Use of visible-near infrared spectroscopy to predict nutrient composition of poultry excreta

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ABSTRACT

Nowadays optimal feed formulation for poultry is sought for available content, which takes into account how the nutrients are digested and metabolized by the animal. The digestibility coefficients of the nutrients are usually obtained in in vivo trials that require feeding the birds with different diets of well-known composition and analyzing a large number of excreta samples. Nutrient excreta composition is usually found by wet analytical methods. This work presents visible-near infrared (Vis-NIR) calibrations for organic matter, protein, fat, gross energy, uric acid and phosphorus in excreta from bioassays involving broiler chickens, laying hens and broiler turkeys carried out between 2017 and 2020. The Vis-NIR spectra (400–2499.5 nm) were pretreated by generalized least squares weighting (GLSW) and partial least squares regression (PLSR) was used to obtain the prediction models. The six parameters were properly predicted with the values of ratio of performance of deviation (RPD) and coefficient of determination of prediction (R^2p) of the validation set ranging from 3.7 to 4.6 and from 0.91 to 0.95 respectively. All but one of the calibrations passed the statistical tests for fit for purpose described in ISO 12099:2017. Despite the global calibrations provided satisfactory results, specific calibrations for broiler chicken excreta and for laying hen excreta were developed to check if their predictions could be even better but the results did not improve. Finally, the root mean square error of prediction (RMSEP) of the global calibrations was compared with the standard error of the reference methods employed for the analysis of these parameters, confirming their high performance and direct applicability.

1. Introduction

Feedstuff is the largest contribution in the cost of poultry production and is a key factor in the animal growth and health. While the

traditional formulation of feedstuff has been based on total content of nutrients, nowadays optimal formulations are sought for available content, which takes into account how the nutrients are digested and metabolized by the animal.

There are different indicators related to feed digestion. The most important in poultry studies are the total tract digestibility co-efficients of the main nutrients (protein, fat or amino acids) and the metabolizable energy (usually determined as apparent metabolizable energy, AME). Other important parameters are the assimilation of calcium and phosphorus because they are needed for the correct formation and maintenance of the skeleton.

The digestibility coefficients of the nutrients and AME are usually obtained through in vivo trials where birds are fed with different diets of well-known composition (Bourdillon et al., 1990). These trials require the collection and analysis of many replicate excreta samples per dietary treatment, trying to minimize external effects such as temperature and humidity in the farm.

Near infrared spectroscopy (NIRS) is a fast analytical technique that may replace the costly wet analytical methods for determining the nutrient content of excreta. Its most important characteristics are the speed, the absence of sample treatment, the null use of solvents and no generation of waste, and the fact that is a multi-parametric technique, that is, a spectrum can be used to predict various parameters simultaneously.

NIR instrument manufacturers commercialize pre-installed "universal calibrations" that are advertised to work well for the routine analysis of the most common raw materials and compound feeds. Nevertheless, commercial calibrations for poultry excreta do not exist currently and only a few calibrations have been reported in the literature. Throughout their different works, Bastianelli et al., (2003, 2004, 2005, 2010) presented calibrations for protein, gross energy, fat, uric acid and starch. Smith et al. (2001) and De la Roza-Delgado et al. (2015) developed also calibrations for gross energy, a key parameter that is required to calculate the metabolizable energy of the feedstuff. All these calibrations were applicable only to broiler chickens. Phosphorus has been determined by NIRS in some works focused on the use of the poultry excreta or manure as fertilizer (Aiken et al., 2005; Reeves, 2001; Ye et al., 2005), while Xing et al. (2008) also presented a calibration model for organic matter.

In summary, there are very few published multivariate determinations from NIR spectra for the nutrient content of poultry excreta and none of them are valid for animals other than broilers. In this work, we present global calibrations for organic matter, protein, fat, gross energy, uric acid and phosphorus developed with excreta samples of broiler chickens, laying hens and broiler turkeys. These calibrations are validated by an external dataset and also by statistical tests. Finally, they are compared with the specific calibrations developed for each poultry species.

