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The possibilities of near infrared reflection spectroscopy to predict total-phosphorus, phytate-phosphorus and phytase activity in vegetable feedstuffs[1]

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Abstract

Two hundred thirty eight samples, representing 19 vegetable feedstuffs, were analyzed for total- and phytate phosphorus (P). Phytase activity was determined on a limited number of feedstuffs with an activity of more than 100 units kg^{-1}. Near infrared reflection spectra (NIRS) were taken between 1100 and 2500 nm in steps of 4 nm. By means of principal component analysis the feedstuffs were grouped in so called starchy (n = 150) and protein-rich feedstuffs (n = 88). NIRS-calibrations were developed using partial least square regression and tested by cross-validation. Total-P varied from 0.22 to 1.25% and phytate-P from 0.05 to 1.02% for starchy feedstuffs and respectively from 0.46 to 1.37% and from 0.22 to 0.56% for protein-rich feedstuffs. Within these broad ranges, NIRS-values were highly correlated to determined total- and phytate-P with prediction errors of 0.08% and 0.08%, respectively for starchy feedstuffs and 0.08% and 0.04%, respectively for protein-rich feedstuffs. However, considering the mostly small deviation of mean or calculated values for the separate feedstuffs, NIRS only makes practical sense to predict total-P for wheat by-products and phytate-P for maize gluten feed. On the other hand, NIRS could be used for unknown or not tabulated products. Spectral signals from total and phytate-P are partly based on direct responses from organic complexes but also on secondary relationships through protein and fat. NIRS seems not sensitive enough to detect phytase.

Keywords: NIRS, phosphorus, phytate phosphorus, phytase, vegetable feeds

Introduction

In some parts of Western Europe with intensive production of pigs and poultry the output of nitrogen and phosphorus from manure heavily threatens soil and water quality. Phosphorus pollution by monogastric animals is mainly due to the very low

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bio-availability of phytate-phosphorus, the most important P-form in cereals, seeds and their by-products (Jongbloed, 1987). In the past, when less attention was paid to the environment, inorganic phosphates were added to mixed feeds with a safety margin. Nowadays, much research is put into fine-tuning of the P-supply from the diet to the animal requirements. A particular topic is the use of phytases to increase the absorbability of phytate-P. Besides certain fungi, also some plant feedstuffs contain powerful phytases (Eeckhout & De Paepe, 1994). Hence, the apparent digestibility of P in feedstuffs of plant origin depends on their origin, the proportion of phytate-P and the presence of intrinsic phytase (Jongbloed *et al.*, 1991). To enable feed companies to formulate low-phosphorus diets, they have to dispose of practical tools to determine the bio-available phosphorus content of their raw materials. Because of laborious and time-consuming reference methods, feed tables with mean values for total- and/or (apparently) digestible phosphorus are currently used.

With the development of the Near Infrared Reflection Spectroscopy (NIRS) technology, new perspectives opened for a fast quantitative analysis of diverse organic materials with an acceptable accuracy. The principle of this physical technique is the selective absorption of electro-magnetic radiation from the 800–2500 nm region in accordance with the characteristic vibration frequencies of mainly AH_y functional groups. There are no absorption bands for minerals in the near-infrared region, but organic complexes and chelates may be detected, whereas ionic forms and salts have no special fingerprint (Shenk *et al.*, 1992). Phosphorus in plants occurs mainly as organic compounds. Its approximate distribution in grain is 50–70% soluble and insoluble phytates, 20–30% phospholipids, phosphoproteins and nucleic acids and 8–12% mineral phosphates (Georgievskii, 1982).

To get specific information for a particular component in a sample, one has first to derive a mathematical relationship between reference values, obtained with a standard method, and the spectral data and this for a large number of samples, representative for the normal variation. However, once the calibration made, one can easily analyze large series of samples in a short time.

In this paper, the possibilities of NIRS to predict total-phosphorus, phytate-phosphorus and phytase activity of currently used raw materials in monogastric feeds will be examined.

Materials and methods

Two hundred thirty eight samples, representing 19 vegetable feedstuffs, were collected during two years from Belgian feed manufacturers. The choice of the feedstuffs and the corresponding number of samples more or less reflected the frequency with which they are used in monogastric feeds. The ground samples were analyzed for total-P by the official EC method (1971) and for phytate-P by the method of Haug and Lantzsch (1983). Phytase activity was determined on undried samples following Eeckhout and De Paepe (1994). A phytase unit is defined as that enzyme activity liberating 1 micromole of inorganic phosphorus per minute from a 0.0015 m Na-phytate solution at pH 5.5 and 37 °C. Data and calibrations are given for about half of

the samples and only for feedstuffs with a mean phytase activity higher than 100 units kg^{-1}.

The precision of the reference methods, calculated as the square root of the sum of the squared differences between duplicates divided by 2 times the number of samples, amounted to 0.009% for total-P and to 0.015% for phytate-P. The repeatability of the phytase activity determination was 38 and 187 units for samples with an activity respectively less and more than 1000 units kg^{-1}.

