Comparison of Spectroscopic Techniques for the Determination of Kjeldahl and Ammoniacal Nitrogen Content of Farmyard Manure

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The feasibility of determining the nitrogen content of farmyard manure using infrared spectroscopy was investigated. Fifteen samples each of cattle, pig, and turkey manure were analyzed by three infrared techniques: Fourier transform mid-infrared (MIR), using attenuated total reflection (ATR); near-infrared reflectance (NIR-R); and near-infrared optothermal photoacoustic (NIR-OT). The near-infrared measurements were made at wavelengths determined respectively by four (NIR-OT) and five (NIR-R) band-pass filters. The total nitrogen (using the Kjeldahl method) and volatile (ammoniacal) nitrogen contents of all samples were measured by wet chemistry. Internally cross-validated (ICV) partial least-squares (PLS) regression was then used to obtain calibrations for the nitrogen content. The data sets obtained by each technique were treated separately. Within these sets, data from each manure type were treated both separately and combined: the best predictive ability was obtained by combining data from all three manure types. From the combined data set, the residual standard deviations and correlation coefficients for the ICV-predicted versus actual Kjeldahl nitrogen content were, respectively, 6772 mg/kg dry wt, 0.862 (MIR); 9434 mg/kg dry wt, 0.771 (NIR-OT); and 8943 mg/kg dry wt, 0.865 (NIR-R). For the ammoniacal nitrogen content, the residual standard deviations and correlation coefficients were 3869 mg/kg dry wt, 0.899 (MIR); 6079 mg/kg dry wt, 0.820 (NIR-OT); and 3498 mg/kg dry wt, 0.961 (NIR-R).

INTRODUCTION

An estimated 41 million pounds of available nitrogen is present in the 40 million metric tons of solid manure produced annually by housed cattle, pigs, and poultry in the UK. Unfortunately, only a small proportion of this material is properly utilized, or given adequate consideration when calculating subsequent inorganic fertilizer applications. This is, in part, because of unawareness of the nutrient content of manure. Although generalized guide values are available, their applicability to individual farm conditions is questionable. Estimation methods for determining nutrient content exist, based upon parameters such as dietary intake and animal performance, but these cannot predict the compositional variability of manure or take account of storage losses. Consequently, to fully utilize solid livestock wastes, suitable methodology and hardware are required for in-field determination of plant nutrient concentrations, and ultimately, for controlling the application of the manure.

Manures from different sources (for example, cattle, pig, turkey) contain markedly different concentrations of total nitrogen. However, only a proportion of this, the "available nitrogen", is taken up by plants within a few weeks of manure application (*1*). The remainder must be broken down into "available" forms by soil microorganisms, possibly over a number of seasons. Plants absorb nitrogen from the soil in the form of either ammoniacal nitrogen (NH_4^+) or nitrate (NO_3^-), the former being converted to the latter by soil bacteria. Different manure types also vary in their content of ammoniacal nitrogen. Moreover, there is substantial variation in the content of each form of nitrogen within manures of the same type, highlighting the need for more specific information regarding nutrient contents. A recent study (*2*) stated that, to optimize the utilization of farmyard manure, the nutrient content of representative samples should be checked on a regular basis. Ideally, a nutrient sensing system would be able to estimate the nitrogen concentrations present within a sample in near "real-time" (maximum, ~tens of seconds), preferably during field spreading.

The work in this paper examines three potential methods for performing this kind of analysis and develops the methodology and calibrations for predicting nitrogen contents in three generic manure types. The methods are all based on infrared spectroscopies, which have the potential to be adapted for use in portable sensors. Near-infrared (NIR, (*3*)), and in particular NIR reflectance (NIR-R), instruments have already been deployed as sensors in hostile industrial processes. Mid-infrared (MIR) spectroscopy (*4*) has been less well-developed for such applications, but MIR spectra can be more readily interpreted than those of NIR, and methods such as attenuated total reflectance (ATR) allow for easy presentation of the sample. A recently developed NIR photoacoustic method, called optother-

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mal spectroscopy (NIR-OT), allows spectra to be collected in a way similar to that of ATR, by placing the sample in contact with a sapphire crystal (*5*).

