CHEMICAL ANALYSIS OF ELEPHANT GRASS (*Pennisetum purpureum*) USING NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIR*)*

Eduardo V. Valdes* B.Sc. Agr., M.Sc., PhD [1,2,3] and Alejandra Renjifo B.Sc.[1]

[1]Disney's Animal Kingdom, Lake Buena Vista, Florida, 32830-1000; [2]University of Guelph, Ontario, Canada; [3]University of Central Florida, Orlando, Florida 32816-2368

Abstract

Samples of *Pennisetum purpureum* (PP) were collected weekly commencing the summer of 2002 and throughout the year 2003. This grass is used in the feeding-enrichment program for the African elephants (*Loxodonta africana*) at Disney's Animal Kingdom (DAK). Samples were submitted to a laboratory where analyses of moisture, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), fat (F), lignin (L) and gross energy (GE) were performed. Twenty-eight samples were used in the NIR calibrations. The accuracy of the calibrations was assessed by calculating the residual standard deviation (RSD) and the bias or average difference between the conventional laboratory methods and the NIR values. The RSD, $Mean_{(NIR)}$ and bias for the NIR predictions of the quality parameters studied were: dry matter (DM %), 0.66, 95.4, 0.01; CP %,.0.17, 8.9, 0; ADF %, 1.27, 42.3, 0.1; NDF %, 1.94, 70.0, 0.30; F %, 0.17, 1.63, 0.02; L %, 0.7, 5.7, 0.5 and $GE_{kcal/g}$ 0.18, 4.5, 0; respectively. The NIR predictions for these quality parameters were acceptable indicating that NIR can be used as an alternative analytical method. However, the accuracy of the predictions can be improved by adding more samples to the calibration set. The main advantage of NIR is that it is quick (seconds) and does not require sample preparation beyond drying and grinding. NIR is also an environmentally friendly technique that minimizes the use of toxic chemicals and can reduce the costs of conventional analyses.

Introduction

NIR has been previously applied in feed quality control programs used in zoos[2, 3]. The technique is fast and easy to use after calibrations are developed and most importantly, NIR is an environmentally friendly technique that minimizes the use of toxic chemicals or the production of toxic elements. In the last few decades, NIR has emerged as a fast and accurate method for measuring quality parameters in agricultural feeds, particularly in forages[5]. At Disney's Animal Kingdom, the African elephants are fed a variety of forages. One important forage being fed is the grass *Penissetum purpureum*, commonly called "elephant grass". This grass is fed daily (500 kg/day) and is obtained from farms in central and south Florida. The cost of the elephant grass is high so rapid monitoring of its quality is important from a nutritional and economical viewpoint.

Methods

Samples of elephant grass were collected weekly starting in the summer of 2002 and continuing through 2003. These samples were submitted for chemical analyses (Cornell University Nutritional & Environmental Analytical Services). Samples were analyzed for moisture (AOAC 934.01, vacuum oven method); CP (AOAC 988.05, Kjeldahl method); ADF (filter bag method, ANKOM Technology, Macedon, NY); NDF (filter bag method, ANKOM Technology, Macedon, NY); GE. (ASTM D240-64). Analyses of soluble carbohydrates (fructose, glucose and starch) were also performed by water extraction followed by determination of sugars by HPLC using a refractive index. Before obtaining the spectra within the near infrared range (400 – 2,500 nm), samples were freeze- dried (Labconco, model 77530) for 72 h and ground using a 1 mm screen (Retsch grinder model ZM100). The spectra were collected using a Foss NIR scanning spectrophotometer model 6500 following a method previously described[2]. The NIR calibrations were developed using the full NIR spectrum by applying partial least squares[1]. Twenty-eight samples were used for developing the preliminary calibrations and six samples were used to validate the calibrations for analytical dry matter (DM), CP, ADF, NDF, F, L, and GE. The DM values used for the calibration samples represented analytical dry matter after samples have been freeze dried. The actual average dry matter for the 28 samples was 24.2 % after harvest. We also developed NIR calibrations for sugars.

Results and Discussion

The NIR predicted values for the quality parameters studied are given in Tables 1,2 and 3. We considered the results preliminary as more samples should be used in the calibration sets. Development of NIR calibrations requires the use of many samples with good chemical data. The more chemical parameters to be predicted the more samples we need to add to the calibration set. Also, the calibration set should encompass all the possible factors that might affect the quality of the crop (e.g. elephant grass), including plant varieties, geographical distribution, soil types, harvesting season, laboratory sample preparation, etc. The latter will be accomplished after a few years of collecting fields samples. However, the preliminary values indicated that DM, CP, ADF, NDF, F, L and GE were well predicted by these equations in both the calibration and validation sets based on previous work.[4, 5,6] ADF showed a high RSD for the validation set samples (7.5%). Ideal RSD for NIR ADF should be lower than 2 %[7]. The potential to predict soluble sugars with NIR was studied with a limited number of samples. Fructose was the only sugar that was well predicted by NIR but we should be cautious, as many samples have to be added to the calibration set to thoroughly assess the technique to measure plant sugars. NIR is a fast technique after calibrations are developed that can be used in the quality control programs. NIR helps to minimize the costs of conventional analyses as only a few samples will have to be analyzed for maintaining / updating the calibrations every year. At an average laboratory cost of $ 150/sample, savings can be significant. Moreover, NIR is a good tool for purchasing feeds, including forage (e.g. elephant grass) as samples have to comply with certain specifications with regards to chemical composition at the time of purchase. The latter is very important for forages such as elephant grass because of its high cost ($ 0.49 – $ 0.75/lb).

