

Near-Infrared Diffuse Reflectance Spectroscopy for the Analysis of Poultry Manures

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The objective was to evaluate near-infrared diffuse reflectance spectroscopy (NIRS) for determining the composition of poultry manures. Poultry manure samples were obtained from a commercial testing laboratory along with conventionally determined analyte values for total N, $\text{NH}_4^+\text{-N}$, organic N (computed as total minus $\text{NH}_4^+\text{-N}$), minerals, and moisture. Samples were blended in a food blender and scanned in polyethylene bags on a FOSS-NIRSystems model 6500 scanning monochromator. Initial results indicated that changes had occurred in $\text{NH}_4^+\text{-N}$ contents from the time of analysis, and samples were reanalyzed for $\text{NH}_4^+\text{-N}$. Using the new analyses, NIRS was able to accurately determine $\text{NH}_4^+\text{-N}$, organic N, total N, and moisture in 128 poultry manures. Results were more accurate when determinations were made on an "as is" as opposed to dry matter basis. Finally, results indicated that NIRS was not suitable for the determination of minerals (P, K, Ca, etc.) in poultry manures.

Keywords: NIRS; poultry manure; ammonia; nitrogen; minerals

INTRODUCTION

Due to environmental regulations, there is an increasing need for rapid methods of analysis for animal manures to more efficiently utilize these materials as fertilizer and to avoid unnecessary environmental contamination. Presently, analysis of poultry and other manures requires the employment of several different assay methods to determine the constituents of interest, that is, nitrogen fractions, minerals, dry matter content, etc. Spectroscopic procedures, such as near-infrared diffuse reflectance spectroscopy (NIRS), offer the possibility of rapid analysis of samples without the generation of the chemical wastes associated with more traditional digestion type assays in use today (1). In addition, spectroscopic methods, which often require little or no sample preparation and can simultaneously determine several analytes, are much more suitable for real-time analysis during the spreading of the manure. For example, real-time NIRS analysis of crops is now being investigated using a spectrometer attached to a combine (Textron Systems, Willington, MA). If manure analysis is to be combined with precision agriculture in order to apply the needed amount of manure (as fertilizer) to the correct field location, a rapid, nonchemical means of manure analysis will be required.

Due to differences in the diets and physiology of livestock species, the manure produced by each has different chemical and physical properties that need to be considered. For example, many dairy manures are slurries with a high moisture (80–95%) content but which may also contain large amounts of straw or other bedding materials, which can cause sampling problems due to their heterogeneous nature (2). Poultry manures, on the other hand, are generally much drier but contain minerals, such as Mn, Zn, and Cu, which are not as much of a problem with dairy manures. Thus, although

work has demonstrated that NIRS can be used to analyze dairy (3) and cattle manures (4), manure composts (5), and swine manure (6), further work is needed to determine the feasibility and limitations for using NIRS to analyze other livestock manures. The objective of this study was to determine the feasibility, limitations, and problems associated with using NIRS to analyze poultry manures.

MATERIALS AND METHODS

Sample Generation and Chemical Analysis. Poultry manure samples were supplied by the University of Maryland Soil Testing Laboratory (College Park, MD). Samples were stored at 4 °C at all times. Samples were obtained starting in September 1999 and continued until June 2000 and thus represent a wide range of samples. Samples were also obtained from both deep pit and floor scrapings and were analyzed for total N, $\text{NH}_4^+\text{-N}$, P, K, Ca, Mg, S, Mn, Zn, Cu, and moisture (organic N computed as total – $\text{NH}_4^+\text{-N}$). Total N and S were determined by combustion on samples dried at 70 °C (Leco Application Bulletin 203-601-073). Ammonium N was determined by distillation (7), total N by addition of organic N, and ammonium N, Ca, Mg, K, and P by perchloric/nitric acid digestion followed by determination on a Technicon Autoanalyzer (8); Cu, Mn, and Zn were determined by perchloric/nitric acid digestion followed by atomic absorption determination (9), and moisture was determined by oven-drying at 70 °C to a constant weight. Due to the nature of the assays used, organic N determinations may include some residual nonvolatilized NH_3 , and moisture determinations include loss of volatiles such as NH_3 . Also, some residual moisture would still be present due to the temperature used. All assay values were computed on an "as is" and moisture, or dry matter (DM), corrected basis.