NIRS-analysis was done with an Infra-alyzer 500 spectrophotometer (IA-500) using IDAS-software (Bran & Luebbe, Norderstedt, Germany). The samples were ground with a Retsch mill provided with a 0.75 mm sieve. Each sample was scanned twice in closed cups and the two scans were averaged. Spectra taken from 1100 to 2500 nm in steps of 4 nm were afterwards corrected for multiplicative scatter, centred and scaled. Because the number of samples was too small to make separate calibrations per feedstuff, spectrally related feeds were grouped following principal component analysis (PCA). Calibrations were developed by means of partial least square (PLS) regression. In contrast with multiple linear regression, these techniques (Unscrambler version 5.01, Camo, Trondheim, Norway) use the information of the whole spectrum by deriving a few new variables or factors, which are linear combinations of the absorbances. The main difference is that PCA extracts the systematic variation from the spectral data only, whereas with PLS the decomposition occurs in relation to the reference data. The optimal number of factors for calibration is determined at the point where the validation variance reaches its first minimum. The models were tested by cross-validation: the samples were split into 30 randomly selected segments, the calibration was repeated 30 times, each time using 1/30 part as validation samples. The accuracy of a calibration was expressed by the prediction error (SEP), calculated as the square root of the average of the squared differences between predicted and reference values. Because feed manufacturers almost solely dispose of cheaper NIRS-filterinstruments, calibrations based on the currently used 19-filterset of an IA-450 will also be discussed.

Results and discussion

Total phosphorus

The total-P content of the feedstuffs is summarized in Table 1. Cereals contain from 0.20 to 0.40% total-P, increasing from sorghum and maize over wheat, rye and triticale to barley and oats. The higher P-content of wheat and maize by-products as compared with whole grain and that of wheat middlings as compared with feedflour can be explained by the predominant occurrence of P in the aleurone layer of the kernel (Reddy *et al.*, 1989). The P-content of legume seeds on average amounts to 0.39% for peas, 0.49% for Vicia beans and 0.55% for soya beans. All studied meals and cakes of the vegetable oil extraction industry are relatively rich in phosphorus, particularly rapeseed and sunflower seed meal. With the exception of wheat by-products, maize gluten feed and sunflower seed meal, the variation within a feedstuff is small.

Table 1. Total-P content (% on fresh matter) and results of NIRS-prediction for separate feedstuffs.

Feedstuff	n	mean ± sd	range	bias	SEP[1]
Cereals					
maize	11	0.27 ± 0.02	0.22–0.30	–0.01	0.03
wheat	11	0.32 ± 0.02	0.30–0.36	+0.01	0.06
barley	10	0.36 ± 0.03	0.30–0.39	–0.01	0.03
sorghum	4	0.25 ± 0.03	0.23–0.29	–0.04	0.05
oats	4	0.37 ± 0.04	0.33–0.40	+0.04	0.10
rye	2	0.34	0.31–0.36	–0.01 +0.05	–
triticale	1	0.34	–	–0.04	–
Wheat by-products					
middlings	35	0.95 ± 0.16	0.49–1.17	+0.01	0.06
feedflour	11	0.49 ± 0.16	0.24–0.73	+0.03	0.07
glutenfeed	19	0.87 ± 0.20	0.62–1.25	–0.02	0.10
Maize glutenfeed	24	0.88 ± 0.11	0.59–1.10	–0.01	0.12
Legume seeds					
peas	14	0.39 ± 0.03	0.36–0.45	+0.02	0.06
Vicia beans	4	0.49 ± 0.06	0.45–0.58	+0.04	0.14
soya beans	7	0.55 ± 0.05	0.46–0.60	+0.02	0.10
Oil meals					
soya bean meal	32	0.65 ± 0.05	0.56–0.73	0.00	0.07
sunflower seed meal	17	1.06 ± 0.15	0.86–1.37	–0.02	0.11
rapeseed meal	13	1.10 ± 0.06	1.02–1.22	0.00	0.05
linseed cake	13	0.79 ± 0.04	0.73–0.87	+0.02	0.05
groundnut meal	6	0.67 ± 0.03	0.62–0.70	+0.01	0.07

[1] root mean square error of prediction.

To obtain a sufficient number of calibration samples and a broad range, spectral relationships between feedstuffs were first examined to enable grouping. In Figure 1 the absorbances in the NIR-region of all samples are projected on a plain according to the principal components 1 and 3, which respectively explained 81 and 3% of the spectral variation. From this, roughly two great groups of feedstuffs can be discerned: above the PC 1-axis in the positive direction of the PC 3-component, cereals, wheat by-products, peas and Vicia beans are located, whereas below the PC 1-axis at the negative side of PC 3 soya bean meal, rapeseed meal, groundnut meal, linseed cake and soya beans are situated. These two groups will further be indicated as 'starchy' and 'protein-rich' feedstuffs, respectively. Maize by-products and sunflower meal are found at both sides of the first PC. Because of nutritional relationships, maize by-products were classified to the starchy feedstuffs, whereas sunflower seed meal was categorized to the so called protein-rich feedstuffs. With these two main feedstuff categories NIRS-calibrations were developed (Table 2). The correlation coefficients of 0.96 and 0.94 for respectively starch and protein-rich feedstuffs indicate the potential of NIRS to predict total-P within a broad range. The SEP of the calibrations for predicting total-P amounted to 0.08% for starchy – as well as for protein-rich feedstuffs. The individual data are visualized in Figure 2 and 3 for