Three different types of infrared instrumentation have been used in this work. The first system is a Fourier transform MIR spectrometer equipped with a horizontal ATR accessory. The second is an optothermal instrument modified for NIR operation and incorporating four pre-selected filters for wavelength selection. The third system is a commercial, process-hardened, NIR-R instrument, incorporating five pre-selected filters.

MATERIALS AND METHODS

Samples. Forty-five samples were available for analysis: fifteen samples each of pig, turkey, and cattle manure. The samples were collected from manure heaps on three farms in East Anglia. For each sample, 1.5−2 kg of manure was collected from different locations across the heap. Samples were sealed and placed in refrigerated storage as soon as possible after collection. Before chemical and infrared analysis, each sample was ground to a coarse paste, by passing through a motorized household mincer. A small amount of each sample was analyzed using standard wet chemistry methods to determine concentrations of nitrogenous compounds (ammoniacal nitrogen ($NH_3 + NH_4^+$) and total ("Kjeldahl") nitrogen), on a dry weight basis.

Spectrometric Analysis. Spectrometric analysis was carried out using three infrared techniques: Fourier transform MIR using ATR sampling; NIR-OT; and NIR-R. MIR spectral acquisition was carried out using a MONITIR spectrometer (Spectra-Tech Applied Systems, Inc., Shelton, CT) equipped with a horizontal ATR accessory, comprising a 45° nominal 10-reflection zinc selenide element. Paraffin film was taped over the surrounding surfaces of the accessory to prevent biological contamination. Samples were gently pressed onto the crystal surface to ensure good contact. The ATR element was cleaned between acquisitions with tissue and Bioguard disinfectant. Loading and cleaning of the accessory was performed within a biological safety cabinet.

All spectral measurements were made at 8 cm^{-1} resolution; triangular apodization was used, and 256 interferograms co-added before Fourier transformation. A single-beam spectrum was collected of each sample and converted to absorbance units using a single-beam background spectrum obtained using a second, nominally identical ATR accessory with a clean, dry crystal. The background spectra were collected during the cleaning and loading of the sample accessory; a time interval of approximately 25 min separated the acquisition of each sample spectrum and its background. All absorbance spectra were truncated to 237 data points in the "fingerprint" region of 896−1806 cm^{-1} (11.16−5.54 mm). Single-point baseline correction at 1806 cm^{-1} was performed. Spectra were retained in this form for subsequent data processing.

NIR-OT analysis was carried out using an instrument from Varilab AB (Sweden) modified for NIR operation, equipped with a sapphire sampling window and four band-pass filters at the wavelengths 1935, 2100, 2180, and 2230 nm. Filter selection was based on known values for absorptions of nitrogen-containing compounds (see below). A modulation frequency of 2 Hz was employed, which corresponds to a sampling depth of approximately 100 μ. For each filter, measurements of two properties were made: the in-phase (*A*) and quadrature (*B*) components of the optothermal signal, in each case averaged over the last 15 s of a 20-s illumination period. For each filter, these measurements were transformed to give a single value, *P*, such that

$$P = k\frac{A + B}{S - B} \qquad (1)$$

where the constant $k = 1000$, and S is the saturation signal obtained from a blank reference sample ($S = 1.5$). This derived

variate has been successfully exploited in previous quantitative analyses of food products (*5* and *6*).

Samples were pressed onto the sapphire window before data collection. The window was cleaned between acquisitions using tissue and Bioguard disinfectant. NIR-OT analysis was carried out on triplicate sub-samples of all manures. The average measurements of *P* from each set of triplicates were retained for subsequent processing.

NIR-R analysis was performed using an MM55 instrument (NDC Infrared Engineering Ltd., Maldon, Essex, UK) equipped with five band-pass filters at the wavelengths 1935, 2050, 2100, 2180, and 2220 nm. The 2050 and 2180 nm filters were selected to correspond to known absorptions of nitrogen-containing compounds; 1935 nm corresponds to water, and 2100 and 2220 nm are reference wavelengths.

