Prediction of nitrogenous compounds in poultry excreta by near infrared spectroscopy

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Introduction

Digestibility of protein is an essential data in the evaluation of nutritional value of feed and raw materials. It is measured by difference between dietary and faecal proteins, which are in fact estimated via the total nitrogen with the classical 6.25 conversion factor. In poultry this procedure is inappropriate because the excreta are in fact a mixture of faeces and urine: the use of total N as an estimate of protein is therefore impossible. Nitrogen in urine is mainly constituted of uric acid (UA) which contains 33% nitrogen. The classical method to overcome this problem[1] is to precipitate proteins of samples after UA solubilisation in formaldehyde. Then true faecal protein is estimated by calculation based on the "precipitable" protein. However this method

- is quite long to perform
- introduces some approximations. For example the ratio between true protein (TP) and precipitable protein (PP) is assumed to be constant (TP = 1.18 × PP)
- uses high quantities of lead: 2.5 g lead acetate per sample which represent 16 g pure lead per digestibility trial (six animals / two replicates)

Near infrared (NIR) spectroscopy has already been used to predict some aspects of poultry excreta / manure composition: moisture, total nitrogen, minerals,[2,3] gross energy,[2] and ammonia nitrogen[3] but, to our knowledge, no significant result has been published on uric acid and TP.

The present study was initiated to investigate the usefulness of NIRS in digestibility studies. Two alternatives can be tested: either produce a calibration of reference TP data, or try to estimate the UA content, which could by the same way improve the pertinence of information because it would avoid the use of the fixed calculation factor. The study was based on a wide database of samples available with known TP and UA values.

Materials and methods

Samples and chemical analyses

A total number of 406 samples of excreta was collected. They originate from many digestibility trials with very variable conditions (type and age of birds, nature and composition of feed, etc.). They were analysed by Kjeldahl method for Total Nitrogen and Terpstra method[1] for True protein. For Uric Acid, a spectrophotometric method adapted from Marquardt[4] was used. This method has been successfully validated against HPLC measurement.[4,5]

Spectra and data treatment

Spectra were collected in reflectance mode on a Foss NIRSystem 6500 spin cell equipment. The spectra were measured in duplicate (with two different cup fillings) and averaged. Various data processing were applied, with several mathematical treatments (derivatives, SNV, Detrend, etc.) in the WINISI package (Infrasoft Intl.). Reliability of prediction models was assessed by cross validation (with 4 subgroups) resulting in the calculation of a *SECV*.

In a second step 50 samples taken at random were discarded from the database and the calibrations were processed again on the remaining samples. The prediction of these 50 samples with the new equations allowed the calculation of a standard error of prediction (*SEP*).

Results and discussion

The best calibrations were obtained with PLS method, on the second derivative of spectra, pretreated with SNV and detrend corrections. The visible wavelengths were discarded because they introduced instability in equations, for example, *SEP* values much greater than *SECV* values.

The performance of predictions obtained in this study (Table. 1) are very encouraging in regard to the extreme variability of samples chosen and to the laboratory errors in reference values (*cv* = 6% and 5% for UA and TP, respectively). Indeed values of *RPD* (= *SD* / *SECV*) ranged from 4.3 to 5.2 for the various compounds which allows a reasonable prediction even if a better accuracy would be required for very precise digestibility experiments.

Table 1. Performance of calibration models

Constituant	*n*	Population		Calibrations statistics				
		Mean	*SD*	*SEC*	R^2	*SECV*	*RPD**	*SEP*
Total N (%DM)	375	5.38	1.13	0.19	0.97	0.26	4.31	0.38
Uric acid (%DM)	355	8.91	2.72	0.38	0.98	0.52	5.20	0.45
True protein (%DM)	379	12.37	2.77	0.53	0.96	0.61	4.41	0.82

*RPD = SD / SECV

The values found in literature[2,3] for total N in poultry excreta are *RPD* = 2.00, and 2.11 to 2.97. Our results have greater *RPD* values, due to the much wider database used, with *SECV* values of the same magnitude. Unfortunately UA and TP calibrations cannot be compared to literature sources.

The better prediction obtained for UA compared to TP measurement (*RPD* = 5.20 vs 4.41) can be due to the fact that UA is a single biochemical compound, which spectral fingerprint is therefore more typical and less dependant of the nature of the animal / feed used in the experiments. Also the drawbacks of the classical true protein method (*cf* introduction) can decrease its biochemical significance and therefore its ability for being accurately predicted.

The validation performed on a randomly chosen subset shows *SEP* values consistently higher than *SECV* except for UA. This is obviously due to the heterogeneousness of the database and will probably be overcome in the future by performing local calibrations on a bigger database. Figure 1 shows that the prediction of UA has a much better R^2 value and a very low bias compared to TP. This is again an important element in favour of the use of the UA criterion in digestibility trials rather that TP in the perspective of use of NIR.

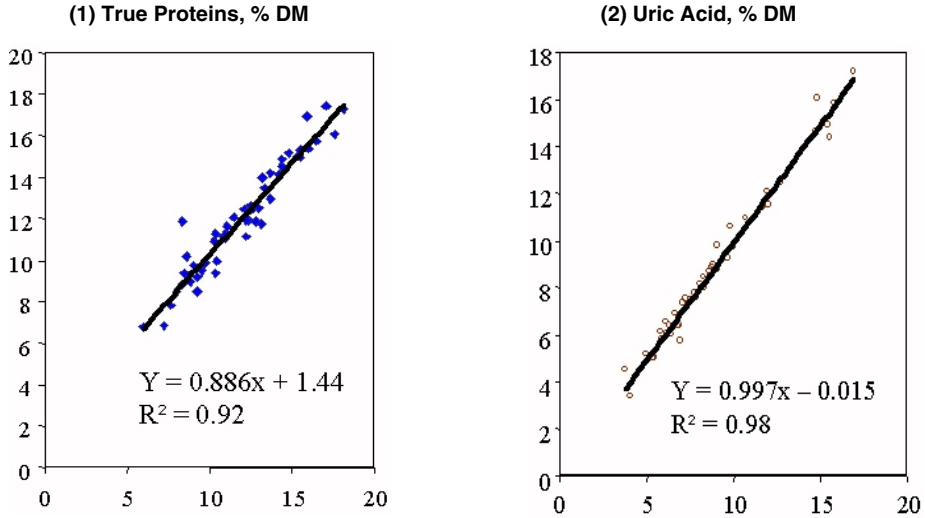

Figure 1. Validation of calibrations: comparison between predicted and reference values on the 50 samples used for validation

NIR appears to be a credible alternative to TP measurement in digestibility experiments in poultry. Although NIRS can be used for the direct prediction of TP in excreta according to the classical method the calculations involving uric acid show promising results. Besides UA is already acknowledged as a good marker to assess dietary protein quality in poultry.[6]

Conclusions and perspectives

These results show that there is a good potential of NIR for the determination of nitrogenous compounds in poultry excreta. The perspectives of using this technique in digestibility experiments are high, especially when one considers that Gross energy—the other major measurement in digestibility trials—can also be predicted very accurately by NIR.[7] A validation of this technique will have to be done in routine digestibility experiments to assess the robustness of the method in extreme situations (particularly atypical diets or animals). The great variability of samples involved in digestibility experiments would suggest to use local calibrations rather than one single general calibration. This will be evaluated in future developments since a higher number of samples are required.

Such calibrations could also be used to estimate the N content of poultry excreta for the management of fertilisation, as proposed by other authors.[2,3,8]

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