2. Materials and methods

2.1. Samples and bioassays

A total of 1025 samples of poultry excreta were collected from 2017 to 2020 at the Institute of Agrifood Research and Technology (IRTA) in Constantí, Tarragona, Spain. The samples had been obtained from 31 bioassays whose objective was to measure the digestibility of diets involving different combinations of raw materials and additives (e.g., enzymes). 17 of the bioassays involved male Ross 308 broiler chickens with an age between 22 and 25 days (620 excreta samples), 11 HyLine Brown laying hens with 18–26 weeks of age (306 excreta samples) and two male (35 and 42 days of age) and one female (24 days) Aviagen Premium broiler turkeys (99 excreta samples), respectively. Diets were based on soybean meal and the main cereal was corn, wheat or barley. Titanium dioxide (TiO_2) was used as indigestible marker in all the studies. The samples of excreta were lyophilized, milled, and stored in sealed bags in a climatic chamber at 17 °C until their analysis. The fact that the excreta samples were obtained from animals of different digestive capacity and involved very different diets provided a wide range of undigested contents of the different nutritional fractions that had to be accounted for by the calibration models.

2.2. Reference values of nutritional parameters

The excreta samples were analyzed in the laboratory with validated methods. Dry matter, nitrogen, fat, ash and phosphorus were determined according to the "AOAC (Official Methods of Analysis of AOAC International) (2016)" methods 925.09, 968.06, 920.39, 942.05, 965.17 respectively. Gross energy was determined by calorimetry using an adiabatic calorimeter (C2000, IKA, Staufen, Germany) according to the DIN 51900 (2005) norm. Uric acid was determined by spectrophotometry following the method described by Marquardt (1983). Organic matter was calculated as the difference between dry matter and ash contents. Protein was obtained subtracting the nitrogen from the uric acid from the total nitrogen and multiplying the result by 6.25. For the six parameters studied in this work the standard error of the laboratory (SEL) were as follows: organic matter (9 g/kg, as fed), protein (7 g/kg, as fed), fat (3 g/kg, as fed), gross energy (40 kcal/kg), uric acid (5 g/kg, as fed), phosphorus (0.4 g/kg, as fed).

Since the digestibility experiments span different years and had different objectives, the number of excreta samples that could be used for modeling was not the same for all the analytical parameters.

2.3. Visible-near infrared (Vis-NIR) spectra acquisition and data analysis

Freeze-dried excreta samples were scanned on a NIRS DS2500 (Foss NIRSystems, Denmark) in reflectance mode with a 7 cm diameter cup where 30 g approximately are introduced each time. Spectra were collected every 0.5 nm from 400 to 2499.5 nm, thus covering the range from visible to NIR. PLS toolbox software (PLS_Toolbox, 2016, Eigenvector Research, Inc., Manson, WA, USA) running in Matlab (MATLAB, Version R2020a, The MathWorks Inc., Natick, MA, USA) was used to carry out all chemometric

treatments.

Partial least squares regression (PLSR) was used to develop the calibration models for organic matter, protein, fat, gross energy, uric acid and phosphorus. Common spectral pretreatments were studied including normalization, standard normal variate (SNV) (Barnes et al., 1989), multiplicative scatter correction (MSC) (Geladi et al., 1985), derivatives (1st and 2nd) (Savitzky and Golay, 1964), orthogonal signal correction (OSC) (Wold et al., 1998) and generalized least squares weighting (GLSW) (Zorzetti et al., 2011). Cross-validation (CV) was used to choose the optimal pretreatments and the optimal number of latent variables for each model.

