104 (2025) 105394

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

Determination of carbohydrate and lignin content in feedstuffs for monogastric animals using near-infrared spectroscopy

Andrés Cruz-Conesa [a,b], Knud Erik Bach Knudsen [c], Itziar Ruisánchez [a], Joan Ferré [a], Anna M. Pérez-Vendrell [b], Samantha Joan Noel [c,*]

[a] Universitat Rovira i Virgili, Faculty of Chemistry, Department of Analytical and Organic Chemistry, Tarragona, Spain
[b] Institute of Agrifood Research and Technology, Animal Nutrition. Mas Bové, Constantí, Spain
[c] Department of Animal and Veterinary Sciences, AU Viborg Research Center Foulum, Aarhus University, DK 8830 Tjele, Denmark

ARTICLE INFO

Keywords:
Cereals
Multivariate calibration
Dietary fiber
Non-starch polysaccharides
Sugars

ABSTRACT

Carbohydrates (CHO) are the principal constituents of the diets given to monogastric animals around the world. They supply the largest part of the energy to the animal, despite the fact only monosaccharides, disaccharides and starch can be enzymatically broken down in the small intestine into monosaccharides that are readily absorbed. In contrast, oligosaccharides (OS), resistant starch, non-starch polysaccharides (NSP) and lignin resist enzymatic digestion and thus cannot be absorbed directly. They compose the so-called dietary fiber. The soluble fraction of fiber is almost completely fermentable and causes benefits to the gastrointestinal tract while the insoluble fibre is less degraded and therefore lead to a decrease in nutrient and energy digestibility. The CHO fraction and its composition is highly variable among feed ingredients but also amongst the different varieties of the same ingredient. To improve the nutrient utilization and to formulate animal diets that are more efficient it is necessary to characterize the CHO fraction of the ingredients. In addition, with the growing use of supplementary enzymes, such as xylanase, β-glucanase or mannanase to mitigate the anti-nutritive effect of fiber the determination of the CHO components is essential. To make a full account of the CHO and lignin constituents, several wet chemical enzymatic, chromatographic and gravimetric methods need to be employed, which are expensive and time-consuming. Near-infrared spectroscopy (NIRS) overcomes these limitations because it is a rapid, non-destructive, multi-parametric technique and requires minimal sample preparation. In this work, around 300 samples of animal feedstuffs, consisting of cereals, cereal co-products, protein-rich and fiber-rich feedstuffs were used to develop and validate near-infrared (NIR) partial least square regression (PLSR) models. Spectral pre-treatment optimization, sample selection and outlier detection were considered for each model. Robust predictions (R^2_P>0.90) were obtained for digestible carbohydrates (sugars and starch) and for most of the total or insoluble components of dietary fiber (lignin, NSP, cellulose, β-glucan, non-cellulosic polysaccharides (NCP), arabinose, xylose, galactose, rhamnose and uronic acids). The OS, fructans were well predicted in cereals (R^2_P=0.94) and linearity (R^2_{CV}>0.70) was also found for α-galactosides (raffinose, stachyose and verbascose) in protein-rich feedstuffs. The obtained results were in general very satisfactory and demonstrate that NIRS is a reliable tool to characterize the carbohydrate fraction of a variety of feedstuffs.

Introduction

Carbohydrates (CHO) are the principal constituents of diets for monogastric animals, accounting for approximately two thirds of the dry matter consumed (Bach Knudsen et al., 2023). The CHO fraction includes compounds with very different chemical structures and properties that define how they are used by the animal. Only monosaccharides are directly absorbed in the small intestine, but monogastric animals have endogenous enzymes that are able to hydrolyze disaccharides and most of the starch to monosaccharides making them available for absorption. Sugars and starch, represent the main energy source in a typical monogastric diet (Bach Knudsen and Laerke, 2018). However, the high specificity of the endogenous enzymes results in a significant fraction of CHO, along with the non-carbohydrate component lignin, not

* Corresponding author.
E-mail address: samantha.noel@anivet.au.dk (S.J. Noel).

