# The use of near infrared spectroscopy (NIRS) to better assess livestock effluents composition. A national experience.

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## Abstract

A total of 202 cattle manure (CM), 165 poultry manure (PM), and 174 pig slurry (PS) samples were collected in France and Reunion Island' major livestock regions. Samples were analyzed for their dry matter (DM), total Nitrogen (N) and ammonium ($NNH_4$) contents. After homogenization of the spectral responses (standardization, removal of interference due to sample holding devices) of the three NIR spectrometers, NIR models were shown to valuably characterize the agronomic potential of fresh manures. As an example, the standard error of the NIR model for N of PM fresh samples, was only twice to three-folds those reported for models developed with finely homogenized (ground, dried) samples. Even done on a small set, direct "on farm" characterization of PM with a portable apparatus after a rough homogenization of the samples showed promising performance. This can represent a valuable alternative to expensive and time-consuming reference methods in the laboratory.

## Introduction

Manures represent 95% of the organic wastes used as fertilizers and amendments in France. They present a wide diversity in composition and nature due to livestock farming systems, stock conditions and subsequent treatments. Even though there is an extended knowledge on manure recycling in France, there is a need to better assess the characteristics of the organic wastes under concern. This is of particular interest for management tools. Near infrared spectroscopy (NIRS) is used for long as an alternative method to characterize manures [1-6], but not on the French national basis. The aims of the present study were thus (i) to build NIRS models for the prediction of the main characteristics of the most abundant manures in France, and (ii), to test the potential of NIRS to assess the composition of manures directly on farm.

## Material and Methods

*Origin, preparation and analyses of livestock effluents*
Sampling, collection route, conservation, subsequent treatments and analyses of the samples were done according to a procedure that was adopted by all participants in order to minimize inter-comparison problems. A total of 202 cattle manure (CM), 165 poultry manure (PM), and 174 pig slurry (PS) samples were collected in France and Reunion Island' major livestock regions of production. Samples were transported in containers with ice packs till their preparation in the laboratory. Fresh slurries were deep-frozen pending for spectra acquisition or analyses. Solid manures were homogenized with a Dito K45 cutter (Electrolux, Senlis, France). Aliquots were made for spectra acquisition on a fresh state or deep-frozen pending for spectra acquisition and analyses. Dry matter (DM), total N (N) and ammonium (N-$NH_4$) were quantified according to French standards. Standard error of laboratory (SEL) were estimated with repeated measures on intern standards (manure, fodder) used to build control charts.

*NIR spectra acquisition (on farm and at lab) and standardization of the spectral responses*
Poultry manure NIR spectra were taken "on farm" with a portable spectrometer (LabSpec, ASD Inc., Boulder, CO, USA) in Reunion Island. Samples were collected in the chicken houses when a batch was removed for slaughtering. Samples were roughly homogenized by hand in a 20 L bucket, and 10 spectra were taken with an optical fibre device on the surface of each sample. Spectra were collected every 1nm, averaged (32 scans per replicate) and recorded as log (1/reflectance). In the laboratories, NIR spectra were taken (i) from homogenized solid (CM, PM) fresh samples in triplicate (3 cup/Petri dish/crystallizing dish fillings) using laboratory NIR spectrometers (two XDS, Foss, Silver Spring, MD, USA for Arvalis and Cirad, and a NIRFlex, Büchi, Flawil, Switzerland for LDAR), and (ii) from hand shaken pig slurry (PS) samples in 4 replicates through the 4 sides of glass vials tightly closed by screw caps. Spectra were collected every 2nm, averaged (32 scans per replicate) and recorded as log (1/reflectance). Replicate spectra were averaged after control of the RMS. Since the spectra were acquired with different spectrometers using different devices, a standardization of the spectral responses of the apparatus was required in order to merge the spectra on a common basis (data not shown). This was done (i) by subtracting the absorption of glass devices, and (ii) by using reference sealed cells whose spectra were acquired on each NIR spectrometer (2 XDS, 1 NIRFlex). Standardization (correction of both X and Y, i.e. lambda and absorbance) was done according to the Win-ISI software (Infrasoft International, Port Matilda, PA, USA).