The spectra and especially the score plots of the PLS models were checked for sample distribution. The sample set was divided into a
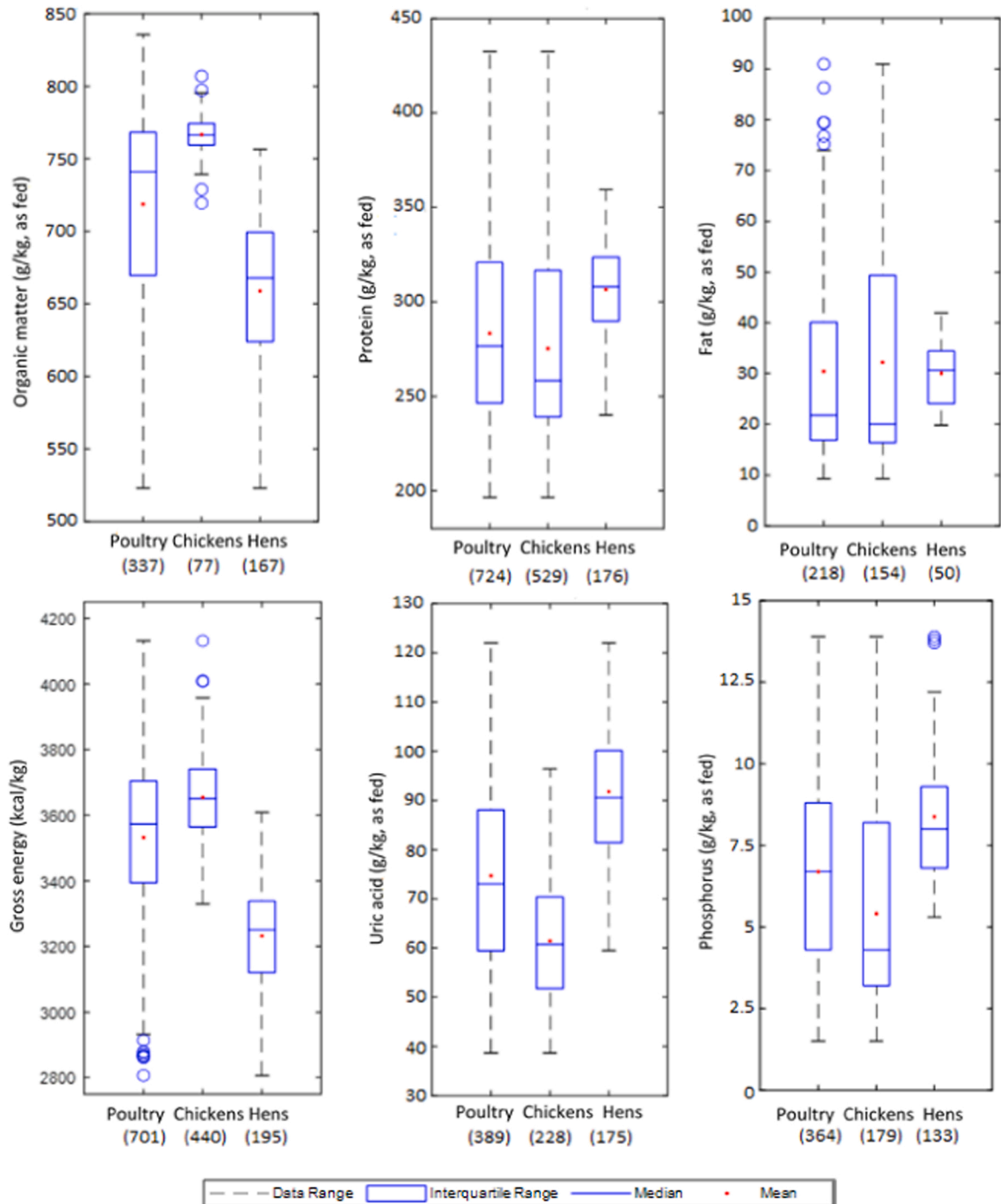

Fig. 1. Box and whisker plot of the measured parameters of the three sample sets: poultry (broiler chickens, laying hens and broiler turkeys), broiler chickens and laying hens. In parentheses the number of samples.

calibration set (75% of the samples) and validation set (25% of the samples) using the Kennard-Stone algorithm applied to the spectra, that allows to retain the spectra distribution (Kennard and Stone, 1969).

Calibration samples were labeled as spectral outliers and thus discarded when the leverage was too high (Hotelling T^2 reduced > 3) or the percentage of residual spectral variance of the sample was too high (Q residuals reduced > 3). Samples were labeled as reference outliers when the difference between the predicted and the reference value was too high (Studentized residuals (t) > 3).

The validity of the predictions from the calibration models was checked with three statistical tests: a *t*-test on bias, a *t*-test on slope and an F-test on standard error of prediction (SEP) according to ISO 12099:2017, whose object is to guide the development and maintenance of NIR calibrations in the agri-food sector (International Organization for Standardization (ISO), 2017).

Finally, because the sample set contained a large number of excreta samples from broilers and hens, we studied if calibrations developed for a single species could predict better than the global calibration that included the three species.

3. Results

Fig. 1 shows the distribution of the reference values for each parameter in the poultry sample set, that included the broiler chickens dataset, the laying hens dataset and the broiler turkeys dataset. The values for broiler chickens and laying hens only are also shown. The variability reflects the large diversity of bioassays that generated the excreta samples. The ranges and the standard deviations of the values of protein, fat and phosphorus were much larger in the broiler chickens dataset than in the laying hens dataset. Only for organic matter were clearly larger in the laying hens dataset. The mean values of the parameters were also quite different in each sample set.