Sample preparation was performed within a biological safety cabinet. Each sample was loaded into a 50-mm disposable Petri dish ensuring that the base of the dish was completely covered to present a nominally uniform surface to the NIR-R instrument. The emerging NIR beam interacted with the sample over a circular region nominally 30 mm in diameter. For each sample, reflectance measurements at the five wavelengths were recorded over 30-s time intervals.

NIR-R measurements were collected from duplicate sub-samples of all turkey and cattle manures (the NIR-R instrument was not available when the pig manure samples were supplied). The logarithm was taken of the average measurements from each pair of duplicates and retained for subsequent processing (it is usual to apply a log transformation to reflectance data in order to linearize the relationship between response and concentration of the absorbing species).

Data Analysis. All data processing was carried out using Matlab 5.3 (The Math Works Inc, Natick, MA) installed on a personal computer.

All data were normalized so that the collection of measurements on each individual sample summed to unity ("area-normalization"). This pretreatment has been shown to mitigate unwanted variability in the data from scattering effects (*7*).

Macros were written for carrying out partial least squares (PLS) regression, and were based on the algorithm for orthogonalized PLS with one dependent variate. In this method, the original predictor variates (spectral data) are transformed to a set of new predictors (the PLS scores) using the dependent variate (concentration data) to determine the parameters of the transformation; a subset of the scores is then regressed onto the concentration data.

To ensure that the PLS models obtained are neither over- nor under-fit, and are able to generalize, we have used "internal cross-validation" (ICV). For each data set under study, one spectrum (or vector of NIR measurements) was omitted and the remainder were used to obtain a regression model which was then applied to the excluded "test" spectrum. This process was repeated as many times as there were spectra in the data set, omitting each spectrum in turn, to produce a complete set of predictions from all the individuals in the data set. ICV predictions obtained in this way have been shown to give a realistic impression of the performance of the model (*8*). In the present work, ICV was carried out for PLS regression models over a range of different dimensionalities (ie., numbers of PLS scores). The optimal subset was identified as that producing the minimum standard deviation of the prediction errors ("residual standard deviation").

RESULTS AND DISCUSSION

The data obtained by all three methods are shown in Figure 1. Only the MIR spectra (Figure 1a) can be readily interpreted. The spectra from the three manure types show considerable inter- and intra-type variability. From visual inspection, spectra from the pig and turkey manures show the greatest variability, those from cattle manure show the least: this is broadly consistent with the variation in the reference nitrogen contents. Regarding inter-type variability, there is
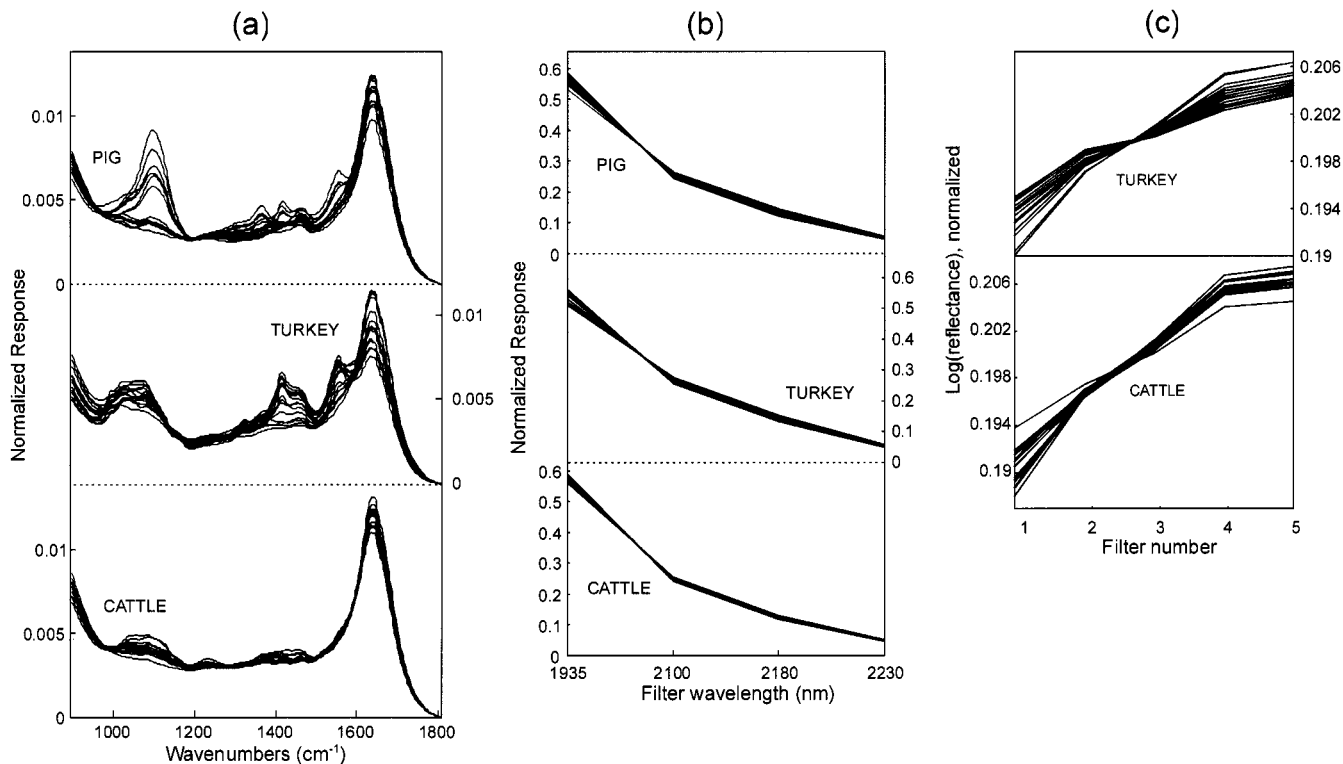
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Figure 1. Data obtained by all three infrared methods: (a) MIR; (b) NIR-OT; (c) NIR-R.

