A Preliminary Study to Measure Protein, Fat and Moisture in Whole Mice and Rats by Near Infrared Reflectance Spectroscopy

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Whole mice and rats were analyzed using near infrared reflectance spectroscopy (NIR) to measure percent protein (Pr), percent fat (FAT) and percent moisture (MST). The NIR calibrations for Pr, FAT and MST were developed using 34 samples including mice and rats of different ages. The accuracy of the calibrations was assessed by calculating the residual standard deviation (RSD) and the bias or the average difference between the laboratory and the NIR values. The prediction of Pr, FAT and MST showed RSD of 1.72%, 1.62% and .22%, respectively, indicating the usefulness of the NIR technique for measuring chemical composition in whole animals. NIR is a technique that is characterized by being fast, accurate and of low operational costs and should be investigated further in nutritional studies in captive feeding programs.

Key words: near infrared, spectroscopy, mice, rats, analysis

INTRODUCTION

The use of chemical analysis is a common procedure for evaluating the nutritive value of feeds. Furthermore, in domesticated species of poultry and fish, nutritional research requires carcass chemical analyses for assessing nutritive value of feeds. In captive feeding programs the formulation of optimal diets also depends on the knowledge of nutrient composition of the diet ingredient(s). However, conventional chemical analyses such as the kjeldhal method for protein and the solvent extraction methods for fat are accurate but are laborious, time consuming, use toxic chemicals and yield toxic waste fluids. In the last decade near infrared reflectance (NIR) analysis has emerged as a rapid and accurate technique for testing feed quality (Norris et al. 1976; Shenk et al. 1979; Valdes et al. 1985). The NIR technique also has been applied to measure crude protein and fat in carcass and breast muscle samples of poultry (Valdes and Summers, 1986) and to measure crude protein, fat and gross energy in poultry and rainbow trout carcasses (Valdes et al. 1989). NIR became an ideal laboratory technique because it is fast (minutes), minimizes the use of chemical reagents, minimizes the production of waste materials and fumes and several constituents in a sample can be analyzed simultaneously.

The objective of this study was to assess the usefulness of near infrared reflectance spectroscopy to measure percent, protein, fat and moisture in whole mice and rats used as feed.

MATERIALS AND METHODS

Thirty three samples with known chemical composition, including 20 rats and 13 mice were used to develop the NIR calibrations for Pr, FA T and MST. Details of the samples, sample preparation and chemical analysis have been reported elsewhere (Douglas et al. 1994). The NIR

spectrophotometer consisted of a scanning instrument (Perstorp Analytical) and 172 wavelengths were used to obtain the spectra in the near infrared region of the spectrum for the 34 samples. Scatter correction due to particle size was necessary during NIR readings. The spectra were stored in a hard disk. Prior to obtaining the spectra, samples were taken from cooler and left in a room where temperature was kept at 20 C for 3 to 4 h. Samples were thoroughly stirred in the holding bottles with a spatula and 4 to 5 g were loaded into a sample cup holder. The sample cup holder was placed into the sample drawer and NIR spectra obtained for the 34 samples. Different mathematical treatments of the NIR spectra were tested before correlating NIR and laboratory values. Within the mathematical treatments, log 1/R (R = reflectance) and first and second derivatives were tested. Partial least squares (PLS) regression, a modified form of principle component regression was used to obtain the NIR equations to predict Pr, FAT and MST. Independent calibrations for Pr , FAT and MST were developed. Simple linear regression analysis relating chemical to the NIR predicted values were calculated. Accuracy of the predictions of the chemical parameters was assessed by the residual standard deviation (RSD). The RSD was calculated from the root mean square error of the regression of the NIR predicted parameter on the laboratory values (Valdes and Leeson, 1992a).

RESULTS AND DISCUSSION

The results of applying NIR to measure Pr, FAT and MST are given in Table 1. The RSD values indicated that these parameters were well predicted by NIR. The best mathematical treatment of the NIR spectra of whole animals was the first derivative. Although good predictions were obtained, the three calibrations showed outliers or samples that did not fit the general population of samples and had to be eliminated from the calibration sets (Table 1). The results of the current study indicated that NIR has potential for measuring chemical parameters in whole animals. Its speed, simplicity of operation and low running costs make NIR an attractive alternative to the more laborious chemical methods and should be investigated further in nutritional studies and feeding programs in zoo's.

REFERENCES

Douglas, T.C., Pennino, M.; Dierenfeld, E.S. Vitamins E and A and proximate composition of whole mice and rats used as feed. COMP. BIOCHEM. PHYSIOL. 107 A, 419-424, 1994.