*Spectral pre-treatment, calibrations and cross validation*
The spectra were transformed by standard normal variate and detrend (SNVD)[7] to minimize interference of particle-size on samples, then 1st derivative was calculated on 5 datapoints and smoothed on 5 datapoints [8] according to the Win-ISI software (Infrasoft International, Port Matilda, PA, USA). Calibrations of DM, N and N-NH$_4$ contents of each effluent set (CM, PM, PS) were performed using the modified partial least square regression (mPLS) of WIN-ISI in order to test the global feasibility of NIR models. Since mPLS is generally performed on sets containing more than 100 data, the multiple linear regression (MLR) was preferably performed for relatively small data sets: PM with 74 N values (laboratory spectra), and PM with 46 DM, N and NNH$_4$ values (on farm spectra). Calibration statistics include the standard error of calibration (SEC), the coefficient of determination (R²), the standard error of cross-validation (SECV), and the coefficient of variation (SECV expressed in % of the mean). Being independent of the parameters units, the RPD ratio (RPDcv= standard deviation/SECV) was used as an evaluation of the general quality of the calibration model developed for each parameter.

**Results**
Table 1 presents the basic statistics of the reference analyses and calibration statistics from NIR models for PS, CM and PM. Depending on the parameter or effluent considered, performance of the models varied. Models for PS presented the least accuracy (R² as low as 0.5) and precision (CV> 20%). For such diluted manures (e.g. DM<1 g 100g$^{-1}$ b.w.), N and NNH$_4$ contents expressed on a DM basis gave unrealistic reference values (e.g. N>50 g 100g$^{-1}$DM). This can partly explain the poor performance of PS models, combined with the nature of the PS itself (a heterogeneous suspension), or to its low reflectivity [3]. It has also been pointed out by several authors [3, 9] that the difference in predictive ability can be attributed to great variability of the sample set. It is particularly true in the present study that combines variability from origin (location, feed), spectra acquisition on several spectrometers with different devices. NIR models for CM were comparable to PS ones for DM and N in terms of R² and RPD. Nevertheless, the CV or the SEL/SECV ratio were better for CM. NNH$_4$ model for CM over-performed the PS one (see SECV and SEL/SECV). Even if NIR models development for CM need improvement (see R², SECV, RPD, CV, and SEL/SECV), the SECV associated with N in this study on fresh CM samples was in the range of those reported for dried ground CM[2, 10]. In contrast, NIR models for PM can be classified among the good ones, their R² approaching or exceeding 0.90, RPD above 3, and CV varying between 2.5 and 14%. Comparable performances were reported elsewhere[11] for smaller data sets. It can be pointed out that the present SECV for fresh samples were just twice to three folds those reported for homogeneous ground dried PM [6]. The SEL/SECV ratios revealed that the DM and even NNH$_4$ models are well-suited for a

utilization of NIRS in the laboratory as fast inexpensive and alternative methods to reference ones. Figure 1 represents an illustration of the PM models developed with "on farm" and "at laboratory" spectra taken from samples that were roughly homogenized, or homogenized by a cutter, respectively. Figure 1 and Table 1 evidenced that even they need improvement, the models developed with "on farm" spectra were not particularly degraded in terms of performance compared to "at laboratory" models. Even for N ($R^2 < 0.7$ for "on farm" model), the SECV, SEL/SECV and CV were comparable. A way of improvement can be an extension of the collection, since the "on farm" set concerned only half the number of "at laboratory" sample set. It would be particularly profitable for the extension of the N range (Fig. 1).

**Conclusion and perspectives**

Dealing with samples from the most represented livestock effluents collected on a national basis (in metropolitan France and Reunion Island), we showed that NIR models could be valuably elaborated for the rapid prediction of dry matter, nitrogen and ammonium contents that characterize the agronomic potential of fresh manures. This was possible provided essential procedures were respected: sampling, conservation, preparation of the samples, spectra acquisition, correction for the spectral interferences from different devices used for collecting the spectra, and standardization of the spectral responses of the different type of spectrometers. DM models for CM and PM can be as performing as reference methods in the laboratory. Models for PM were shown to be the most performing for the 3 parameters under concern. The standard error of the model that was developed for N with fresh samples, was only twice to three-folds those reported for models developed with finely homogenized (ground, dried) samples. This can represent a valuable alternative to expensive and time-consuming reference methods in the laboratory. Even done on a small set, direct "on farm" characterization of PM with a portable apparatus after a rough homogenization of the samples showed promising performance. "On farm" and "at laboratory" NIR technique seem to be good candidates for rapidly providing a valuable information that can be used as input data in livestock effluents management tools. As an extension of the present study, we are currently working on CM characterization in a comparable way to that described for PM.