clearly a marked effect arising from differences in sample water content. The ratio of the water (O−H bend) peak at 1650 cm^{-1} to other bands clearly varies considerably between the manure types. This was reflected in the physical form of the samples, with cattle and pig manures being noticeably wetter and softer than turkey manure. An additional complicating factor is the variability in the amount of bedding (straw) that was present in the samples; this is likely to give rise to intensity at around 1000 cm^{-1}. Nitrogen-related absorbances are expected at (*9*) 1650−1590 cm^{-1} (N−H deformation, primary or secondary amine); 1220−1020 cm^{-1} (C−N, primary or secondary amine); 1650 and 1550 cm^{-1} (C=O, amide I and C−N amide II); 1680−1650 or 1625−1613 cm^{-1} (N−O stretch of nitrites); 1640−1626 cm^{-1} (NO$_2$ symmetrical stretch of nitrates, also at 1280−1270 cm^{-1}); 1390−1430 cm^{-1} (NH$_4^+$ deformation); and 1550−1430 cm^{-1} (NH$_3^+$ deformation). In all the MIR spectra, features can clearly be seen in the regions where nitrogen compounds are expected to absorb, and it is likely that a number of such compounds are present in all samples. Variability in the amount of these compounds present may arise from variation in the age of the manure stacks or in the positions from which samples were obtained. Heterogeneity within stacks may also result from dietary differences, which may explain much of the inter-species variability.

In the first instance, the data arising from the three different manure types were treated separately. PLS (incorporating ICV) was applied to the data obtained by each technique, from each manure type, and for the nitrogen contents determined by each of the two reference methods. This entailed sixteen separate calibration processes: in all cases, ICV predictions were calculated for models using from one to the maximum number of nontrivial PLS scores (which is equal to min(*d*, *m*-1), where *d* is the number of variates and *m* is the number of individuals used in the calibration). The optimum

model dimensionality was identified as that producing the minimum in the standard deviation of the prediction errors: these are given, along with the correlation coefficient between the ICV predicted and actual nitrogen concentrations, in Table 1.