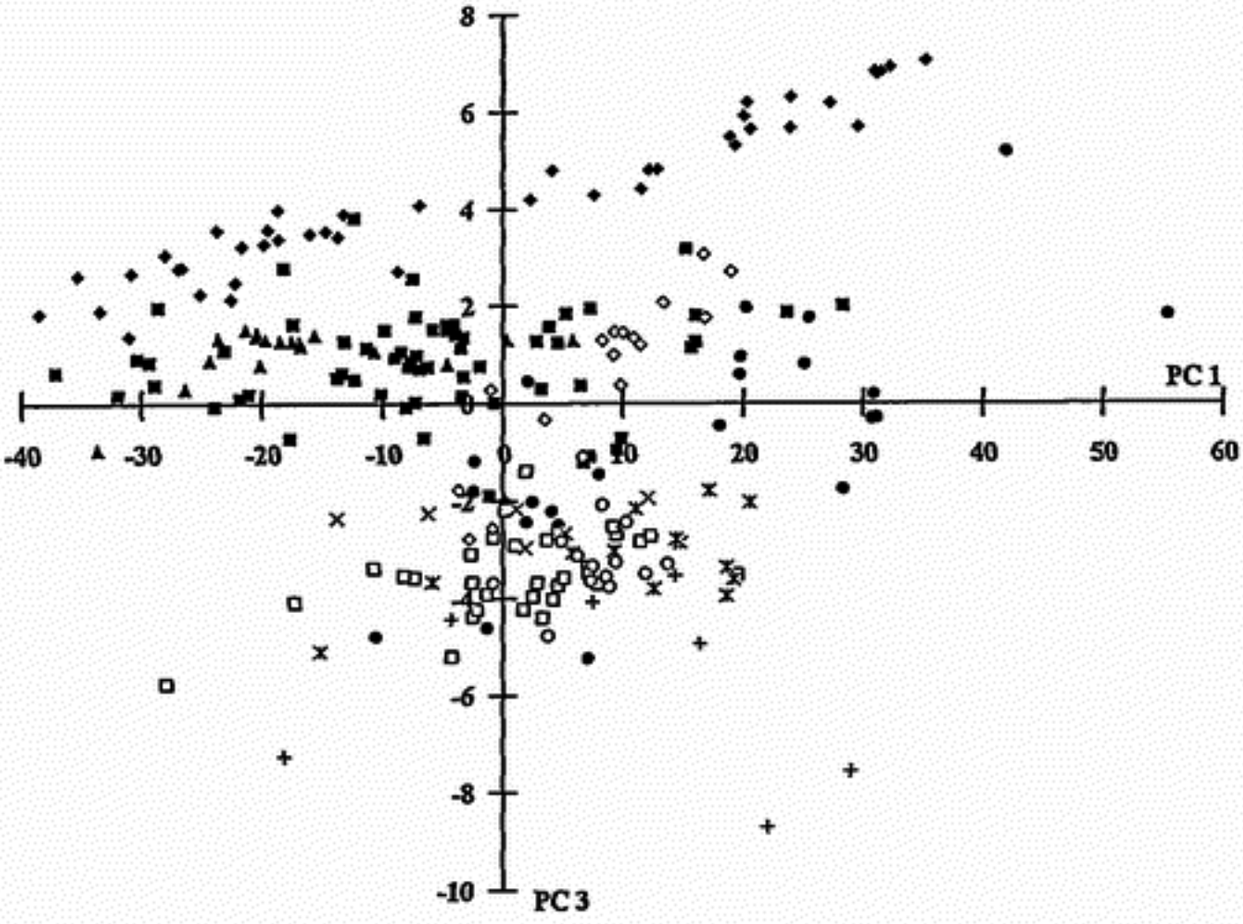

Figure 1. Projection of absorbances of feedstuffs in NIR-region on a plain with principal components 1 and 3 as axes. ■ wheat by-products, • cereals, ▲ peas/beans, ● maize by-products, □ soya bean meal, ○ sunflower seed meal, ✱ rapeseed meal, × groundnut meal, ○ linseed cake, + soya beans

starchy and protein feedstuffs, respectively. The results of the calibrations, derived from the 19-filterset of an IA-450, were somewhat less accurate with prediction errors of 0.13% for starchy feedstuffs and 0.09% for protein-rich feedstuffs.

Table. 2 Results of NIRS-calibrations for total – and phytate phosphorus.

	Reference analysis (%)		IA-500			IA-450		
	mean ± sd	range	PLS[1]	r	SEP[2]	PLS	r	SEP
Starchy feedstuffs (n = 150)								
Total-P	0.65 ± 0.30	0.22–1.25	10	0.96	0.08	5	0.91	0.13
Phytate-P	0.41 ± 0.23	0.05–1.02	9	0.95	0.08	9	0.92	0.09
Protein feedstuffs (n = 88)								
Total-P	0.81 ± 0.22	0.46–1.37	10	0.94	0.08	8	0.88	0.10
Phytate-P	0.38 ± 0.07	0.22–0.56	8	0.78	0.04	6	0.77	0.04

[1] number of partial least square factors.
[2] root mean square error of prediction.

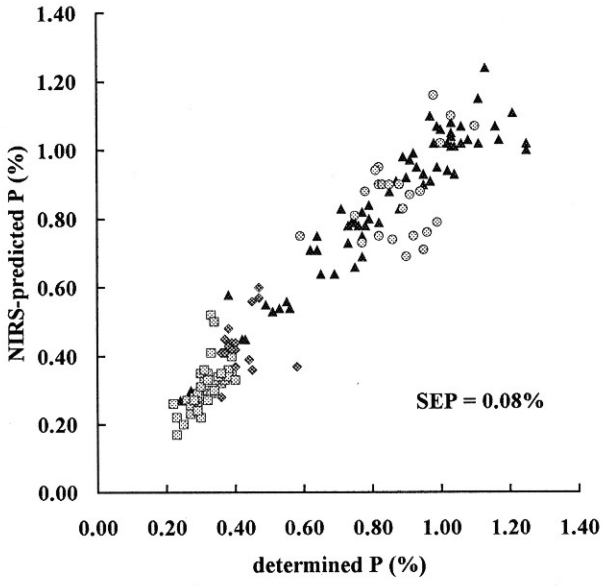

Figure 2. Relationship between determined and NIRS-predicted total-P for starchy feedstuffs.
▲ wheat by-products, ▨ cereals, ◆ peas/Vicia beans, ⊗ maize by-products