https://doi.org/10.1016/j.psj.2025.105394
Received 19 March 2025; Accepted 4 June 2025
Available online 5 June 2025

being digested by the endogenous enzymes in the small intestine. This fraction, commonly referred to as dietary fiber (DF), can be fermented to some extent by the microbiota at all sites of the gastrointestinal tract, but, primarily in the large intestine. The DF fraction include: oligosaccharides (OS), resistant starch (RS), non-starch polysaccharides (NSP) and lignin (Cummings and Stephen, 2007). In plant materials, OS consist mainly of α-galactosides (raffinose, stachyose and verbascose) deriving from protein rich crops and feedstuffs and fructans deriving predominantly from cereals. RS is the non-digestible starch fraction present in most feedstuffs, but at low concentration (0.1-1 % of the total starch) (Bach Knudsen and Laerke, 2018). The NSP group is the main constituent of the DF and includes cellulose, and non-cellulosic polysaccharides (NCP) whose monomers are arabinose, xylose, glucose, galactose, mannose, rhamnose, fucose and uronic acids (Navarro et al., 2019). In cereals, arabinose and xylose are markers for arabinoxylan and glucose is the marker for β-glucan, whereas uronic acids, arabinose and galactose are markers for pectic polysaccharides. Lignin is a phenolic polymer, linked to cellulose and NCP and with a strong effect on the physicochemical properties and the degradation of NSP in the gastrointestinal tract (Bach Knudsen et al., 2023). For these reasons, it is common to include lignin in the studies related to CHO and most analytical methods for fiber determination include lignin as part of the measurement (Bach Knudsen and Laerke, 2018).

A factor that plays an important role for the function of the CHO fraction is the solubility. OS are fully and NCP partially soluble and the components therefore in general more readily fermented by the microbiota inhabiting the gastrointestinal tract of monogastric animals. Insoluble NCP together with cellulose and lignin, on the other hand, represent a fraction of the diet that is not digested and slowly fermented leading to a low digestibility of nutrients and energy (Jha and Berrocoso, 2015). A part of these insoluble CHO can be solubilized, particularly in the presence of enzymes, allowing for greater utilization. Nowadays, supplementing feeds with carbohydrases such as xylanase, β-glucanase or β-mannanase is a common practice in the swine and poultry industry (Ward, 2021; Petry and Patience, 2020).

Presently, diets are designed using table values for chemical composition of the individual feedstuffs. Nonetheless, the entire carbohydrate fraction does not appear in these tables. Most tables, only refer to starch and to the traditional measurements of crude fiber (CF), neutral detergent fiber (NDF) or acid detergent fiber (ADF). However, these parameters do not give a full account of the nutritional effects of the fiber fraction. The CF values only account for 15-30 %, depending on the feedstuffs, of TF and ADF and NDF fail to account for most, or almost all, soluble NSP in the feed (Bach Knudsen and Laerke, 2018). Moreover it is not totally clear which fractions of the fiber matrix the gravimetric methods represent and hence the relevance of these values for monogastric animal nutrition is called into question (Choct, 2015). Examining the individual sugar residues of NCP, cellulose and lignin and solubility of structural carbohydrates provides a more comprehensive insight into the fiber makeup of feedstuffs. In addition, the widespread use of supplementary enzymes has emphasized the need for determining the CHO where these enzymes act. Unfortunately, the analysis methods to characterize the fiber fraction by enzymatic-chemical-gravimetric methods are laborious and expensive and, as a result, relatively few data are available in the literature (Bach Knudsen and Laerke, 2018). In addition, it has been shown that CHO and lignin may be highly variable between and within different feedstuffs, hence it is not recommended to assume that an individual batch of feedstuff will have the same fiber profile as another (Bach Knudsen, 2014; Rodehutscord et al., 2016) .

Near-infrared spectroscopy (NIRS) offers several advantages over traditional analytical methods, including its rapidity, non-destructiveness, multiparameter nature, and minimal sample preparation. C–H and O–H bonds, which are abundant in carbohydrate structures, strongly absorb in the NIR region. These absorptions correspond to vibrational transitions, including stretching (the elongation and contraction of chemical bonds), deformation (changes in bond angles), and their respective overtones (multiples of fundamental vibrations) and combination bands (simultaneous excitation of two or more vibrational modes). These features produce broad and overlapping spectral signals that are approached by chemometrics to extract meaningful chemical information and develop calibration models (Osborne and Fearn, 1988). The proven ability of NIRS to determine the nutrient content of a multitude of feedstuffs has promoted this technique for routine analysis in the agri-food sector (Badaró et al., 2022; García-Sánchez et al., 2017; Cheli et al., 2012). However, few authors have presented NIR calibration models to determine CHO content beyond starch, and those that have focused on only one type of feedstuff (e.g. cereals) or limited CHO fractions (e.g. NSP) (Nieto-Ortega et al., 2022; Gomes et al., 2020; Blakeney and Flinn, 2005). Moreover, the papers published so far have only evaluated the predictive precision by using cross-validation and not by using an external dataset for validation.