No useful calibrations were obtained for cattle manures by any of the techniques, or for pig manures by NIR-OT or NIR-R. The MIR data, however, yielded an acceptable calibration for the pig manure and ammoniacal nitrogen content. In contrast, the ICV predictions for the turkey manures were encouraging for all three infrared techniques. The difference in the ability to calibrate probably reflects the differences in the ranges of nitrogen contents of each manure type; turkey manures exhibit the widest range of reference values. Plots of the predicted versus reference nitrogen contents for the turkey manure calibrations are given in Figure 2. The relatively large prediction errors in all calibrations are likely to be due to sample inhomogeneity: even after the sample preparation step, the manures cannot be regarded as homogeneous systems and are certainly heterogeneous on the micron scale (the order of magnitude over which the infrared radiation interrogates the sample, in the MIR and NIR-OT techniques). Inevitably, the problem of sub-sampling and interrogating representatively, particularly in the infrared analysis but also in the reference methods, will introduce error, and it may be quite large. Gross heterogeneity of the samples may lead not only to variability in the measurements obtained by any one technique, but also means that the samples used for reference analysis may be chemically different from those used in the spectroscopic analysis. Furthermore, considering that chemical and spectroscopic analyses were carried out at different sites and at different times, sample handling and aging may represent additional sources of variability.

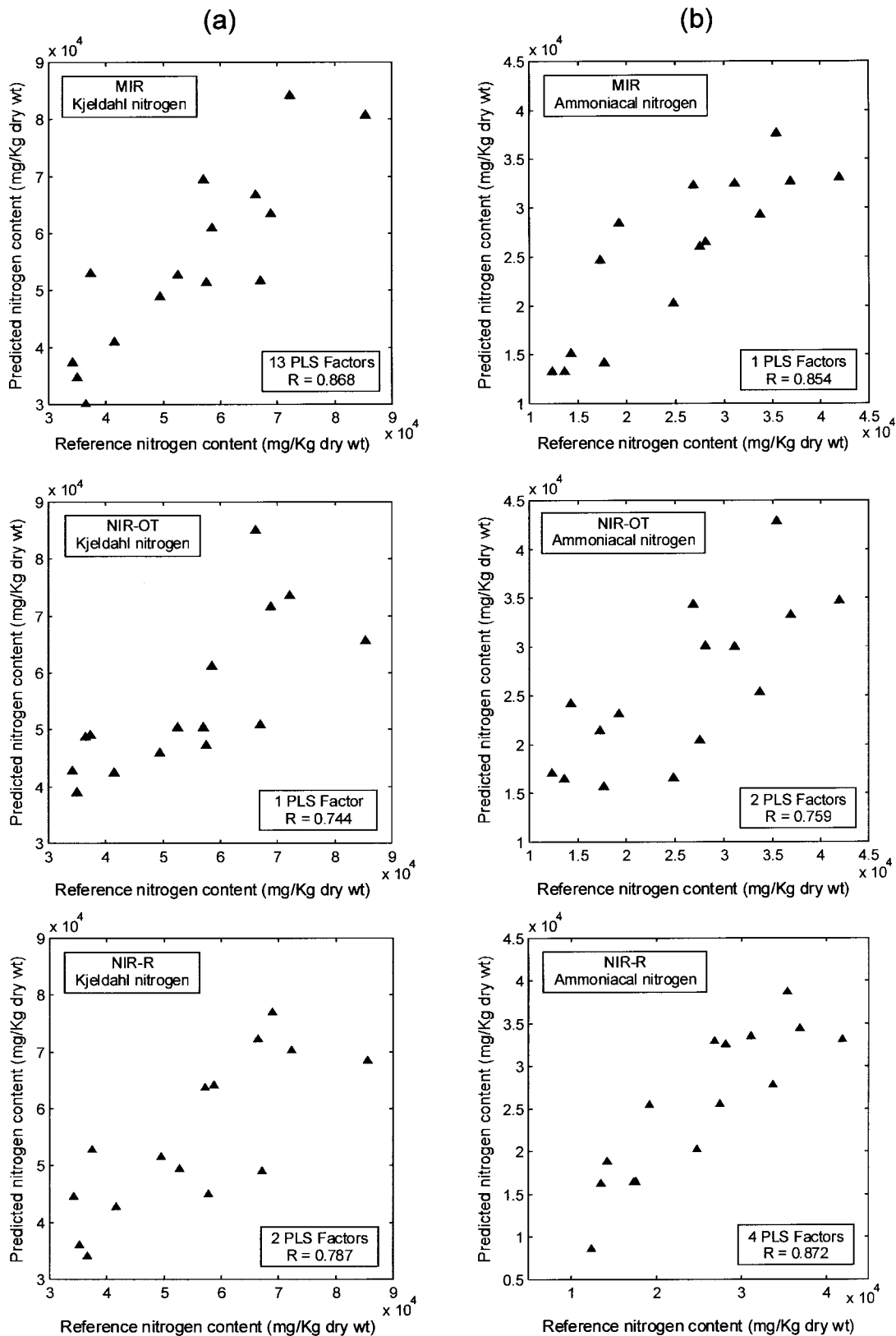Next, the data from all manure types obtained by each infrared technique were concatenated to give three