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**Tab.1 NIR models developed for "PS" (pig slurry), "CM" (cattle manure), "PM" (poultry manure) with spectra taken from fresh homogenized samples in the laboratories, or for "PM-farm" with poultry manure spectra taken from fresh roughly homogenized samples in the chicken houses.**

| Constituent | set | n | Mean | SD | SECV | SEL/SECV | R² | λ | RPDcv | CV% | regression |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DM in % b.w. | PS | 150 | 4.72 | 3.39 | 1.436 | 1.05 | 0.819 | 691 | 2.4 | 30 | mPLS |
|  | CM | 166 | 20.9 | 3.88 | 1.361 | 1.00 | 0.876 | 691 | 2.8 | 7 | mPLS |
|  | PM | 155 | 59.8 | 12.4 | 1.511 | 1.11 | 0.985 | 691 | 8.2 | 2.5 | mPLS |
|  | PM-farm | 46 | 64.73 | 12.44 | 1.499 | 1.10 | 0.985 | 5 | 8.3 | 2.3 | MLR |
| N in % DM | PS | 144 | 9.81 | 3.53 | 2.482 | 22.1 | 0.501 | 691 | 1.4 | 25 | mPLS |
|  | CM | 166 | 2.45 | 0.44 | 0.285 | 2.53 | 0.579 | 691 | 1.5 | 12 | mPLS |
|  | PM | 74 | 4.93 | 1.22 | 0.382 | 3.40 | 0.901 | 8 | 3.2 | 8 | MLR |
|  | PM-farm | 39 | 4.10 | 0.69 | 0.392 | 3.49 | 0.667 | 4 | 1.8 | 9.5 | MLR |
| NNH₄ in % DM | PS | 142 | 5.93 | 2.7 | 1.780 | 26.8 | 0.563 | 691 | 1.5 | 30 | mPLS |
|  | CM | 170 | 0.25 | 0.18 | 0.074 | 1.12 | 0.834 | 691 | 2.5 | 30 | mPLS |
|  | PM | 153 | 1.03 | 0.43 | 0.141 | 2.12 | 0.892 | 691 | 3.1 | 14 | mPLS |
|  | PM-farm | 45 | 0.96 | 0.41 | 0.097 | 1.46 | 0.942 | 5 | 4.2 | 10 | MLR |

n is the number of spectra used for modeling; SD standard deviation; SECV standard error of cross-validation; SEL/SECV ratio standard error of laboratory / SECV; R² coefficient of determination; λ is the number of wavelengths used by the models; RPDcv ratio SD / SECV; CV% coefficient of variation; regression type is modified partial least square or multiple linear
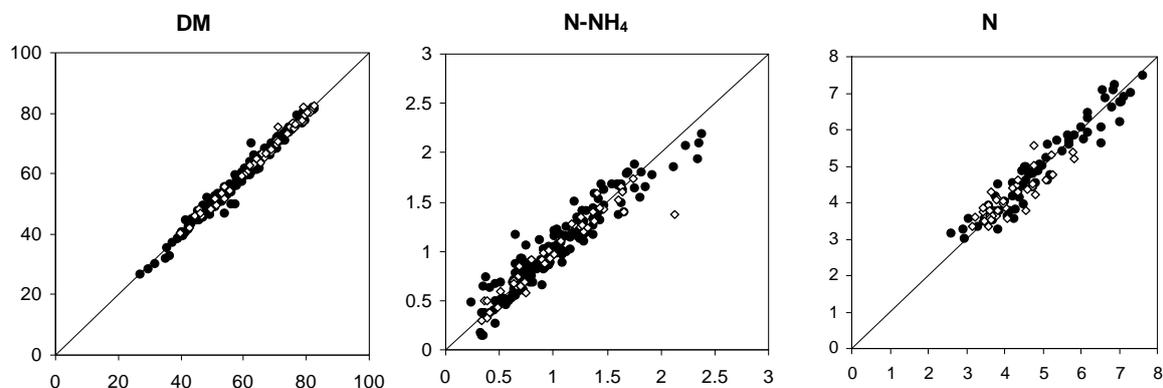


**Figure 1. Reference and NIR-predicted Dry Matter (g 100g⁻¹ b.w.), N-NH₄ and total N (g 100g⁻¹ DM) contents for Poultry Manures (dots for laboratory samples; circles for "on farm" samples).**