In this work, a large and diverse dataset comprising cereals, cereal co-products, protein-rich, and fiber-rich feedstuffs for monogastric animals was used to study the capability of NIRS to characterize all CHO and lignin fractions.

Materials and methods

Samples

The feedstuff dataset (304 samples) consists of the dataset described by Bach Knudsen (1997), high-fiber feedstuffs published by (Serena and Bach Knudsen, 2007), and other samples that have been analyzed by essentially the same analytical procedures and used in different published and unpublished nutritional experiments.

The samples represent feedstuffs for monogastric animals with diverse CHO composition. The feed samples were collected since 1988 and come from the Danish and European feedstuffs market. The samples have been stored dry and in the dark at −20°C to preserve their chemical coposition. Similar samples that have been used as reference samples for the wet chemical analysis have been stored in the same conditions since the mid nineties without any systematic change in analytical values. Samples were grouped according to their characteristics and consisted of whole grain cereals (168 samples; barley (33), corn (5), wheat (59), oats (2), rice (1), rye (34), sorghum (1) and triticale (32)); cereal co-products (58 samples; barley husk (1), brewers spent grain (10), corn flour (3), maize bran (1), maize gluten (5), oat hull (1), oat meal (1), rice flour (1), rye bran (14), rye meal (1), wheat bran (18), wheat aleurone (1) and wheat pericarp/testa (1)); protein-rich feedstuffs (44 samples; soybean meal (7), sunflower meal (3), rapeseed meal (9) and cake (1), fava beans (1), peas (6), lupins (4), red clover (4), rye grass (4), cotton seed cake (3), coconut cake (1), flaxseed cake (1) and palm cake (1)) and fiber-rich feedstuffs (34 samples; alfalfa (3), grass meal (2), apple pomace (1), pea hull (10), sugar beet pulp (9) and potato pulp (9)). A summary of the principal constituents (dry matter, crude protein, fat, crude fiber and ash) determined for each group of samples are given in the supplementary materials (Supplementary Table S1).

Reference analysis

The CHO and lignin constituents determined with wet chemistry methods are illustrated in Fig. 1. Not all the parameters were analyzed in all the samples and some CHO were present at very low concentrations. For instance, β-glucan were only analysed in cereals and cereal co-products while α-galactosides were primarily present in protein-rich feedstuffs. The Supplementary Tables S2-S9 display the samples that were analyzed and used to develop each calibration model.

Sugars and α-galactosides were extracted together and detected using a colorimetric assay for sugars and a HPLC method for α-galactosides (Bach Knudsen and Li, 1991). Fructans were determined separately by an enzymatic assay described by Larsson and Bengtsson (1983). Starch was analyzed by the enzymatic-colorimetric method

