Eucalyptus polyanthemos* and *E. rossii* foliage for four weeks and then switched to *E. polyanthemos* only for the two weeks before the experiment started. The experiment was designed as three separate 6×6 digram-balanced Latin squares, as described by Ratkowsky *et al.* (1993). Foliage from three groups of six trees was collected into plastic bags and stored at 4°C with the ends in water. At 1700 hours on each experimental day a bundle of foliage weighing 200–250 g was placed in the feeding tube of water attached to each cage. A smaller (~150 g), but otherwise identical bunch of 'control' foliage, was placed in a similar tube in front of each cage. Starting at 0900 hours the next day we weighed the uneaten intact foliage and collected the spilled foliage into a paper bag and dried it to constant mass. The control foliage was used to monitor changes in hydration during the feeding period and a sample (~10 g) was dried to constant mass to determine the dry matter of the foliage offered. Dry-matter determinations of all foliage were carried out at 40°C to avoid the loss of volatile oils. A separate sample (~40 g) of the control leaf was frozen pending analysis by NIR spectroscopy.

We estimated the potential food intake using the model described by Lawler *et al.* (2000). This model was derived by partial least-squares regression of the NIR spectra of 36 samples of *E. polyanthemos* foliage against the actual consumption of that foliage by common ringtail possums in captivity. Many different regression methods have been used to relate NIR spectra to factors of interest. For example, multiple linear regression uses a few selected wavelengths as predictors of the attribute of interest. However, the advantage of the partial least-squares procedure is that it uses all the wavelengths collected and should reflect the composition of the plant in a more holistic way. The model predicts the food intake of common ringtail possums under standardised laboratory conditions and does not claim to predict the absolute amount of foliage that an animal might consume in the field.

Samples of all 18 foliages were freeze dried and ground to pass a 1-mm mesh in a Cyclotec 1093 Mill (Tecator, Sweden). All samples were held at 40°C overnight to ensure comparable moisture content. Spectra were collected on a NIR Systems (Silver Springs MD) 6500 scanning spectrophotometer in reflectance mode using a spinning-cup sample holder. This instrument was spectrally matched to the instrument used by Lawler *et al.* (2000). We carefully followed the procedures described by Lawler *et al.* (2000), which were based on a recommended standard (Anon. 1995). All the spectra used to construct the model of Lawler *et al.* (2000) were available to us and so we were able to compare the measured food intakes in our study against the values predicted from the model of Lawler *et al.* (2000) by linear regression.

Results and Discussion

The common ringtail possums ate 14–42 g DM $kg^{-0.75}$ day^{-1} of the *E. polyanthemos* foliage. Fig. 1 shows the relationship of the voluntary dry matter intake measured in this experiment with the predicted dry matter intake derived from the model of Lawler *et al.* (2000). The standard error of prediction was 3.6 g DM $kg^{-0.75}$ day^{-1} ($r^2 = 0.81$; slope = 1.04). These data show that NIR spectroscopy is a valid and highly accurate method for predicting the potential feeding rates of ringtail possums eating *Eucalyptus* foliage.

This finding has at least two important consequences. First, measurements of potential feeding rates derived in this way can be rapidly applied to a large number of samples in the field to quantify the degree of nutritional heterogeneity in a habitat. Secondly, because this approach is based on spectral measurements and not on chemical entities it can be applied from other spectral platforms such as portable field spectrometers and also airborne hyperspectral remote-sensing platforms (Ebbers *et al.* 2002). This potentially opens the way for remotely sensing critical nutritional determinants of animal habitat on a landscape scale.

Models based on NIR spectra can be continuously updated, as new values become available. In the case of common ringtail possums, we have combined the present data set with that of Lawler *et al.* (2000) to produce a new model predicting potential food intake. This new model was derived by partial least-squares regression of spectra from 54 samples of foliage – 36 samples (Lawler *et al.* 2000) plus the 18 observations reported here – against measured food intake. The error of prediction (measured as the standard error of the regression or standard error of calibration) of this new model (3.2 g DM $kg^{-0.75}$ day^{-1}; $r^2 = 0.94$; slope = 1.04) is similar to that described by Lawler *et al.* (2000) for 36 samples (3.4 g DM $kg^{-0.75}$ day^{-1}). The size of the prediction errors reflects the precision with which