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Figure 2. ICV-predicted versus reference values for turkey manure for (a) Kjeldahl, and (b) ammoniacal, nitrogen contents.

"combined" data sets. PLS (incorporating ICV) was applied to each of these, for the nitrogen content determined by each of the two reference methods. Details of these six calibration processes are also given in Table 1. Plots of the standard deviation of the prediction errors versus the number of PLS scores used in the regression are given in Figure 3. For the MIR data, the best calibration performance is obtained from model dimensionalities of 12 and 16, respectively, for the Kjeldahl and ammoniacal nitrogen contents. There is a clear onset of overfitting once model dimensionalities exceed around 20; this behavior is as expected from a high-dimensional data set. Figures 3b and 3c show that PLS is also advantageously used to analyze the low-

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Table 1. Standard Deviation of Prediction Errors, Model Dimensionality, and Correlation between Predicted and Reference Values for All "Useful" Calibrations[a]

infrared method	manure type	number of samples	Kjeldahl nitrogen calibrations			ammoniacal nitrogen calibrations		
			residual standard deviation (mg/kg dry wt)	number of PLS factors	correlation between actual and ICV- predicted values	residual standard deviation (mg/kg dry wt)	number of PLS factors	correlation between actual and ICV- predicted values
MIR	pig	15	no useful calibration obtained[a]			2209	7	0.853
MIR	turkey	15	8143	13	0.868	4860	1	0.854
MIR	cattle	15	no useful calibration obtained			no useful calibration obtained		
NIR-OT	pig	15	no useful calibration obtained			no useful calibration obtained		
NIR-OT	turkey	15	10578	1	0.744	6182	2	0.759
NIR-OT	cattle	15	no useful calibration obtained			no useful calibration obtained		
NIR-R	turkey	15	9729	2	0.787	4627	4	0.872
NIR-R	cattle	15	no useful calibration obtained			no useful calibration obtained		
MIR	combined data set [pig; turkey; cattle]	45	6772	12	0.862	3869	16	0.899
NIR-OT	combined data set [pig; turkey; cattle]	45	9434	3	0.771	6079	3	0.820
NIR-R	combined data set [turkey; cattle]	30	8943	3	0.865	3498	4	0.961