Fig. 2 shows the mean Vis-NIR spectra of the excreta of the three poultry species. Spectra presented some differences in magnitude in the region from 400 nm to 1200 nm (which includes the visible region and the beginning of the NIR region) but they were all similar in shape and intensity in the NIR region from 1200 nm to 2499.5 where the most characteristic bands related with the nutrient content are found. Since the NIR bands are highly-overlapped it is difficult to find correlations between the spectra and the components of complex samples such as the excreta samples. Organic matter could be correlated with the intensity of the bands corresponding to the C-H, N-H and O-H bonds. The principal bands related to fat would be those for C-H, whose first overtone appears around 1700 nm, the second at 1200 nm and the combination bands around 2300 nm. Gross energy content is correlated with fat content, hence the C-H bands are also representative for this parameter. Protein and uric acid are correlated with C-H and N-H bands. The first overtone at 1900 nm and the combination band at 2100 nm are overlapped with the O-H bands. The second overtone around 1500 nm would be more easily assignable. Phosphorus can be determined through its association with organic components such as the phytate molecule.

The spectral differences reflect the differences in the metabolism of the three species and the substantial differences in their diets used in the bioassays. For example, the diets given to laying hens are less energetic and have a higher inorganic content than the diets for broiler chickens. This ultimately affects the composition of the excreta.

Since the spectra of the different species are similar the calibration models were first developed including the three species. Of the different preprocessing methods tested, autoscaling followed by GLSW performed the best for all the parameters. Three samples were clearly identified as spectral outliers and were removed from subsequent analyses because of their high values of Hotelling T^2 and Q residuals. Fig. 3 shows the grafic of scores of the PLS model for the protein content in the excreta with code color. The scores show that there are no clear clusters of spectra due to the species, as already suggested by the spectra in Fig. 2. One can also observe how the values of the property vary with the scores, as it is to be expected from a PLS model. Despite the fact that the birds received different diets and their digestive performance is different, similar score plots are obtained for the PLS models of the other parameters (not
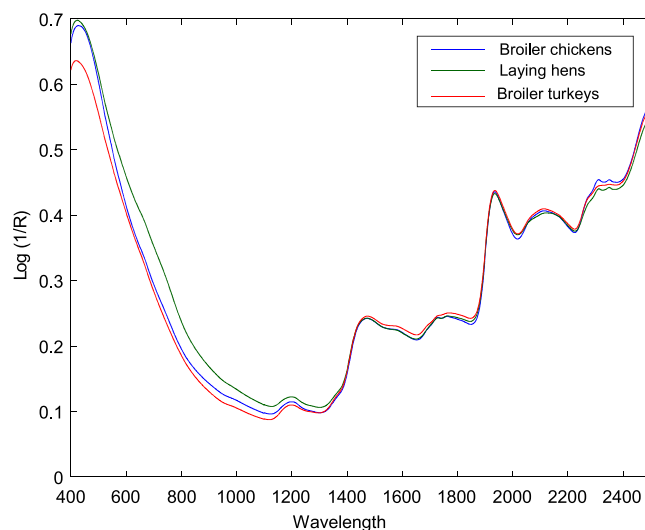

Fig. 2. Mean Vis-NIR spectra of the excreta samples for broiler chickens, laying hens and broiler turkeys.
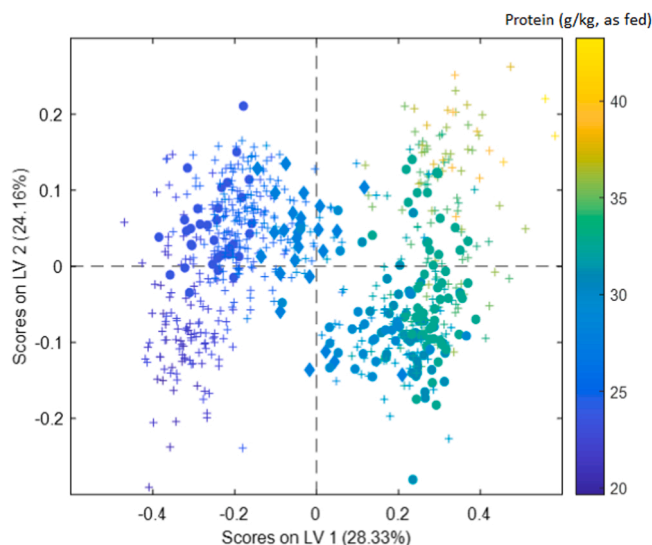
Fig. 3. Score plot of the protein PLS model considering the animal species: broiler chickens (crosses), laying hens (circles), broiler turkeys (rhombus) and the concentration of protein measured in each sample (color bar).

shown).