In addition, 124 samples were reanalyzed for $\text{NH}_4^+\text{-N}$ in our laboratory. The ammonia was extracted by shaking 2.5 g of manure with 250 mL of solution consisting of 0.1 N HCl and 2 M KCl for 1 h (conditions determined by experimentation) using a wrist shaker. The resulting extract was then filtered using a serum filter and analyzed on a Technicon Autoanalyzer

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Table 1. Composition of Poultry Manures Used for NIR Studies

207 Samples Analyzed at University of Maryland				
component	mean	SD	minimum	maximum
% NH ₄ ⁺ -N	0.74	0.22	0.0042	1.31
% organic N	3.04	0.98	0.19	5.42
% total N	3.78	1.02	0.20	6.48
% P as P ₂ O ₅	2.85	0.77	0.018	4.92
% K as K ₂ O	2.18	0.53	0.13	3.39
% Ca	1.85	1.86	0.50	11.65
% Mg	0.68	0.19	0.11	1.56
% S	0.60	0.29	0.066	1.75
ppm of Mn	392	95.4	62.7	768
ppm of Zn	413	124	6.58	921
ppm of Cu	364	173	2.19	974
% moisture	28.0	11.3	12.0	65.1

124 Samples Reanalyzed for Ammonium N and Dry Matter at Beltsville

component	basis	mean	SD	minimum	maximum
% NH ₄ ⁺ -N	as is	0.47	0.12	0.012	0.75
	DM	0.70	0.23	0.028	1.40
% organic N	as is	2.97	0.88	0.19	5.33
	DM	4.17	0.88	0.44	6.88
% total N	as is	3.44	0.88	0.20	5.90
	DM	4.87	0.86	0.47	7.62
% dry matter	as is	69.9	10.4	25.2	84.7

for NH₄⁺-N. Total N was recomputed for these samples using the original organic N value and the new NH₄⁺-N. A new DM was also determined by drying the scanned samples at 100 °C in a forced-air oven for 72 h (determined by experimentation to yield constant weights).

Spectra. Spectra from 400 to 2498 nm were obtained using a NIRSystems model 6500 scanning monochromator equipped with silicon (400–1098 nm) and PbS (1100–2498 nm) detectors with data collected every 2 nm (1050 data points) at a nominal bandwidth of 10 nm. Samples were scanned using a sample transport module with the sample placed in polyethylene bags. This allowed a sample path ~12 cm long to be scanned. A ceramic standard was used for the background spectra, and spectra were computed as log(1/R), where *R* = reflectance. Each spectra consisted of 64 co-added scans for both the sample and the background.

Compositional Statistics and Partial Least Squares Analysis (PLS). Means and other summary statistics were computed using SAS version 7 (Statistical Analysis Systems, Cary, NC). Calibrations for all analytes were performed using PLS under GRAMS/32 (10). A one-out cross-validation was performed and a final calibration developed and applied to the samples in question. In addition, outliers were sometimes removed on the basis of being labeled as compositional outliers by the software in question using the criteria that the predicted–actual difference for the sample was three standard deviations from the mean difference. The number of factors used for any calibration was determined using the *F* statistic from the PRESS error. In addition, spectra were pretreated by applying either mean and variance scaling or multiplicative scatter correction in addition to a first or second derivative. Finally, calibrations were developed using either all data points (400–2498 nm), only the region from 400 to 1098 nm (visible and shortwave near-infrared), or only the near-infrared (NIR) region from 1100 to 2498 nm only.