LITERATURE CITED

1. Martens, H. and T. Naes. 1987. In: Near infrared technology in the agricultural and food industries. P. Williams and K. Norris eds. Amer. Assoc.. Cereal Chem. St. Paul. Minn.
2. Valdes. E.V., E.S. Dierenfeld and M.P. Fitzpatrick. 1997. Application of near infrared reflectance spectroscopy (NIR) to measure protein, fat and moisture in fish samples. Proceedings of the 2nd NAG/AZA conference, October 16-19, Fort Worth, Texas.
3. Valdes, E.V., E.S. Dierenfeld and S.E. Oyarzun. 1995. A preliminary study to measure protein, fat and moisture in whole mice and rats by near infrared reflectance spectroscopy. Proceedings of the 1st NAG/AZA conference, Toronto, Ont. May 1-2.

4. Valdes, E. V., L. G. Young I. McMillan and J.E. Winch. 1985. Analyses of hay, haylage and corn silage samples by near infrared reflectance spectroscopy. Can. J. Anim. Sci. 65: 753-760.
5. Valdes, E.V. 1993. Alternative methods to measure metabolizable energy and other quality parameters in poultry feeds. Ph.D. Thesis. University of Guelph, Ontario, Canada.
6. Valdes, E.V., G.E. Jones and J. Hoekstra. 1990. Effect of growing year and application of a multi-year calibration for predicting quality parameters by near infrared reflectance spectroscopy in whole-plant corn forage. Can. J. Plant Sci. 70:747-755.
7. Valdes, .V., D.M. Smith, D.M. Brown and S. Leeson. 1990. Measuring alfalfa quality by near infrared reflectance under experimental conditions. Third International Symposium on NIRA. June 23-30. Brussels, Belgium.

Table 1. Prediction of quality parameters in elephant grass (*Pennisetum purpureum)* using near infrared reflectance spectroscopy [a].

Parameter	DM %	CP %	ADF %	NDF %	F %	L %	GE kcal/g
n	27	27	27	26	26	26	26
RSD	0.66	0.17	1.27	1.94	0.17	0.7	0.18
Mean lab	95.5	8.9	42.4	69.7	1.65	5.2	4.5
Mean NIR	95.4	8.9	42.3	70.0	1.63	5.7	4.5
Bias	0.01	0	0.1	0.3	0.02	0.5	0
Range lab	93.5 – 97.1	4.4 – 18.6	35.4 – 50.4	58.6 – 77.6	1.3-2.3	3.3-8.3	4.1-4.9
Outliers	1	1	1	2	1	2	2

[a] Calibration set; DM: analytical dry matter; bias: non-random error or the average difference between the NIR predicted and the laboratory values; outliers: samples whose spectra were different from the population of samples.

Table 2. Prediction of quality parameters in elephant grass (*Pennisetum purpureum)* using near infrared reflectance spectroscopy [a].

Parameter	DM %	CP %	ADF %	NDF %	F %	L %	GE kcal/g
n	6	6	6	6	6	6	6
RSD	0.10	0.90	7.5	4.3	0.09	0.8	0.001
Mean lab	94.3	10.9	44.5	64.7	1.14	7.7	4.2
Mean NIR	94.6	10.0	44.8	67.6	1.42	6.6	4.5
Bias	0.3	0.9	0.3	3.1	0.32	1.1	0.3
Range lab	92.1-94.6	8.1-17.4	40.1-48.5	59.8-68.1	0.1-2.1	5.0-10.6	3.9-4.4
Outliers	0	0	0	0	0	0	0

[a] Validation set; bias: non-random error or the average difference between the NIR predicted and the laboratory values; outliers: samples whose spectra were different from the population of samples.

Table 3. Analyses of soluble carbohydrates in elephant grass (*Pennisetum purpureum)* using near infrared reflectance spectroscopy [a].

Parameter	Fructose		Starch		TSC	
	C	V	C	V	C	V
	%		%		%	
n	10	6	10	6	12	6
RSD	0.7	0.2	0.3	0.02	1.9	0.8
Mean lab	3.6	2.5	0.7	1.1	4.9	3.8
Mean NIR	3.9	2.4	0.7	1.0	5.1	3.1
Bias	0.3	0.1	0	0.1	0.2	0.7
Range lab	1.8-6.4	1.4-4.8	0.1–1.2	0.6-2.0	0.1-9.0	0.1-8.5
Outliers	2	0	1	0	0	0

[a] bias: non-random error or the average difference between the NIR predicted and the laboratory values; outliers: samples whose spectra were different from the population of samples; TSC: total soluble carbohydrates; C :calibration set; V: validation set.