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Prediction of the nutritional composition of the crop contents of free-living scarlet macaw chicks by near-infrared reflectance spectroscopy

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Abstract

Context. It is difficult to determine with accuracy the nutrition of bird diets through observation and analysis of dietary items. Collection of the ingested material from the birds provides an alternative but it is often limited by the small sizes of samples that can be obtained.

Aims. We tested the efficacy of near-infrared reflectance spectroscopy (NIRS) to assess the nutritional composition of very small samples of growing-parrot crop content.

Methods. We used 30 samples of the crop content of free-living scarlet macaw (*Ara macao*) chicks. Samples were scanned with a near-infrared reflectance analyser, and later analysed by traditional wet laboratory methods for crude protein/N, fat, ash, neutral detergent fibre, P, K, Ca, Mg, Cu, Zn and S. A calibration model was developed using principal components analysis.

Key results. Coefficients of determination in the calibration (R^2) and standard errors of cross-validation (SECV) for most of the nutrients showed a good performance (mean R^2 of 0.91 ± 0.11 s.d., $n = 10$) when excluding Zn (R^2 of 0.15, SECV = 25.37).

Conclusions. The present results established NIRS as a valid technique for the non-destructive, low-cost prediction of a variety of nutritional attributes of avian crop contents as small as 0.5-g dry weight.

Implications. The use of NIRS expands the possibilities of wild-animal nutrition research.

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Introduction

Knowledge of avian nutrition, as a component of both their ecology and management, is central to understanding the survival and productivity of the wild populations (Robbins 1993), and has a direct application in *ex situ* husbandry (Thomas 1987). Through foraging observations, it is often possible to identify the main food sources of species , but it is difficult to determine the less common items in the diet and quantify the exact proportion of each food type consumed (Snyder *et al*. 1987; Rosenberg and Cooper 1990). As a result, it is usually impossible to determine the nutritional content of diets through just observation and analysis of dietary items. However, a variety of techniques exists to collect samples directly from the crops and stomachs of individual birds (Rosenberg and Cooper 1990; Enkerlin-Hoeflich *et al*. 1999). Unfortunately, the small size of the individual samples greatly limits the traditional wet laboratory analyses that can be carried out. Pooling samples of similar characteristics is often necessary to achieve the minimum sample sizes needed for analysis; however, this reduces statistical power needed to address ecological hypotheses (Cottam *et al*. 2006; Brightsmith *et al*. 2010).

Despite the high percentage of psittacine taxa threatened (BirdLife International 2000), there is a dearth of studies regarding the nutrition of free-ranging psittacines (Koutsos *et al*. 2001). This is in part because the bird's extensive food manipulation and processing makes it very difficult to gather quantitative food-intake data. Parrots feed their chicks undigested regurgitate, so tube sampling the crop provides an opportunity to determine the composition of the diet as fed and unaffected by differential digestion (Enkerlin-Hoeflich *et al*. 1999; Brightsmith *et al*. 2010). However, individual crop samples are usually <1.5 g

in dry weight, whereas analyses such as proximal and mineral composition commonly require 2.5 g of sample each.

Near-infrared reflectance spectroscopy (NIRS) is an indirect method that estimates chemical composition by comparing spectra of samples with known composition to spectra of samples with unknown composition (Shenk 1992; Shenk and Westerhaus 1994). Near-infrared radiation (750–2500 nm) is absorbed mainly by organic bonds (Miller and Rutzke 2003). The frequencies that match the vibrational waves of these bonds are absorbed, whereas other frequencies are reflected or transmitted, resulting in NIR spectra that contain detail on the chemical composition of the material (Shenk 1992). The advantages of NIRS are that it is non-destructive, has high precision, produces no waste, requires no costly reagents, needs minimal sample preparation and requires a very small sample size (Blanco and Villarroya 2002).