RESULTS AND DISCUSSION

Chemical Analysis. Summary statistics for the two sets of samples (all samples and those reanalyzed for NH₄⁺-N) are shown in Table 1. As can be seen, the range of values for all analytes was quite good with ranges of >10-fold for all but moisture. The central difference between the two sets of samples was in the range of the NH₄⁺-N values, particularly for the high end values

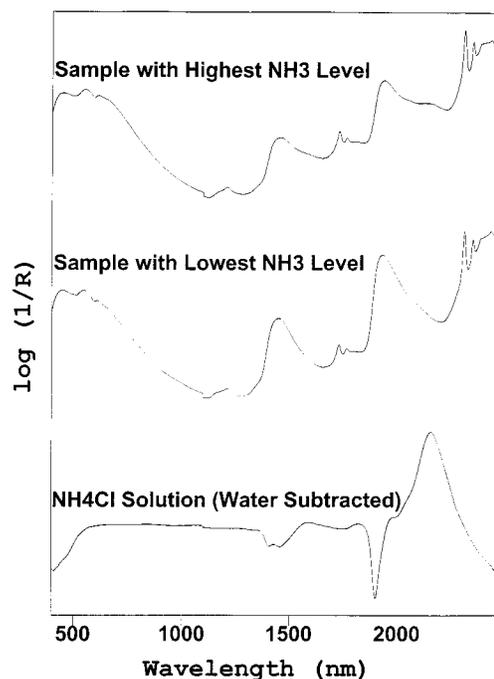


Figure 1. NIR spectra of poultry manure samples with highest and lowest ammonium N concentrations and of saturated ammonium chloride solution (water spectrum subtracted out).

on the set of 124 samples. This is believed to be due changes in the NH₄⁺-N content between the time the samples were analyzed at the testing laboratory and the time they were reanalyzed at Beltsville. Examination of samples for which both analyses were available supported this conclusion, although a small study in which samples were analyzed and then reanalyzed 1 and 2 weeks later after storage at 4 °C, or in a freezer, also showed some samples in which NH₄⁺-N increased. These changes in NH₄⁺-N also are reflected to a lesser degree in the total-N and organic-N contents of the two sample sets.

Spectra. The spectra of the poultry manures from set 2 with the lowest (0.012 mg/g) and highest (0.747 mg/g) concentrations of NH₄⁺-N are shown in Figure 1. Also shown is the spectrum of NH₄Cl in water after subtracting the spectrum of water (not presented). The dips below baseline in the NH₄Cl spectrum are due to changes in the spectrum of water in the presence of NH₄Cl. Thus, the spectrum of water in salt solutions is not the same as that of pure water (11). As can be seen in Figure 1, there is a large peak at ~2150 nm due to NH₄⁺. This peak can also be seen in the poultry manure with the highest level of NH₄⁺-N, but not in the other sample. The sharp bands present in several places are due to the spectrum of the polyethylene bag used to hold the samples as shown in Figure 2. It should be noted that changes in the polyethylene bags due to source or production batch might cause errors in sample determinations, something not examined in this study. As can also be seen in Figure 2, there are two large bands at 1450 and 1920 nm due largely to water. The sharp discontinuity seen at 1098–1100 nm is due to the changeover in detectors, which occurs at this point.

Calibration Results Using Samples Scanned in Triplicate. Sample heterogeneity can affect calibration accuracy in that a nonrepresentative sample is scanned, just as sampling problems can affect analyte determi-

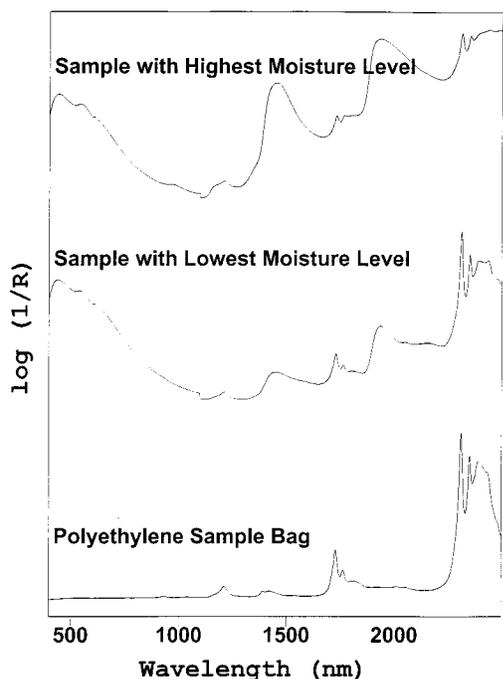


Figure 2. NIR spectra of poultry manure samples with highest and lowest moisture concentrations and of polyethylene bag used to contain samples for scanning.