The optimal number of PLS latent variables ranged from 3 in the model for fat to 8 in the model for phosphorus. Once the models had been calculated, the following samples were detected as clear reference outliers and were removed from subsequent modeling: one sample for protein and uric acid, three for fat, four for phosphorus and six for gross energy. Other 11 samples, all with the largest values of protein (350–450 g/kg, as fed), were removed from the protein model after being predicted with large errors. Removing these samples from the calibration and validation set improved greatly the performance of the calibration for all the other samples. Nevertheless, if such high values of protein are found in future assays, one may consider reintroducing those samples to expand the calibration range.

Removing the part of the spectra corresponding to the visible radiation did not improve the performance of the calibration models. Therefore, the full spectral range was used in all models.

The performance of the global calibrations for poultry excreta is summarized in Table 1. The six studied parameters (organic matter, protein, fat, gross energy, uric acid and phosphorus) were well predicted. The ratio of performance of deviation (RPD) for the validation set ranged from 3.7 to 4.6 and the coefficient of determination of prediction (R^2p) from 0.91 to 0.95. These values were good enough to accept the calibrations for routine use. The measured versus predicted values are shown in Fig. 4. The *t*-test for the bias concluded that none of the calibrations had a significant bias. The *t*-test for the slope was passed by all the calibrations easily. Lastly, the results of the F-test for the standard error of prediction (SEP) suggested that there is not overfit in all the models except for the model for fat, probably due to the fact that is the model constructed with less samples.

Despite the global calibrations provided satisfactory results, specific calibrations for broiler chicken excreta and for laying hen excreta were developed to check if predictions could improve (Table 2). The statistics in this case were obtained using cross-validation (CV). In the broiler chickens sample set, four of the six studied parameters (protein, fat, gross energy, and phosphorus) were well predicted (RPDcv > 3 and R^2cv > 0.90). However, the results of the calibrations for laying hen excreta were poor (RPDcv < 3 and R^2cv < 0.90) in all cases. The results of the specific calibrations comparing to the global were much worse in the case of the laying hens calibrations and similar or worse for some parameters and much worse for others in the case of broiler chickens.

Table 1
Statistics of the poultry excreta calibration models: number of samples used to calibrate (Ncal) and validate (Nval), root mean square error of calibration (RMSEC) and prediction (RMSEP), coefficient of determination of calibration (R^2c) and prediction (R^2p), ratio of performance of deviation in prediction (RPDp), bias and slope of the predicted vs measured regression line.