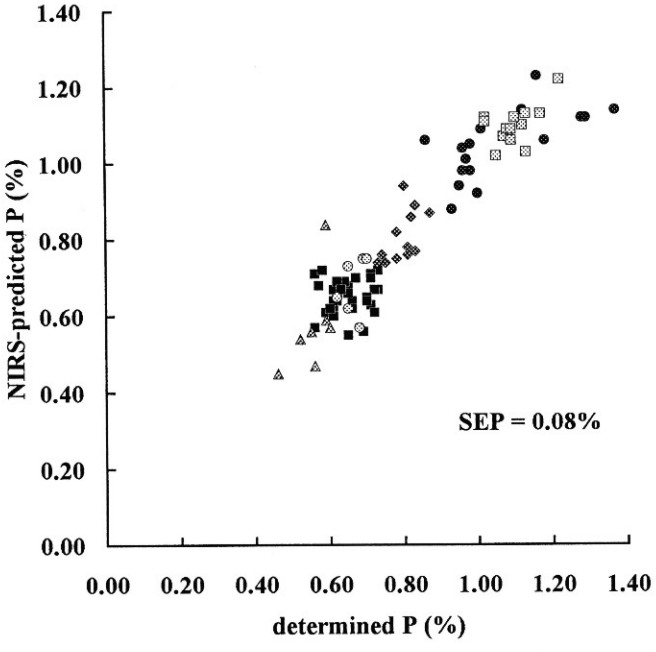

Figure 3. Relationship between determined and NIRS-predicted total-P for protein feedstuffs.
■ soya bean meal, ● sunflower seed meal, ▨ rapeseed meal, ⊗ groundnut meal, ◆ linseed cake, ▲ soya beans

The bias, being the predicted minus reference value, and the prediction error calculated for the separate feedstuffs are given in Table 1. The mean difference between predicted and determined phosphorus content was small in all cases. Compared with the global prediction error, the separate error was somewhat higher for oats, wheat and maize gluten feed, for Vicia and soya beans and sunflower seed meal. Considering the standard deviation on the mean P-content, prediction by NIRS would only make sense for wheat by-products and to a lesser extent for sunflower seed meal. Literature about the use of NIRS to predict the content of phosphorus in feedstuffs and minerals in general is scarce. Shenk and Westerhaus (1985) found a SEP of 0.04% for maize grain, whereas Convertini *et al.* (1990) mentioned calibration errors of 0.10% for wheat grain and 0.02% for soya beans with separate equations. NIRS-calibrations to predict P-content of forages (Shenk *et al.*, 1979; Shenk & Westerhaus, 1985; Valdes *et al.*, 1985; Jones *et al.*, 1987; Clark *et al.* ,1987) always show prediction errors of 0.05% or smaller. In all cases feedstuff-specific equations with a relatively narrow P-range were developed, so that determination coefficients were smaller than in our study.

The interpretation of the underlying causes of the spectral response in the NIR region is very speculative because of overlapping and complex absorption bands and the more as the concentration of the constituent is lower. In an attempt, the correlation plot was studied, being the result of single-term regressions, one at each available wavelength, of the reference P-content against absorbance. For starchy feed-