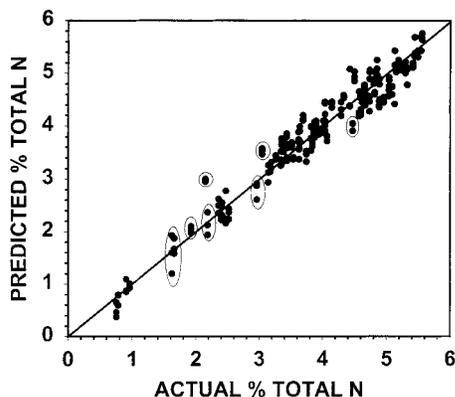


Figure 3. Final calibration results for total N for 91 poultry manure samples with triplicate subsamples scanned (total $n = 273$). Results for a few typical triplicates are circled.

nation by conventional means. Initially, 91 samples were scanned in triplicate (three different subsamples scanned in three different polyethylene bags). The results are shown in Figure 3 for total N. Some typical sample results are shown by the circled triplicate results. As shown, some variation does occur but does not appear to be too great a problem. However, scanning of more samples either by scanning more replicates or by scanning a larger sample with a different spectrometer setup would likely improve calibrations, assuming that equally accurate reference determinations were available. Similar results were previously obtained for dairy manures (3).

Results Using 247 Samples and Testing Laboratory Analyses. *Organic, Total, and Ammonium N and Moisture Determinations.* The data in Table 2 show the calibration results obtained for 247 samples analyzed at the University of Maryland Soil Testing Laboratory using spectral data from 400 to 2498 nm, from 1100 to 2498 nm, and from 400 to 1098 nm only. As shown, results using the visible–shortwave NIR region (400–

Table 2. Final Calibration Results for 207 Samples Analyzed at University of Maryland Soil Testing Laboratory Based on Various Spectral Ranges

component	visible–NIR (400–2498 nm)		NIR (1100–2498 nm)		visible (400–1098 nm)	
	R^2	RMSD ^a	R^2	RMSD	R^2	RMSD
% $\text{NH}_4^+\text{-N}$	0.725	0.116	0.775	0.105	0.469	0.162
% organic N	0.894	0.317	0.889	0.325	0.722	0.513
% total N	0.886	0.343	0.884	0.345	0.647	0.603
% P as P_2O_5	0.616	0.477	0.585	0.496	0.341	0.625
% K as K_2O	0.556	0.351	0.625	0.323	0.430	0.398
% Ca	0.799	0.831	0.800	0.827	0.353	1.49
% Mg	0.398	0.144	0.458	0.136	0.458	0.137
% S	0.594	0.187	0.545	0.198	0.228	0.258
ppm of Mn	0.573	62.2	0.537	64.8	0.504	67.1
ppm of Zn	0.497	87.5	0.401	95.5	0.262	106
ppm of Cu	0.480	124	0.524	119	0.411	132
% moisture	0.843	4.44	0.847	4.39	0.838	4.53

^a RMSD = root-mean-squared deviation = (sum of squared residuals/ n)^{1/2}; units indicated by component.

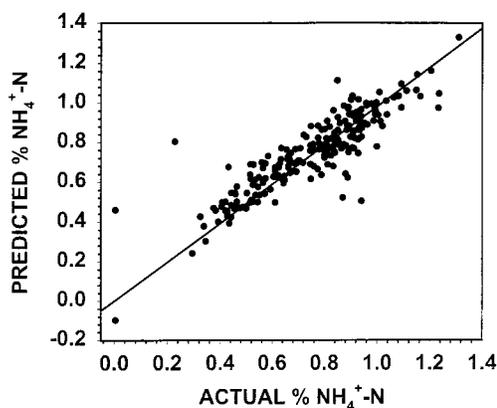


Figure 4. Final calibration results for ammonium N for 207 poultry manures using original analyses provided by testing laboratory (performed several weeks prior to scanning).