[a] A calibration is designated "useful" if the regression of the ICV predictions onto the reference values has $R > 0.514$ (separate manure data sets), $R > 0.292$ (combined data sets, MIR and NIR-OT), and $R > 0.357$ (NIR combined data set). These conditions equate to F-tests at the 5% level.
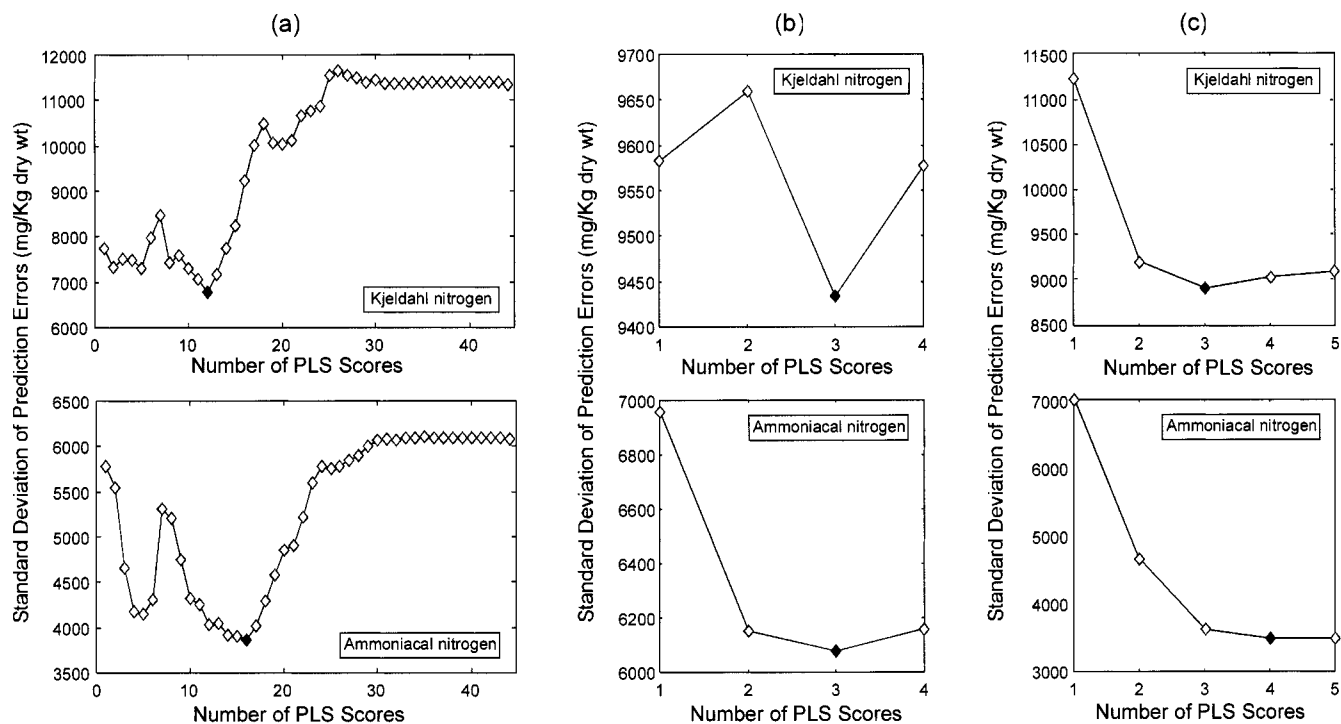

Figure 3. Standard deviation of the prediction errors versus the number of PLS scores used, for (a) MIR, (b) NIR-OT, and (c) NIR-R "combined" data sets. The solid diamonds ◆ indicate the optimum model dimensionality.

dimensional data sets. In all cases, the optimum model dimensionality (again, defined as that producing the minimum in the standard deviation of the prediction errors) is lower than the maximum number of PLS scores (calibrations that use all nontrivial PLS scores produce results effectively identical to those of OLS applied to the original data).

Plots of the ICV-predicted versus reference nitrogen contents are presented, for the optimum model dimensionalities and all three infrared methods, in Figure 4. For the Kjeldahl reference data, the calibration performances of the MIR and NIR-R techniques were similar. For the ammoniacal nitrogen, NIR-R performed best, giving an encouraging correlation coefficient between

the predicted and reference values of 0.961. However, direct comparison of the infrared techniques is perhaps unjust, because the NIR-R data set did not include measurements from the pig manures as the instrument was not available at that time. The calibration performance of the NIR-OT data was consistently poorer; however, useful calibrations were obtained.

