Predicting variations in total and phytic phosphorus in raw materials of plant origin

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Introduction

Phytic phosphorous (PP) constitutes the major proportion of the total phosphorus (P) of many raw materials of plant origin, usually accounting for 60 to 85%. PP concentrations depend on genotypes, pedo-climatic conditions or harvest year and show substantial degrees of variation within the same ingredient as well as between different feed ingredients. Such variations are increased when considering processed raw materials, since the location of phytate in the seeds varies significantly.[1, 2] PP is usually regarded as an antinutrient due to its potential for chelating positively charged cations and proteins. Its bioavailability for pigs and poultry is also very low, leading to a requirement for supplementation of diets with inorganic phosphorus and/or with exogenous phytase activities. Thus, assessment of the phosphorus value of ingredients contributes to the optimisation of phosphorus nutrition and a reduction of its release into the environment. The main objectives of this study were to measure PP in relation to P in a range of ingredients and to investigate the prediction of the variations using near infrared (NIR) spectroscopy.

Materials and Methods

Samples and analysis

Samples (n=350) of plant origin used in monogastric feeding and covering a range of 21 ingredients, were collected worldwide during the past two years. All raw materials were ground using a rotor mill (Retsch ZM200, 1 mm) and were analysed for their concentrations of total P using a mineralisation method (pR NF EN 15510) and inductively coupled plasma-optical emission spectrometry (ICP-OES) detection. The limit of quantification (LOQ) for total P was 20mg/kg with a 10% confidence interval. Additionally, ingredients were analysed for their concentrations in phytic P by an acid extraction of inositol phosphates (IPs) followed by treatment with a 6-phytase and subsequent treatment with alkaline phosphatase. Method precision, estimated from the differences between duplicates, was 0.01%.

Near infrared spectroscopy

For all ground samples, reflectance spectra were measured in a NIRSystems 5000 scanning monochromator (Foss, Dk.) equipped with a sample transport module (2nm steps, 32 scans per sample). Absorbance values were recorded as log 1/*R*, with *R* as the reflectance of the sample. Data were processed using SNV, Detrend with 1,4,4,1 settings and 2 outlier passes. Additionally, a FT-MPA (Bruker) was used to acquire absorbances from 8000 to 4000 cm^{-1} with 16 cm^{-1} resolution. Spectral data were primarily subjected to vector normalisation and first derivative prior to calibration development. Prediction models for P and PP contents in raw materials were developed using mPLS regression. Calibration performances were not different between the two NIR instruments, and consequently were globalised for both sets of databases. They were characterised by standard error of calibration (SEC), calibration R^2 (R^2C), standard error of cross-validation (SECV) and cross-validation R^2 (R^2CV).

Validation

The main ingredients used in monogastric feed, i.e. corn, wheat and soybean meal, were also analysed for their P and PP reference values. Some of these raw materials were selected as external samples in order to cover the range of values observed in the calibrations. The accuracy of the calibrations was validated with these external samples and expressed by the prediction error (RMSEP). Repeatability and reproducibility of the calibrations were also evaluated by running 10 replicate NIRS analyses at same time or within a 2 monthperiod respectively and using one NIR instrument. Finally, the performances of transferability within a network of 20 NIR instruments, including 15 NIRSystems 5000 and 6500 (Foss) models and 5 MPA (Bruker), were verified.

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Results and Discussion

Results (Table 1) showed wide variations in the total P content of raw materials, with the lowest concentrations found in cereals, particularly in corn and sorghum. The highest total P concentrations were usually observed for processed raw materials, such as cereal by-products, since the extraction (e.g in wheat bran) or the conversion (e.g. in dried distillers' grains, DDGS) of the starch fraction leads to a concentration of total P. Oilseed meals also showed high P levels, with the highest variability observed within sunflower meals, in which a high proportion (81%) was in PP form. Similar P to PP ratios and high degrees of variation were found in the other ingredients (cereals, oilseeds and meals) whereas corn DDGS and wheat DDGS contained potentially more available P for animals.[3] Prediction models (Table 2) developed using NIR spectroscopy explained 97 and 93% of the variations measured in total and phytic P respectively with corresponding standard errors of 0.054 and 0.063%. First indications of calibration accuracy were given by running cross-validations which revealed SECV values in agreement with SEC figures (0.054 vs. 0.060% and 0.063 vs. 0.071%). With respect to the ranges (0.08-1.34% and 0.01-1.05%) and the standard deviations (0.30 and 0.23%) measured over the full range of ingredients, this validated the calibrations to discriminate total and phytic P content in the ingredients studied.

Complementary and external validations (Table 3) carried out with corn, wheat and soybean meals confirmed the accuracy previously reported with SECV values found in the range of the RMSEPs, and also showed the capability of the calibrations to predict and discriminate phosphorus values within the same ingredient populations whether they presented low (such as in corn) or high concentrations (such as in soybean meal) of phosphorus. Calibrations developed on subset populations of raw materials i.e. specific equations for cereals (not shown) led to SECV values lower than those developed on the full range of raw materials (0.052 vs.0.060% and 0.036 vs.0.071% respectively for total and phytic P).