NIRS is widely accepted for compositional and functional analyses in agriculture and manufacturing (Association of Official Analytical Chemists 1996; Huang *et al*. 2008). It is widely used to predict a variety of nutrients in leaves, grasses and grains (Givens and Deaville 1999; Cozzolino 2009), including amino acids (Wu *et al*. 2002), tannins and alkaloids (Clark *et al*. 1987*b*) and mineral elements (Clark *et al*. 1987*a*; Cozzolino and Moron 2004). It has been applied in the study of the foraging ecology and nutrition of several wild and domestic herbivorous mammals through the analysis of their diets, excreta and oesophageal extrusa (Volesky and Coleman 1996; Woolnough and Foley 2002; Rothman *et al*. 2009). In avian nutritional studies, it has been used only to predict the nutritional composition of feed and excreta (Smith *et al*. 2001; Landuar *et al*. 2006; Tyagi Pramod *et al*. 2009; Ankra-Badu *et al*. 2010). In the present study, we evaluated NIRS as a tool to assess the nutritional composition of small dietary samples collected from wild avian species, by using samples of crop content from wild scarlet macaw chicks.

Materials and methods

Sample description

The present study analysed the crop-content samples from free-living scarlet macaw chicks previously collected by Brightsmith *et al*. (2010). Samples were collected during the 2005 breeding season at the Tambopata Research Center, in the lowland forests of south-eastern Peru (13°07′S, 69°36′W; 250 masl). Crop contents were sampled following Enkerlin-Hoeflich *et al*. (1999). In this technique, the bird is restrained by hand, the crop is massaged, a flexible and lubricated plastic tube is inserted into the crop trough the oesophagus, the crop contents are pushed up into the tube, and the tube is removed. In total, 48 individual samples were obtained from 10 chicks found in seven nests (mean ± s.d. dry weight per sample 1.5 ± 0.9 g). Because of the small samples, 13 of the samples were analysed as independent samples, whereas the remaining 35 were grouped in 17 composite samples for analysis (mean dry weight 2.5 ± 1.6 g). Composite samples were created by combining samples from chicks in the same nest collected on the same day or from chicks of similar age. During pre-process examination of the crop samples, it was determined that they contained seeds, wood or bark, fruit pulp, insect larvae and 19% of them contained clay (Brightsmith *et al*. 2010). All samples were placed in refrigerator at 4°C within 30 min of collection. The samples were dried to a constant weight in an oven at ~55°C (Association of Official Analytical Chemists 1996), ground to a fine powder (<1-mm particle size), and stored in airtight containers until analysis.

Standard nutritional analysis

Proximate laboratory analyses were performed at the Palmer Research Center at the University of Alaska. Nitrogen was calculated using the Dumas method (Association of Official Analytical Chemists 1996) in a LECO CHN-1000 analyser (LECO, St Joseph, MI, USA) for carbon, hydrogen and nitrogen. Crude protein was estimated by multipliying N × 6.25 (Pellet and Young 1980). Crude fat was calculated using the ether extraction method (Randall 1974). Concentrations of Ca, K, P, Mg, Zn, Cu and S were determined by mass spectroscopy (Miller and Rutzke 2003) after wet ashing (Michaelson *et al*. 1992). Neutral detergent fibre (NDF) was calculated by Van Soest's detergent analysis system (Goering and Van Soest 1970). Ash was calculated by heating the sample to 550°C for 12 h. All results are presented on a dry matter basis (Association of Official Analytical Chemists 1996).

Collection of spectral data

Each sample was scanned once with a Perten DA 7200 IR spectrometer (Perten Instruments AB, Hägersten, Sweden). A mirror module was used to accommodate the small sample. The window is made of sapphire, with a surface area of 25 cm^2, with a 256-pixel indium–gallium–arsenide (InGaAs) detector operating in the wavelength range 900–1700 nm. The spectra were stored in optical sensitivity units log (1/R), where R represents the percentage of energy reflected (Fig. 1).

Calibration set and model development

The multi-variant chemometrics package 'Unscrambler' (CAMO Software Inc., Woodbridge, NJ, USA) was used to process the spectral data from the samples. An independent calibration model

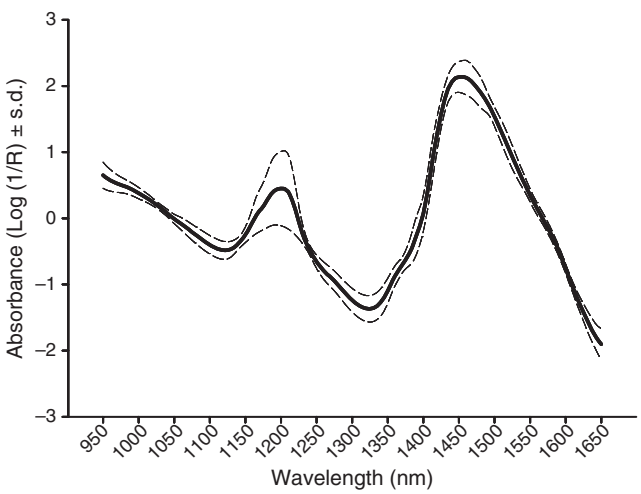

Fig. 1. Near-infrared reflectance spectra (950–1650 nm) of crop samples from free-ranging scarlet macaws (*Ara macao*) in south-eastern Peru.