1098 nm) were never very good and were comparable to the other results only for moisture determination. These results match those found previously for dairy manures (Reeves and Van Kessel, unpublished data). Unfortunately, the least expensive instruments are those that operate in this region due to the ability to utilize inexpensive Si detectors. From a comparison of the full spectra results (400–2498 nm) to those achieved using only the NIR region (1100–2498 nm), it is shown that the NIR data did better for moisture and $\text{NH}_4^+\text{-N}$ and virtually the same for organic and total N, indicating that the visible–shortwave NIR spectral data add little or nothing to calibrations for the analytes in question. Final calibration results using NIR spectra for $\text{NH}_4^+\text{-N}$ are presented in Figure 4. As shown, there were a group of samples that were determined with considerably less accuracy than the majority. Removal of these samples ($n = 16$) resulted in a greatly improved calibration, $R^2 = 0.889$ and $\text{RMSD} = 0.071$. These results supported the conclusion that some values for $\text{NH}_4^+\text{-N}$ had changed over time and that accurate calibrations for $\text{NH}_4^+\text{-N}$ were possible. Finally, overall these results indicated that the samples in question were generally in good condition and acceptable for other determinations such as minerals, indications being that only the values for the most volatile fraction, that is, $\text{NH}_4^+\text{-N}$, were significantly affected by storage.

Determination of Minerals in Poultry Manure by NIR. The remaining data in Table 2 show the results of efforts to determine minerals in the poultry manures. Overall,

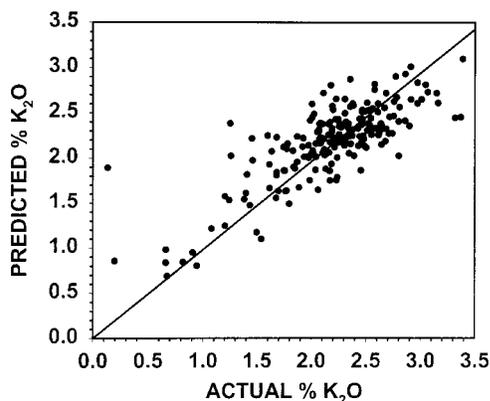


Figure 5. Final calibration results for K as K_2O for 207 poultry manures using original analyses provided by testing laboratory (performed several weeks prior to scanning).

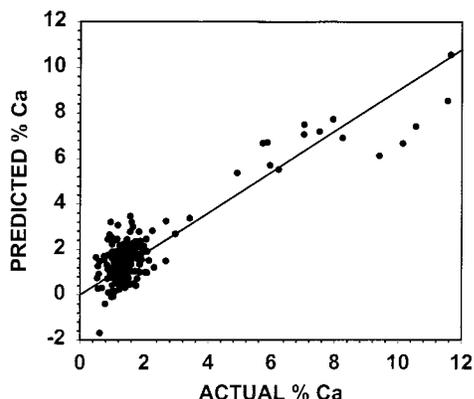


Figure 6. Final calibration results for Ca for 207 poultry manures using original analyses provided by testing laboratory (performed several weeks prior to scanning).

examination of the calibrations for minerals shows the results to be unsatisfactory regardless of the spectral region used, the best results being for the determination of Ca and K using NIR spectra. However, as shown in Figure 5, the results achieved for K would not be very useful if an accurate value for a specific sample was needed. Even removal of eight samples indicated by the software to be concentration outliers did not produce a very satisfactory calibration ($R^2 = 0.708$ and $RMSD = 0.254$). To obtain calibration results similar to those achieved for NH_4^+-N , etc., considerably more samples would need to be removed, and there was no rational basis indicated for such an effort. Except for Ca, the results for the other minerals examined followed patterns similar to those shown for K. Although the best

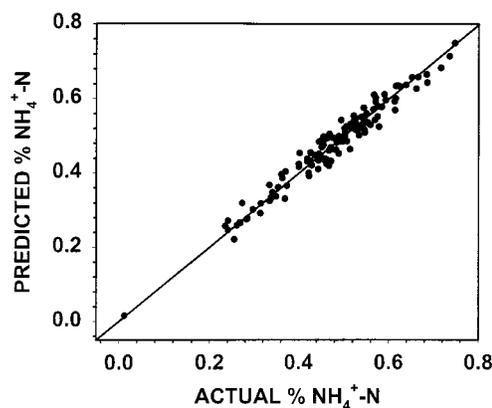


Figure 7. Final calibration results for ammonium N for poultry manures reanalyzed for ammonium N on the same day. Samples were scanned after removal of three concentration outliers ($n = 121$).

results, at least by R^2 , appeared to be for the determination of Ca, as shown in Figure 6, the Ca values were non-evenly distributed, with most samples having values between 0 and 2% and a little over a dozen samples having higher values (up to 12%). These results indicate that NIRS could be useful in determining poultry manures with abnormally high Ca levels, but the reason for these higher level samples is unknown.