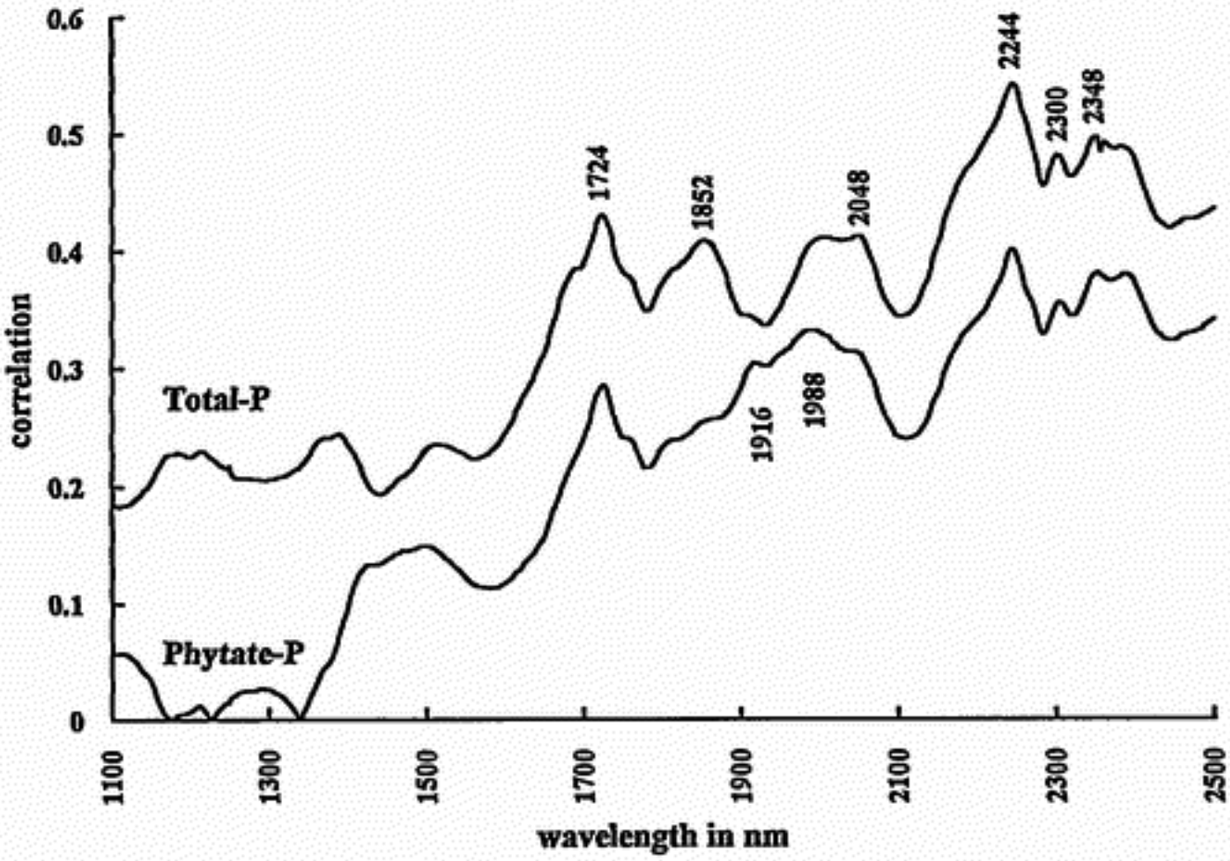

Figure 4. Correlation plot between absorbances in near-infrared region (4 nm spacing) and total-P and phytate-P for starchy feedstuffs.

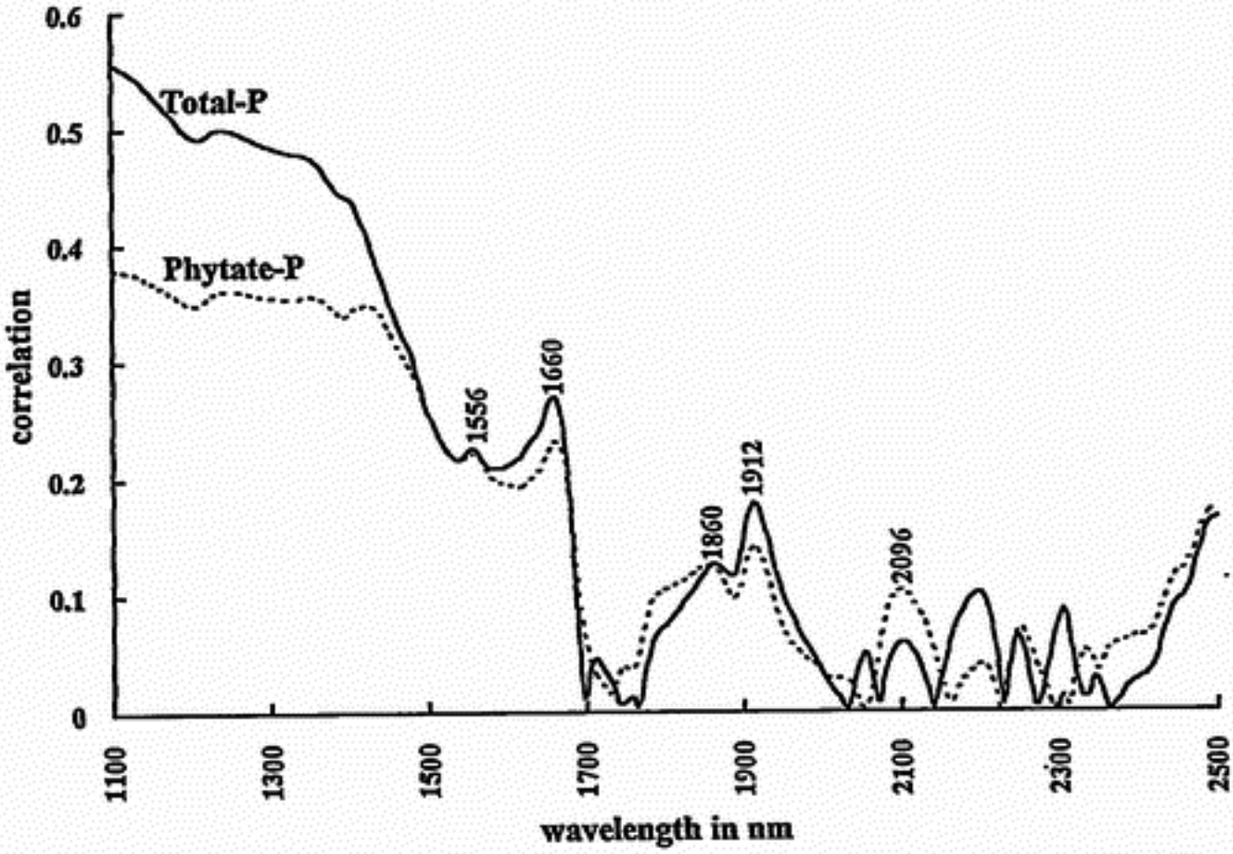

Figure 5. Correlation plot between absorbances in near-infrared region (4 nm spacing) and total-P and phytate-P for protein-rich feedstuffs.

stuffs (Figure 4), the highest correlation (r = 0.54) was found at 2244 nm, which is characteristic for basic amino acids (Osborne & Fearn, 1988) and probably referring to protein-phosphorus links. Peaks at 2348 (r = 0.50), 2300 (r = 0.48) and 1724 nm (r = 0.43) are typical for lipids, as shown by Law and Tkachuk (1977) and may be traced back to phospholipids. The peak at 1852 nm (r = 0.41) could be assigned to P-OH bounds (Murray & Williams, 1990), present in phytate-P or myoinositol hexaphosphate. In the correllellogram for protein-rich feedstuffs (Figure 5), the peak at 1660 nm (r = 0.27) is characteristic for mono-unsaturated fatty acids, at 1556 nm (r = 0.23) for peptide bounds, at 1912 nm (r = 0.18) and 1860 nm (r = 0.13) for P-OH-bounds (Osborne & Fearn, 1988; Murray & Williams, 1990). The two correllellograms resembled only in the peak at 1852–1860 nm, which could indicate that phosphorus is differently bound in starchy and protein-rich feedstuffs. On the other hand, one has to be careful with directly assigning spectral signals to the studied constituents because of the low concentrations. Clark *et al.* (1987) found no similarities in wavelengths chosen to predict P in forages and those highlighted in phytate or phosphate spectra. The use of NIRS to predict the ash content of white flour as a purity index was ascribed to secondary correlations between ash and either oil or protein, which prevail in much higher amounts in bran than in endosperm, rather than to potassium orthophosphate, the main component of flour ash, which was shown to have no distinctive NIR absorption bands (Osborne and Fearn, 1988). This phenomenon could be confirmed in our study, since for starchy feedstuffs protein content