CONCLUSIONS

All the techniques studied were able to produce calibrations for Kjeldahl and/or ammoniacal nitrogen content from at least some of the data sets, although the best predictive ability was obtained from MIR and
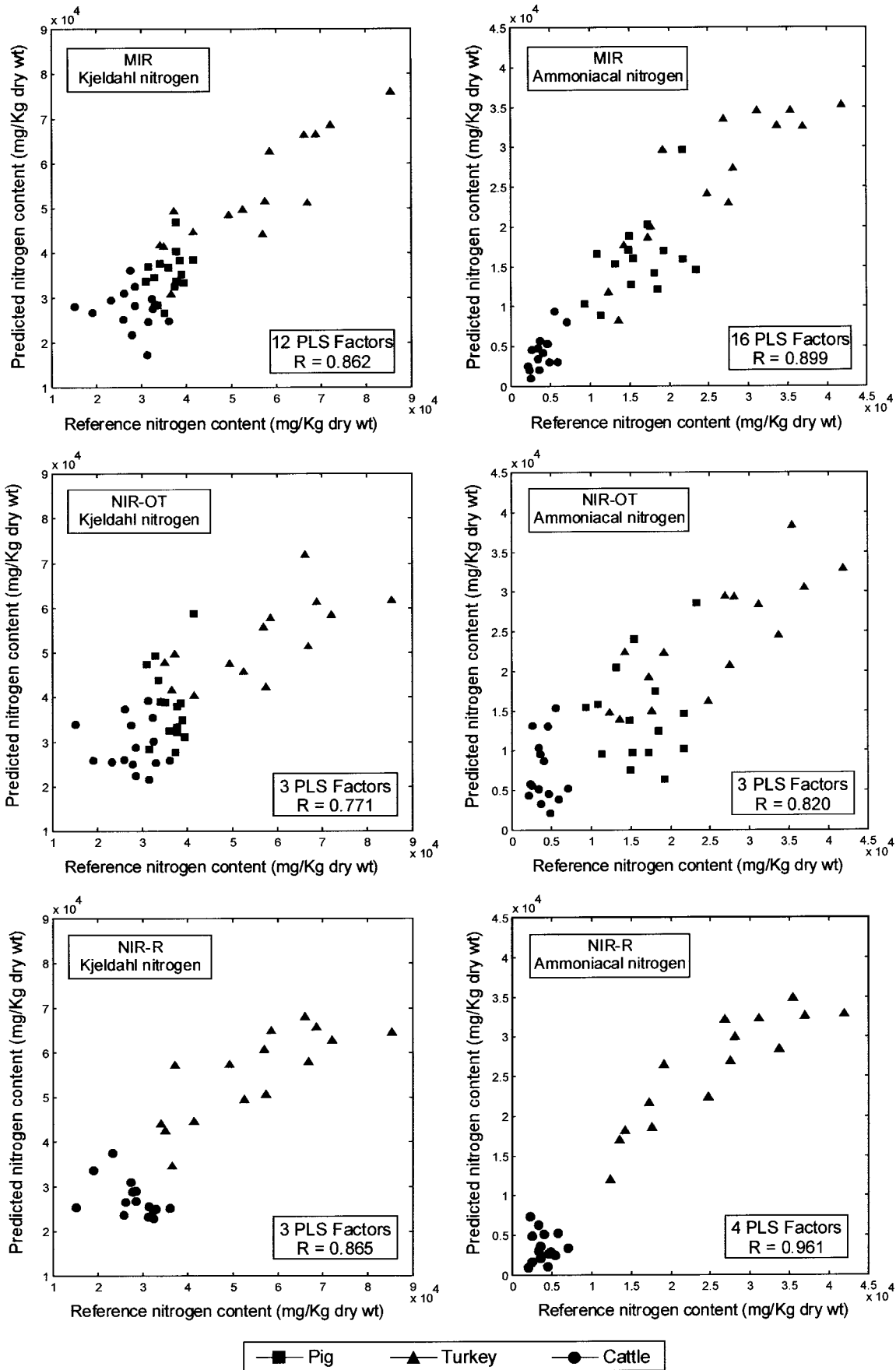
Figure 4. ICV-predicted versus reference values for all three infrared methods, for (a) Kjeldahl, and (b) ammoniacal, nitrogen contents.

NIR-R. Of the manures tested, turkey exhibits the highest and most variable nitrogen content; probably

for these reasons, the data from turkey manures also produced the best calibrations. A large range of refer-

ence values is helpful because the representative examination of such heterogeneous samples is difficult, and substantial error may be introduced. If the range of reference values is small (such as for cattle), the error due to unrepresentative interrogation may altogether prevent a useful calibration from being established.

For a practical implementation of the method on the farm or on a muckspreader, the NIR-R method would be the technique of choice. First, the instrumentation could be readily adapted to make continuous measurements of sample reflectance at the spreader outlet. Second, NIR radiation penetrates further into samples than MIR and, therefore, offers more representative interrogation. Improved calibrations may result from further instrumental development, for example through optimization of filter selection or an increased sampling volume. We believe the NIR technique has the potential to form the basis of a farm-based nitrogen control system.

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