Norris, K.H., Barnes, R.F., Moore, D.E., Shenk, J.S. Predicting forage quality by infrared reflectance spectroscopy. JOURNAL OF ANIMAL SCIENCE. 43:889- 897, 1976.

Shenk, J.S., Westerhaus, M.O., Hoover, M.R. Analysis of forages by infrared reflectance JOURNAL OF DAIRY SCIENCE.62:807-812, 1979.

Valdes, E.V., Young, L.G., McMillan, I. and Winch, J.E. Analysis of hay, haylage and corn silage samples by near infrared reflectance spectroscopy. CANADIAN JOURNAL OF ANIMAL SCIENCE 65:753-760, 1985.

Valdes, E.V., Summers, J.D. Determination of crude protein and fat in carcass and breast muscle samples of poultry by near infrared reflectance spectroscopy (NIR). POULTRY SCIENCE. 65: 485-490, 1986.

Valdes, E.V., Atkinson, J.L., Hilton, J.W., Leeson, S. Near infrared reflectance analysis of fat, protein and gross energy of chicken and rainbow trout carcasses. CANADIAN JOURNAL OF ANIMAL SCIENCE 69: 1087-1090, 1989.

Valdes, E. V ., Leeson, S. Near infrared reflectance analysis as a method to measure metabolizable energy in complete poultry diets. POULTRY SCIENCE 71: 1179- 1187,1992a.

TABLE 1. Relationship between conventional chemical and near infrared predicted values in whole animals.

ID/	SPECIES	PROTEIN (%)		FAT (%)		MOISTURE (%)	
		LAB	NIR	LAB	NIR	LAB	NIR
2	SM MOUSE	61.10	58.86	26.72	28.85	2.89	3.06
4	SM MOUSE	53.05	52.46	23.19	24.62	3.09	3.12
5	SM MOUSE	53.07	54.57	21.26	20.61	2.98	2.97
6	MD MOUSE	53.96	57.92	26.08	22.92	3.50	3.58
7	MD MOUSE	65.05	64.94	21.74	21.27	3.10	3.44
10	MD MOUSE	61.11	58.55	13.69	15.27	4.17	3.59
11	MD MOUSE	56.79	56.80	26.02	24.96	3.70	*
12	LG MOUSE	47.20	47.31	39.95	38.39	1.49	1.89
13	LG MOUSE	30.98	*	50.92	*	1.58	*
14	LG MOUSE	53.30	52.79	17.03	17.05	2.38	2.49
15	LG MOUSE	38.25	34.45	37.00	*	1.70	*
16	LG MOUSE	45.94	48.08	26.64	26.48	2.27	2.08
17	LG MOUSE	54.25	51.99	29.91	22.20	1.88	1.80
28	RAT	61.46	62.72	25.81	25.50	3.50	3.62
29	RAT	60.33	60.06	27.77	28.09	3.50	3.54
30	RAT	53.83	53.85	34.36	31.63	3.50	3.47
31	RAT	58.74	57.53	28.88	28.81	3.50	3.67
32	RAT	59.48	58.92	28.36	30.38	3.50	3.21
33	RAT	63.52	*	35.23	37.04	2.09	2.08
34	RAT	47.73	49.04	32.47	31.38	2.98	2.65
35	RAT	54.78	56.30	21.90	24.88	4.18	4.26
36	RAT	55.70	57.59	16.66	19.22	4.68	4.36
37	RAT	58.97	57.20	31.17	*	3.88	4.23
38	RAT	67.73	*	22.25	22.11	3.19	3.18
39	RAT	57.57	55.44	23.81	25.02	3.07	3.41
40	RAT	59.01	62.15	24.87	*	3.58	*
41	RAT	58.94	59.40	25.95	24.51	3.89	3.62
42	RAT	57.23	57.74	23.01	23.72	3.57	3.57
43	RAT	53.34	52.96	31.44	30.98	4.39	4.41
44	RAT	53.14	52.23	31.45	31.22	4.49	4.45
45	RAT	40.09	39.71	50.55	51.46	2.58	2.75
46	RAT	54.94	56.86	34.63	31.82	2.48	2.54
47	RAT	54.94	54.33	34.63	37.27	2.48	2.28

Statistical analysis

	PROTEIN(%)		FAT (%)		MOISTURE (%)	
	LAB	NIR	LAB	NIR	LAB	NIR
n	33	30	33	29	33	29
outliers(*)	3		4		4	
Mean	54.78	54.76	27.32	27.50	3.22	3.22
d (lab-NIR)	.02		-.18		0	
Range(lab)	38.25-65.05		13.69-50.55		1.88 - 4.68	
slope	.91**		1.01**		1.0**	
RSD	1.72		1.62		.22	
R^2	.92		.95		.92	