and to a lesser extent fat content were positively related to total-P with respective correlation coefficients of 0.71 and 0.38. In contrast, protein and fat content were negatively related ($r = -0.64$ and -0.30, respectively) to total-P for protein-rich feedstuffs. This discrepancy could be explained by the heterogeneous nature of this product group with protein rich soybean meal and oil rich soya beans, both relatively poor in phosphorus.

Phytate phosphorus

The phytate-P content of the feedstuffs is presented in Table 3. Roughly two-thirds of phosphorus in cereals is present as phytate-P, but this ratio varies from 0.53 for oats to 0.77 for sorghum. Compared with the whole wheat (0.61), more phosphorus is bound as phytate in feedflour (0.63) and certainly in middlings (0.76). On the other hand, wheat and maize glutenfeed contain relatively less phytate-P, 0.58 and 0.50, respectively. Some hydrolysis of phytate-P may have occurred during soaking before starch extraction (Jongbloed *et al.*, 1988). The low phytate-P content in one wheat and one rye sample could be explained by germination, during which phytase activity increases (Reddy *et al.*, 1989). In legume seeds about half of the phosphorus is

Table 3. Phytate-P content (% on fresh matter) and results of NIRS-prediction for separate feedstuffs.

	n	Phytate phosphorus %				Phytate/total-P	
Feedstuff		mean ± sd	range	bias	SEP[1]	mean ± sd	range
Cereals							
maize	11	0.19 ± 0.02	0.16–0.21	0.00	0.05	0.68 ± 0.06	0.61–0.77
wheat	11	0.20 ± 0.05	0.05–0.24	0.00	0.04	0.61 ± 0.16	0.15–0.72
barley	10	0.21 ± 0.02	0.17–0.23	+0.01	0.04	0.59 ± 0.02	0.55–0.62
sorghum	4	0.19 ± 0.02	0.17–0.22	–0.09	0.10	0.77 ± 0.03	0.74–0.80
oats	4	0.20 ± 0.03	0.16–0.23	+0.12	0.14	0.53 ± 0.06	0.48–0.59
rye	2	0.14	0.08–0.20	–0.04+0.02	–	0.41	0.26–0.56
triticale	1	0.22	–	–0.10	–	0.65	–
Wheat by-products							
middlings	35	0.73 ± 0.16	0.29–1.02	–0.02	0.07	0.76 ± 0.06	0.59–0.87
feedflour	11	0.33 ± 0.16	0.06–0.54	–0.01	0.10	0.63 ± 0.17	0.25–0.76
glutenfeed	19	0.49 ± 0.09	0.31–0.69	+0.01	0.08	0.58 ± 0.13	0.39–0.90
Maize glutenfeed	24	0.44 ± 0.13	0.17–0.62	0.00	0.08	0.50 ± 0.14	0.20–0.68
Legume seeds							
peas	14	0.19 ± 0.04	0.13–0.27	+0.02	0.06	0.47 ± 0.07	0.36–0.60
Vicia beans	4	0.23 ± 0.01	0.21–0.24	–0.01	0.06	0.47 ± 0.08	0.36–0.53
soya beans	7	0.27 ± 0.04	0.22–0.32	+0.01	0.05	0.49 ± 0.05	0.39–0.53
Oil meals							
soya bean meal	32	0.34 ± 0.04	0.27–0.39	0.00	0.04	0.52 ± 0.03	0.46–0.57
sunflower seed meal	17	0.43 ± 0.04	0.37–0.51	0.00	0.04	0.41 ± 0.06	0.30–0.47
rapeseed meal	13	0.38 ± 0.04	0.34–0.48	+0.01	0.04	0.35 ± 0.02	0.32–0.41
linseed cake	13	0.48 ± 0.05	0.39–0.56	–0.01	0.06	0.60 ± 0.04	0.52–0.67
groundnut meal	6	0.33 ± 0.02	0.30–0.37	0.00	0.03	0.50 ± 0.03	0.46–0.54

[1] root mean square error of prediction.

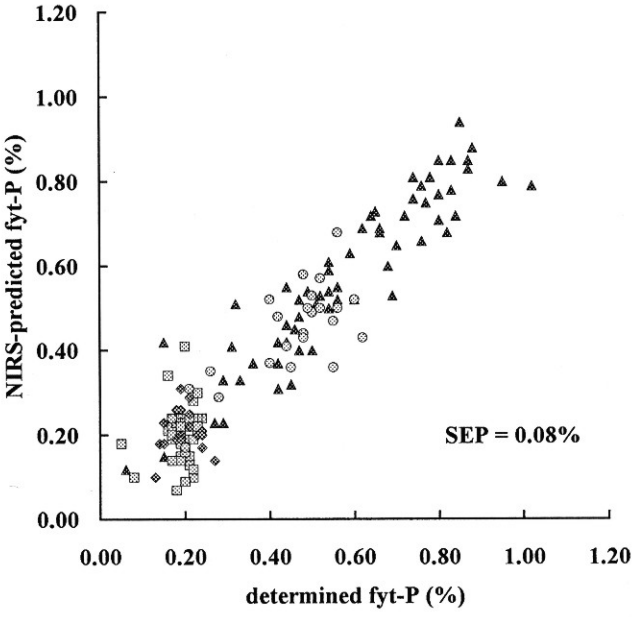

Figure 6. Relationship between determined and NIRS-predicted phytate-P for starchy feedstuffs.
▲ wheat by-products, ▨ cereals, ◆ peas/Vicia beans, ⊗ maize by-products