Table 1. Nutritional values and calibration-equation performance for crop samples from free-ranging scarlet macaw (*Ara macao*) chicks collected in south-eastern Peru

NDF = neutral detergent fibre

Parameter	NDF (%)	Ash (%)	Crude fat %	Crude protein (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Zn (ppm)	S (%)
Mean	42.8	7.15	19.0	17.3	0.34	0.92	0.88	0.29	14.4	39.8	0.31
s.d.	26.46	5.71	13.4	11.8	0.22	0.50	1.06	0.28	4.99	10.7	0.45
R^2	0.86	1.00	0.92	0.93	0.93	0.91	0.98	0.99	0.63	0.15	0.99
SECV	10.5	0.35	3.99	3.28	0.06	0.15	0.13	0.02	3.81	25.4	0.03
N	30	19	24	30	29	24	22	28	21	27	19

for each nutritional attribute was developed through principal component analysis (PCA) (Christy *et al*. 2004; Roggo *et al*. 2005). To evaluate the predictive power of the chosen model, we determined the coefficients of determination (R^2) for each nutritional attribute as a measure of the proportion of variability explained by the regression model.

Validation set

Because of the reduced sample size, we used the cross-validation method with samples from the initial dataset, for validation of the calibration accuracy (Shenk and Westerhaus 1994), avoiding the need to set aside samples for a validation set. The pooled residuals of each prediction (SECV) were calculated to evaluate the precision of the chosen equations for each nutritional attribute.

Results

The NIRS equations for all nutrients except Zn showed strong predictive power. The R^2-values between the NIRS predictions and laboratory analyses were above 0.85 for all nutritional variables tested, except Cu ($R^2 = 0.63$) and Zn ($R^2 = 0.15$) (Table 1).

Discussion

Our study found that NIRS accurately predicted the nutrient contents of the crop contents of scarlet macaw nestlings for 10 of 11 nutrients tested. These results mirror those achieved with commercial plant species such as rice (Kong *et al*. 2005) and oats (Redaelli and Berardo 2007), as well as wild plants consumed by mountain gorillas (*Gorilla beringei*) (Rothman *et al*. 2009), African ungulates (Woolnough and du Toit 2001) and wombats (*Lasiorhinus krefftii*) (Woolnough and Foley 2002).

Minerals are usually not well predicted by NIRS, unless they are part of organic complexes or chelates, or if concentrations are correlated with other constituents of the sample (Clark *et al*. 1987*a*; Cozzolino and Moron 2004). However, six of seven minerals in our study showed good correlations, and only Zn showed R^2 of <0.60, suggesting that most of the minerals are bound organically.

Our results showed that NIRS is a valid technique for the non-destructive, low-cost prediction of the nutritional composition of avian crop contents. NIRS can be used with samples as small as 0.5-g dry weight, expanding the possibilities of research in the nutrition of parrots and other animals where only small samples are available. In our research, we are using NIRS to increase the amount of ecologically relevant information from our samples by (1) scanning individual small samples with NIRS (as small as 0.5 g), (2) combining samples with similar spectra into composite samples large enough for traditional laboratory analysis, (3) scanning these composite samples with NIRS, and (4) conducting laboratory analyses on these composite samples. We can then use the laboratory analyses on the composite samples to create the NIRS calibration curves and predict the nutritional content of the individual small samples. In this way, we can look at the samples in individual scales, and test for nutritional differences among e.g. nest mates, habitats, times of day collected and chick age, with much finer resolution than possible without the NIRS. Further studies should explore the possibilities of using NIRS to identify the actual ingredients consumed by the birds (Volesky and Coleman 1996). Determining the key food resources on which avian species depend will help understand their ecology and develop better management and conservation strategies.

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