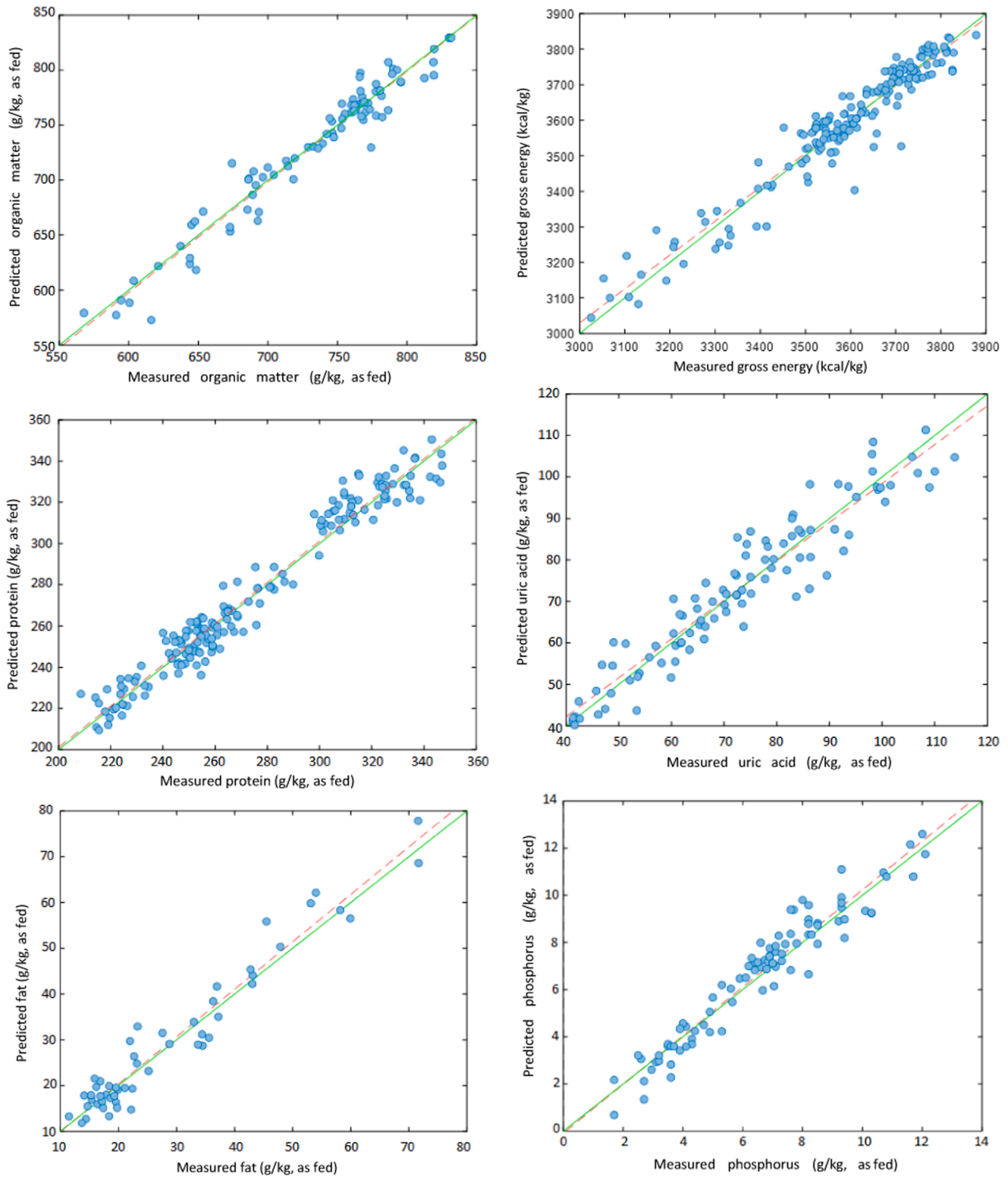| Parameter	Ncal	RMSEC	R^2c	Nval	RMSEP	R^2p	RPDp	Bias	Slope
Organic matter (g/kg, as fed)	255	13	0.97	85	14	0.95	4.6	-1.2	1.01
Protein (g/kg, as fed)	522	9.1	0.96	174	9.2	0.95	4.6	0.7	1.00
Fat (g/kg, as fed)	159	3.2	0.96	53	4.0	0.94	3.8	-0.4	0.95
Gross energy (kcal/kg)	513	46	0.94	171	49	0.93	3.7	2.7	0.95
Uric acid (g/kg, as fed)	294	4.9	0.93	98	5.2	0.91	4.5	0.1	0.94
Phosphorus (g/kg, as fed)	264	0.6	0.94	83	0.7	0.93	3.9	0.1	0.94

Fig. 4. Predicted vs measured values of organic matter, protein, fat, gross energy, uric acid and phosphorus obtained with the poultry (broilers chickens + laying hens + broiler turkeys) data set. The green continuous line is the 1:1 line and the dashed red line is the fitted straight line.

Table 2

Number of samples (N) and performance of the PLS models (SECV, R2cv and RPDcv) for the specific calibrations: broiler chickens dataset and laying hens dataset.

Parameter	Broiler chickens set				Laying hens set			
	N	SECV	R^2cv	RPDcv	N	SECV	R^2cv	RPDcv
Organic matter (g/kg, as fed)	77	7.7	0.74	1.9	167	20	0.87	2.7
Protein (g/kg, as fed)	529	10	0.95	4.8	176	10	0.87	2.8
Fat (g/kg, as fed)	154	4.1	0.96	4.9	50	5.0	0.34	1.2
Gross energy (kcal/kg)	440	38	0.90	3.1	195	80	0.73	1.9
Uric acid (g/kg, as fed)	228	4.7	0.86	2.0	175	7.0	0.74	1.9
Phosphorus (g/kg, as fed)	178	0.8	0.93	3.8	133	1.0	0.76	1.9

4. Discussion

4.1. Validation and quality of the global calibrations for poultry excreta

The global calibrations for poultry excreta were validated with an external sample set obtaining good results (Table 1 and Fig. 4). The exception is the model for fat, that will require more samples to improve the model and obtain a SEP similar to the SEC.