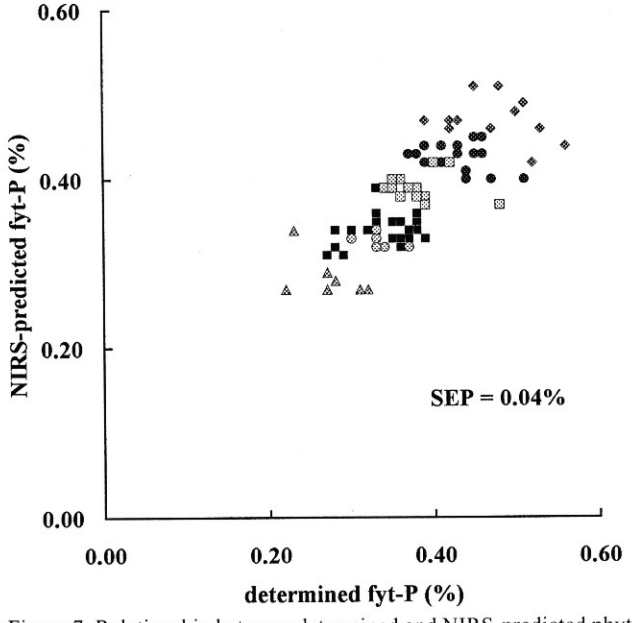

Figure 7. Relationship between determined and NIRS-predicted phytate-P for protein-rich feedstuffs.
■ soya bean meal, ● sunflower seed meal, ▨ rapeseed meal, ⊗ groundnut meal, ◆ linseed cake, ▲ soya beans

present in phytate form. Concerning oil meals the percentage of phytate-P varies from 0.35 for rapeseed meal, 0.41 for sunflower seed meal, 0.50 for groundnut meal, 0.52 for soya bean meal to 0.60 for linseed cake.

The results of the NIRS-calibrations for phytate-P are given in Table 2. For starchy feedstuffs, the correlation coefficient (0.95) and prediction error (0.08%) were identical as those obtained for total-P. For protein-rich feedstuffs, correlation was lower (0.78) but prediction error was smaller (0.04%) as compared with the total-P equation, which is explained by the smaller range in reference values. The individual data are visually presented in Figure 6 and 7. The filter-calibrations showed a similar accuracy as the monochromator ones.

The biases and SEP-values for the separate feedstuffs are shown in Table 3. There appeared a systematic underestimation of sorghum and the triticale sample and an overestimation of oats, causing a high prediction error. Separate errors, being greater than the global error, were further observed for wheat feedflour, linseed cake and soya beans. Considering the mostly small within-feedstuff variation, NIRS-prediction seems only meaningful for wheat middlings and feedflour and maize gluten feed. Moreover, phytate-P of certain feedstuff groups can be calculated from total-P by means of linear equations (Simons *et al.*, 1981; Eeckhout & De Paepe, 1994). From a visual observation of the phytate-P/total-P ratio for all samples two feedstuff groups showing a similar relationship could be distinguished, whereas other feedstuffs behaved somewhat different:
Cereals and wheat mill products (n = 89):

$$\text{phytate-P\%} = 0.850 \times \text{total-P} - 0.08 \quad (R^2 = 0.98\ ;\ \text{RSD} = 0.04\%)$$

Legume seeds, linseed cake and groundnut meal (n = 76):

$$\text{phytate-P\%} = 0.684 \times \text{total-P} - 0.10 \quad (R^2 = 0.90\ ;\ \text{RSD} = 0.03\%)$$

For the concerning feedstuffs the residual standard deviations are smaller than the prediction errors with NIRS, so that regression equations are more appropriate. Thus, NIRS could only be useful for maize gluten feed. Parrish *et al.* (1990) recommended NIRS to predict phytate-P in dehulled cottonseed, for which they obtained a standard error of 0.12%.

The correlations between each wavelength and phytate-P being generally lower than those for total-P, the curve showed a similar shape (Figure 4 and 5). The only remarkable difference was the appearance of a peak at 1988 nm (r = 0.33) and 1916 (r = 0.30) for starchy feedstuffs, which could be ascribed to P-OH bounds from myoinositol hexaphosphate (Osborne & Fearn, 1988; Murray & Williams, 1990). The increase of the peak at 2096 nm (r = 0.10) for protein-rich feedstuffs is more difficult to clarify.

Phytase activity

From the above studied feedstuffs only a few showed a phytase activity of more than 100 units kg^{-1} (Table 4). Phytase activity varied considerably within a feedstuff, particularly for wheat middlings, wheat feed flour and corn distillers (above classified

Table 4. Phytase activity (units kg^{-1}) and results of NIRS-prediction for separate feedstuffs.

Feedstuff	n	mean ± sd	range	bias	SEP[1]
rye	1	6127	–	–3922	–
wheat feedflour	8	3492 ± 1067	1234–4364	288	872
wheat middlings	26	2938 ± 1317	120–5208	–12	1092
wheat	8	1192 ± 221	928–1526	–188	769
barley	6	549 ± 155	454–862	529	711
corndistillers	3	385	141–850	139	1385
peas	11	116 ± 54	36–183	49	973

[1] root mean square error of prediction.

under maize gluten feed). In the case of wheat by-products, which were pelleted or not, this variation may be due to the partial destruction of phytase by heat, developed during pelleting (Eeckhout & De Paepe, 1994).