Table 1. Concentrations of total and phytic P (g/100g, as fed) and their ratios in ingredients examined

Ingredients	Total P				Phytic P			Total to phytic P (P/PP)	
	N	mean	range	CV%	mean	range	CV%	mean	range
Wheat	36	0.31	0.21-0.37	12	0.23	0.14-0.28	16	73	57-85
Corn	47	0.24	0.19-0.34	14	0.20	0.14-0.30	16	81	64-96
Barley	15	0.34	0.27-0.37	8	0.23	0.17-0.26	11	66	58-74
Sorghum	26	0.24	0.17-0.32	19	0.20	0.14-0.27	18	83	70-92
Full fat soybean	15	0.51	0.42-0.56	8	0.36	0.27-0.42	11	71	64-84
Rapeseed	7	0.68	0.61-0.74	8	0.56	0.48-0.63	9	82	78-85
Rapeseed meal	18	1.06	0.89-1.22	9	0.83	0.73-0.98	9	79	66-91
Canola meal	11	1.02	0.89-1.16	9	0.82	0.73-0.91	6	81	75-93
Soybean meal	54	0.65	0.49-0.83	9	0.42	0.36-0.57	10	66	59-80
Sunflower meal	17	1.03	0.81-1.71	22	0.83	0.65-1.37	23	81	73-89
Corn DDGS	20	0.76	0.40-0.91	13	0.33	0.17-0.43	23	44	22-63
Wheat DDGS	16	0.84	0.73-0.93	7	0.30	0.14-0.51	36	36	15-66
Wheat bran	16	0.98	0.69-1.29	14	0.81	0.46-1.22	20	82	67-95

Table 2. Database description and statistics of calibrations (full range of ingredients)

	Total P	Phytic P
Units	g/100g	g/100g
N	350	350
Outliers	1	2
Min	0.08	0.01
Mean	0.57	0.39
Max	1.34	1.05
SD	0.30	0.23
SEC	0.054	0.063
R^2C	0.97	0.93
SECV	0.060	0.071
R^2CV	0.94	0.90
Wavelength range/step	1100-2500/2nm	1100-2500/2nm
Pre-treatment	SNV-DE +1st derivative	SNV-DE +1st derivative
Regression method	mPLS	mPLS

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Table 3. Statistics of validation of the results with respect to major ingredients used in monogastric feeds

	Wheat		Corn		Soybean meals	
	Total P	Phytic P	Total P	Phytic P	Total P	Phytic P
Units	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g
N	50	50	50	50	30	30
Min	0.22	0.15	0.19	0.15	0.58	0.37
Max	0.38	0.30	0.30	0.25	0.74	0.52
SD	0.04	0.03	0.03	0.02	0.04	0.04
Average, lab reference	0.31	0.22	0.25	0.19	0.65	0.43
Average, NIRS prediction	0.32	0.23	0.24	0.18	0.65	0.44
RMSEP	0.04	0.04	0.04	0.03	0.05	0.04
% of outlier samples	0%	0%	4%	4%	2%	2%

Table 4. Description of robustness of calibrations with respect to major ingredients used in monogastric feeds

	Wheat		Corn		Soybean meal	
(g/100g)	Total P	Phytic P	Total P	Phytic P	Total P	Phytic P
Concentration, lab reference	0.33	0.26	0.22	0.18	0.64	0.46
Repeatability (N=10):	0.34	0.25	0.23	0.19	0.66	0.47
NIRS prediction average (and SD)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Reproducibility (N=10):	0.34	0.25	0.23	0.19	0.66	0.48
NIRS prediction average (and SD)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.01)
Transferability (N=20):	0.35	0.25	0.24	0.19	0.66	0.48
NIRS prediction average (and SD)	(0.01)	(0.01)	(0.01)	(0.00)	(0.01)	(0.01)

As previously reported in the literature,[4] this decreased the limits of detection of the NIR calibrations with respect to low P concentrations found in cereals and to increase calibration robustness, especially when considering the transferability among a network of instruments involving both FT and monochromator sytems located in different areas worldwide. Table 4 reports statistics related to the validation of the calibration robustness by running prediction replicates with a single instrument (MPA, Bruker and NIRSystems 5000 using their respective equations). Thus, data on soybean meals showed SD values from 0.00 to 0.01% for repeatability and reproducibility. Robustness was not affected when transferring equations within 20 different NIRS instrument as shown by the SD (0.01%).

Conclusion

There is considerable interest in dietary phosphorus and its accurate assessment could lead to better PP utilisation by animals, particularly when supplementing diets with phytase. Our results showed that total and phytic P contents vary highly between raw materials and this may have implications on daily feed formulation and efficiency. The NIR technique seems sensitive enough to detect P and PP even in ingredients such as cereals exhibiting low P concentrations. Calibrations can explain more than 90% of the variations with accuracy levels allowing quality discrimination between different feed ingredients as well as within the same ingredient. This suggests that using NIR could contribute to accurate monitoring of these nutrients.

References

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