Considering the implementation of the calibrations for routine analyses, it can be seen that the prediction errors (Table 1) are close to those of the reference methods given in the materials and methods section.

The calibrations were compared with other calibrations for excreta found in the literature. Bastianelli et al. (2010) predicted several parameters related with the nutritional content of excreta. Unlike in our work, their sample set only contained broiler chicken excreta. The results presented in this work were similar to Bastianelli's results for protein, slightly worse for fat and gross energy and better for uric acid. These authors did not predict organic matter and phosphorus. The reason of their better performance on fat and gross energy could be due to the fact that their dataset has a larger range of fat and energy values. Smith et al. (2001) predicted nitrogen, gross energy and phosphorus among other parameters in broiler excreta but their models did not achieve RPD > 3 in none of these three cases. De la Roza-Delgado et al. (2015) developed a gross energy calibration for poultry although no information was given about the number of samples or the poultry species in their dataset. Their prediction errors were similar to the results presented here but their R^2 and RPD values were worse. The performance of the organic matter calibration reported in our work is better than the single study reported so far by Xing et al. (2008). The current results for phosphorus were also better than those that had been presented previously in any type of poultry excreta or manure and also better than those reported for raw materials (Aureli et al., 2017) or feedstuffs (Swart et al., 2012; Khaleduzzaman et al., 2017). The explanation for these significant results could be that phosphorus in excreta is mainly in form of phytate phosphorus. An important fraction of the phosphorus that raw materials such as corn or wheat contain is in this form. Due to the poor availability of phytate in poultry intestine, the percentage of phytate phosphorus digested by the animal is small. Thus, the fraction of phosphorus, which will be more concentrate in the excreta, should be easier to predict by NIRS since phytate is an organic molecule with C-H and O-H bonds that reflect in the near infrared region.

4.2. Global calibrations versus specific calibrations

The models developed for protein, fat, gross energy, and phosphorus with the broiler chickens dataset would be acceptable (Table 2). However, the predictions of organic matter and uric acid are not good enough. In the first case, the small number of samples compared with the other parameters and the narrow range of values (Fig. 1) could cause the low regression coefficient. In the second, the inaccuracy in the reference method, which is quite similar in value to the standard deviation of the values in the sample set.

The poor results for the laying hen excreta could be due to the fact that the laying hens sample set contains fewer samples and also that they belong to trials were the diets tested were very different. Nevertheless, this variability does not translate into a great standard deviation in the reference values. Moreover, the high ash content in these samples is thought that it could affect the predictions. However this higher variability in the ash content makes that the calibration for organic matter exhibited a regression coefficient higher than the one of the specific calibration for broilers chickens because the range of values is wider.

The results suggest that the global calibrations developed with the complete poultry data set, besides being the only valid ones for organic matter and uric acid, are preferable over the specific calibrations for each poultry species. On one side, global calibration models are based on a larger number of samples and include both the spectral variability and the parameter variability found in different animals and diets, and are expected to be applicable to a wider variety of future samples than the particular models. On the other hand, using only a few global calibration models will require less effort for monitoring and updating the models than using several species-specific calibration models.

5. Conclusions

We have shown a new series of Vis-NIRs calibration models that can replace the slow laboratory determinations of organic matter, protein, fat, gross energy, uric acid and phosphorus in poultry excreta. This will help to reduce the costs of digestibility studies and improve the discovery of optimal feedstuff diets.

It was also found that, despite the fact that broiler chickens, laying hens and broiler turkeys are different species, the spectra of their excreta could be modeled together. Joining the three datasets together increased the range of each parameter that had to be predicted and lead to models that are much widely applicable than those that are specific for each species.

CRediT authorship contribution statement

Andrés Cruz-Conesa: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Joan Ferré:** Conceptualization, Methodology, Software, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Anna M. Pérez-Vendrell:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation**,** Writing – review & editing, Supervision**,** Project administration**,** Funding acquisition. **M. Pilar Callao:** Conceptualization. Writing – review & editing. **Itziar Ruisánchez:** Conceptualization, Methodology**,** Writing – review & editing, Supervision**,** Project administration**,** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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