A global NIRS-calibration was developed for the 63 samples with 8 partial least square factors. Although the correlation coefficient of 0.76 between predicted and reference values suggested some relationship, the prediction error of 1101 units kg^{-1} indicated the calibration to be of little practical value. Considering the bias and SEP-values for the separate feedstuffs in Table 4, feedstuff-specific calibrations would bring little improvement. Searching for the reason of this bad spectral response the precision of the reference method was calculated since the calibration error can not be smaller. Repeatability was moderate, but typical for determinations of enzyme activity and was certainly not the main reason of this high prediction error. Starr *et al.* (1981), who also found a very weak NIR-response when trying to predict α-amylase in grain, stated that the concentration of phytase is too small and almost likely indistinguishable from other proteins by spectrophotometric methods. Following Iwamoto and Kawano (1992), the sensitivity limit of NIRS is about 0.15% for most constituents. The significant correlation was probably due to a subsidiary relationship through protein.

Acknowledgements

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References

Clark, D.H., H.F. Mayland & R.C. Lamb, 1987. Mineral analysis of forages with near infrared reflectance spectroscopy. *Agronomy Journal* 79: 485–490.

Convertini, G., D. Ferri, F. Lanza & A.M. Cilardi, 1990. Determination by N.I.R.S. of P, K, Na, Ca and Mg contents in the various plant organs of sugar beet, wheat, sunflower, soybean and sorghum in continuous cropping and two-year rotations. In: R. Biston & N. Bartiaux-Thill (eds.), Proceedings of the Third International Conference on Near Infrared Spectroscopy. Agricultural Research Centre Publishing, Gembloux, pp. 522–540.

Eeckhout, W. & M. De Paepe, 1994. Total-phosphorus, phytate-phosphorus and phytase activity in vegetable feedstuffs. *Animal Feed Science and Technology* 47: 19–29.

Georgievskii, V.I., 1982. The physiological role of macroelements. In: V.I. Georgievskii, B.N. Annenkov & V.T. Samokhin (eds.), Mineral Nutrition of Animals. Butterworths, London, pp. 91–170.

Haug, W. & H.-J. Lantzsch 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science in Food and Agriculture* 34: 1423–1426.

Iwamoto, M. & S. Kawano 1992. Advantages and disadvantages of NIR applications for the food industry. In: I. Murray & I.A. Cowe (eds.), Making Light Work: Advances in Near Infrared Spectroscopy. VCH, Weinheim, pp. 367–375.

Jones, G.M., N.S. Wade, J.P.Baker & E.M. Ranck, 1987. Use of near infrared reflectance spectroscopy in forage testing. *Journal of Dairy Science* 70: 1086–1091.

Jongbloed, A.W., 1987. Phosphorus in the feeding of pigs. Doctoral thesis. Agricultural University, Wageningen, The Netherlands, 343 pp.

Jongbloed, A.W., H. Everts & P.A. Kemme, 1991. Phosphorus availability and requirements in pigs. In: W. Haresign & D.J.A. Cole (eds.), Recent Advances in Animal Nutrition. Butterworth-Heinemann, Oxford, pp. 65–80.

Jongbloed, A.W., P.A. Kemme & G.F.V. van der Peet, 1988. Digestible P in feedstuffs for pigs. (In Dutch). Institute for Livestock Feeding and Nutrition Research, Lelystad, The Netherlands, 33 pp..

Murray, I. & P.C. Williams, 1990. Chemical principles of near-infrared technology. In: P.C. Williams & K.H. Norris (eds.), Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists, St. Paul, pp. 17–34.

Law, D.P. & R. Tkachuk, 1977. Near infrared diffuse reflectance spectra of wheat and wheat components. *Cereal Chemistry* 54: 256–265.

Osborne, B.G. & T. Fearn (eds.) 1988. In: Near Infrared Spectroscopy in Food Analysis. Longman Scientific & Technical, New York, 200 pp.

Parrish, F.W., J.P. Madacsi, B.Q. Phillippy, A.G. Wilfred & S.M. Buco, 1990. Determination of phytic acid in cottonseed by near-infrared reflectance spectroscopy. *Journal of Agriculture and Food Chemistry* 38: 407–409.

Reddy, N.R., M.D. Pierson, S.K. Sathe & D.K. Salunkhe (eds.), 1989. Phytates in Cereals and Legumes. CRC Press, Boca Raton, 152 pp.

Simons, P.C.M., K. Terpstra, G.R. Teunis & H.M. Zweers-Japing, 1981. Phytate phosphorus and total phosphorus in feedstuffs. (In Dutch). Communication 335. Institute for Poultry Research, Beekbergen, 9 pp.

Shenk, J.S. & M.O. Westerhaus, 1985. Accuracy of NIRS instruments to analyze forage and grain. *Crop Science* 25: 1120–1122.

Shenk, J.S., M.O. Westerhaus & M.R. Hoover, 1979. Analysis of forages by infrared reflectance. *Journal of Dairy Science* 62: 807–812.

Shenk, J.S., J.J. Workman & M.O. Westerhaus, 1992. Application of NIR spectroscopy to agricultural products. In: D.A. Burns & E.W. Ciurczak (eds.), Handbook of Near-Infrared Analysis. Marcel Dekker, New York, pp. 383–431.

Starr, C., A.G. Morgan & D.B. Smith, 1981. An evaluation of near infrared reflectance analysis in some plant breeding programmes. *Journal of Agricultural Science* (Cambridge) 97: 107–118.

Valdes, E.V., L.G. Young, I. McMillan & J.E. Winch, 1985. Analysis of hay, haylage and corn silage samples by near infrared reflectance spectroscopy. *Canadian Journal of Animal Science* 65: 753–760.