Overall, the results seen for the other minerals are more typical for NIRS and mineral determinations. Due to the lack of spectral absorptions for minerals in the NIR region, calibrations are generally not very good and depend on relationships between organic components and the minerals, that is, indirect or surrogate calibrations (12, 13). The problem with such surrogate calibrations is that they are only as accurate as the correlation between the organic component and the mineral of interest and can easily produce erroneous results if other sources of the mineral, that is, limestone, etc., are added to the sample. In this case, such information was not provided with the samples, and the reasons for the calibration seen can only be speculated upon. In summary, the results achieved using 207 poultry manures do not support the use of NIRS for the accurate determination of minerals in poultry manures.

Determinations Using 124 Freshly Analyzed Manures. On the basis of the conclusion that the NH_4^+-N levels may have changed in at least some samples between the initial analysis and the NIRS scanning, 124 samples were reanalyzed for NH_4^+-N just prior to scanning. Total N was computed for these samples using the new NH_4^+-N values and the previ-

Table 3. Final Calibration Results Using NIR Spectra (1100–2498 nm) for 124 Samples Reanalyzed for Ammonium N

component	all samples ($n = 124$)						with concentration outliers removed					
	F^a	1-out \times validation		final calibration		n	F	1-out \times validation		final calibration		
		R^2	$RMSD^b$	R^2	$RMSD$			R^2	$RMSD$	R^2	$RMSD$	
Analytes Calculated on an As Is Basis												
NH_4^+-N	13	0.910	0.037	0.947	0.028	121	13	0.927	0.033	0.960	0.024	
organic N	11	0.853	0.337	0.911	0.261	118	12	0.910	0.256	0.944	0.200	
total N	11	0.855	0.337	0.911	0.263	118	12	0.908	0.261	0.943	0.204	
dry matter	8	0.966	0.019	0.977	0.016	119	8	0.980	0.014	0.986	0.012	
Analytes Calculated on a Dry Matter Basis												
NH_4^+-N	10	0.868	0.083	0.932	0.060	118	10	0.899	0.064	0.933	0.053	
organic N	13	0.728	0.463	0.837	0.353	116	10	0.780	0.391	0.853	0.320	
total N	13	0.710	0.467	0.823	0.362	116	10	0.753	0.407	0.838	0.331	

^a Number of calibration factors used. ^b $RMSD = \text{root-mean-squared deviation} = (\text{sum of squared residuals}/n)^{1/2}$; units indicated by component.

ously determined organic N values. The results are shown in Table 3 for analyte values computed on as is and DM bases. As can be seen, the results were considerably improved, especially for $\text{NH}_4^+\text{-N}$, the analyte for which the analytical values were in question (Table 3). Removal of three samples identified as concentration outliers produced the results in Figure 7. Overall, the results for all measures, after the removal of a few outliers, were excellent, demonstrating that accurate calibrations for these analytes in poultry manure were possible. As previously discussed, a short study indicated that the organic N values can change over time, and this may also account for some of the outliers found for total and organic N determinations.

Finally, calibrations were performed using DM adjusted analyte values. Although previous work with silages (14) indicated that equally good calibrations can be obtained whether using analyte values on an as is or a DM basis, this does not appear to be the case with poultry manures as demonstrated in Table 3. Comparing the as-is-based and DM-based calibrations (data not presented) showed that it was not just a case of lowered R^2 due to different ranges of analyte values, but rather less accurate overall determinations as indicated by increased scatter in the data.

In conclusion, results from NIRS studies on >200 poultry manures showed that (1) accurate calibrations for ammonium, organic, and total N and moisture can be developed using NIR spectra from 1100 to 2498 nm, (2) inclusion of spectral information in the visible–shortwave-NIR region (400–1098 nm) offers little or no additional benefit, (3) calibrations based on only the visible–shortwave-NIR region are not viable, and (4) determination of minerals in poultry manures using any of the spectral regions discussed is also not viable.

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