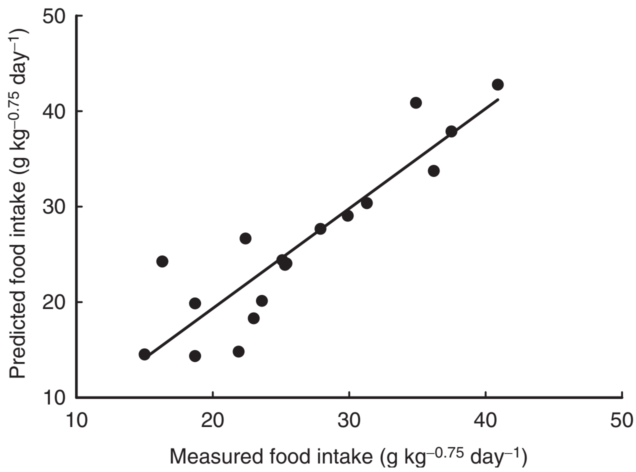

Fig. 1 The relationship between mean voluntary dry matter intake for 18 samples of *E. polyanthemos* foliage by common ringtail possums and the intake predicted by an existing regression model (Lawler *et al.* 2000) relating the near-infrared spectra of each sample to the measured food intake ($r^2 = 0.81$, s.e. = 3.6, $n = 18$, $F_{1,17} = 71$, $P < 0.0001$).

we can measure food intake in common ringtail possums and dominance of foliar chemistry in determining how much the animals are willing to eat (Lawler *et al.* 2000; McIlwee *et al*. 2001). However, the new combined model with 54 samples should be more robust because it incorporates more variation in trees, animals, and experimental procedures.

Acknowledgments

We thank Dr Ivan Lawler for useful discussions and comments on this note and for supplying the spectra from his original model and Ms Miranda Ebbers for help in acquiring spectra and for drawing the figure. The work was supported by a grant from the Australian Research Council to WJF.

References

Anon. (1995). ‘Standard Practices for Infrared, Multivariate, Quantitative Analysis’. (Designation: E1655-94). (American Society for Testing and Materials: West Conshohocken, PA.)

Davies, A.M.C. (1998). Cross validation: do we love it too much? *Spectroscopy Europe* **10**, 24–25.

Ernest, K. (1994). Resistance of creosote bush to mammalian herbivory: temporal consistency and browsing-induced changes. *Ecology* **75, 1**684–1692.

Ebbers, M. J., Wallis, I. R., Dury, S., Floyd, R., and Foley, W. J. (2002). Spectrometric prediction of secondary metabolites and nitrogen in fresh *Eucalyptus* foliage: towards remote sensing of the nutritional quality of foliage for leaf-eating marsupials. *Australian Journal of Botany* **50**, 761–768.

Foley, W. J., McIlwee, A., Lawler, I. R., Aragones, L., Woolnough, A. and Berding, N. (1998). Ecological applications of near-infrared spectroscopy – a tool for rapid, cost-effective prediction of the composition of plant tissues and aspects of animal performance. *Oecologia* **116**, 293–305.

Lawler, I. R., Foley, W. J., Eschler, B. M., Pass, D. M. and Handasyde, K. (1998). Intraspecific variation in secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116**, 160–169.

Lawler, I. R., Foley, W. J. and Eschler, B. M. (2000). Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327–1338.

McIlwee, A. M., Lawler, I. R., Cork, S. J. and Foley, W. J. (2001). Near infrared spectroscopy as a predictor of *Eucalyptus* foliar nutrients and of the feeding rates of folivorous marsupials. *Oecologia* **128**, 539–548.

Ratkowsky, D. A., Evans, M. A. and Alldredge, J. (1993). ‘Cross-over Experiments, Design, Analysis and Application.’ (Marcel Dekker: New York.)

Shenk, J. S., and Westerhaus, M. O. (1991). Population structuring of near infrared spectra and modified partial least squares regression. *Crop Science* **31**, 1548–1555.

Snyder, M. A. (1992). Selective herbivory by Albert’s squirrel mediated by chemical variability in Ponderosa pine. *Ecology* **73**, 1730–1741.

Manuscript received 8 May 2002; accepted 12 February 2003