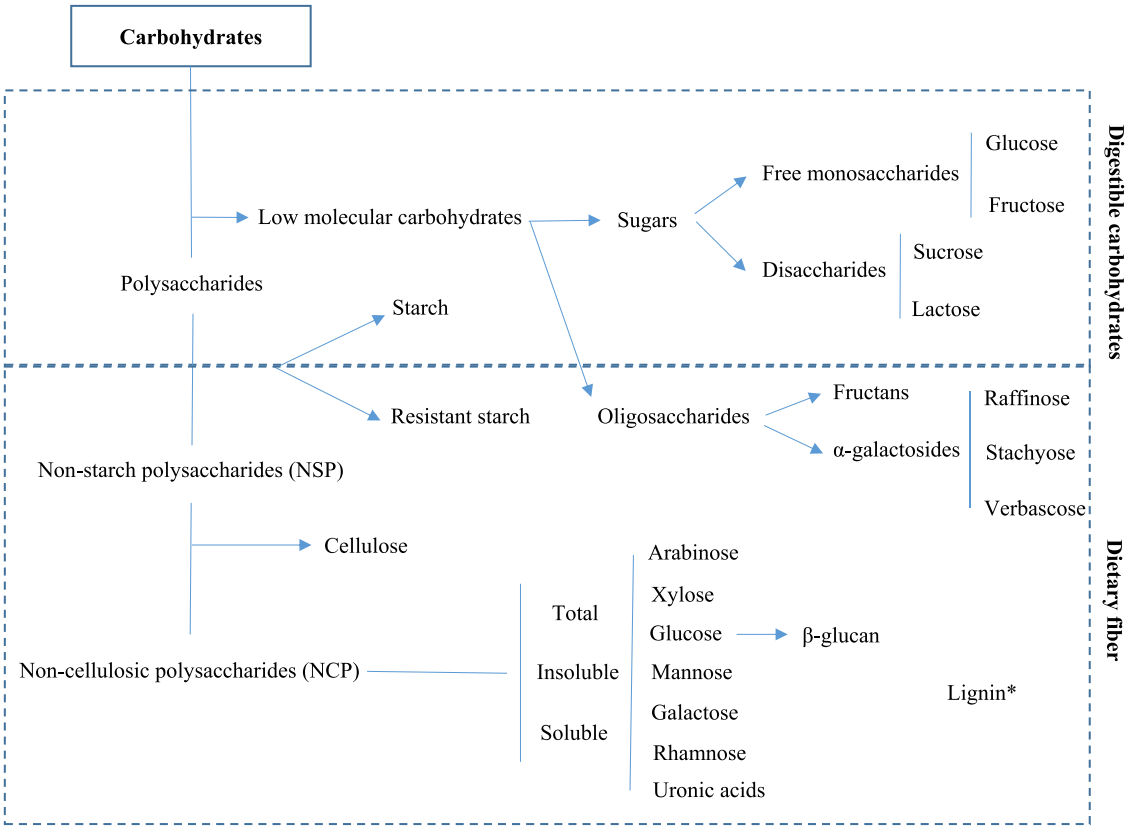

Fig. 1. Carbohydrates in feedstuffs. Digestible carbohydrates are represented by sugars and starch. Dietary fiber is represented by non-starch polysaccharides, lignin, oligosaccharides and resistant starch.
*Lignin is not a carbohydrate but it is treated together with non-starch polysaccharides because it is strongly linked to this component in the cell walls.

described by Bach Knudsen (1997), resistant starch by the enzymatic-colorimetric method of McCleary and Monaghan (2002) and β-glucan by the enzymatic-colorimetric method of McCleary and Glennie-Holmes (1985). Total and insoluble NSP constituents (cellulose, and NCP residues) were determined as alditol acetates by gas-liquid chromatography for neutral sugars and by colorimetry for uronic acids using a modification of the Uppsala (Theander and Aman, 1979) and Englyst et al. (1982) procedures as described by Bach Knudsen and Laerke (2018). Lignin (Klason lignin) was determined gravimetrically as the residue resistant to hydrolysis by 2 mol/L H_2SO_4 (Theander and Aman, 1979).

Cellulose content was estimated by comparing the amount of glucose released after swelling with 12 mol/L H_2SO_4 followed by hydrolysis with 2 mol/L H_2SO_4, and direct hydrolysis with 2 mol/L H_2SO_4 without the initial swelling. The cellulose value was calculated as the difference in glucose released between these two treatments:

$$\text{Cellulose} = \text{NSP}_{\text{glucose(12mol/L H2SO4)}} - \text{NSP}_{\text{glucose(2mol/L H2SO4)}} \quad (1)$$

Total or insoluble non-cellulosic polysaccharides (T-NCP or I-NCP) were obtained before and after extraction of the soluble NSP components by a phosphate buffer at neutral pH (0.2 mol/L, 100°C, 60 min, pH 7.0) as:

$$\text{NCP} = \text{rhamnose} + \text{arabinose} + \text{xylose} + \text{galactose} + \text{mannose} + \text{glucose} + \text{uronic acids} \quad (2)$$

soluble NCP (S-NCP) was calculated as:

$$S - \text{NCP} = T - \text{NCP} - I - \text{NCP} \quad (3)$$

total fibre (TF) was calculated as:

$$\text{TF} = T - \text{NSP} + \text{lignin} \quad (4)$$

and dietary fiber (DF) as:

$$\text{DF} = T - \text{NSP} + \text{Lignin} + \text{OS} + \text{RS} \quad (5)$$

with oligosaccharides calculated as

$$\text{OS} = \alpha - \text{galactosides (raffinose} + \text{stachyose} + \text{verbascose)} + \text{fructans} \quad (6)$$

The carbohydrate distribution across the four groups of feedstuffs is shown in Supplementary Tables S10-S11. Supplementary Table S12 shows the results of a study to calculate the precision of the reference analysis.

Near-infrared analysis

Feedstuff samples were ground through a 1 mm sieve and stored at −20°C in airtight containers until needed. The samples were dried at 60°C in a forced air oven for 48 hour before scanning, to prevent moisture buildup in the containers that could spoil the samples on thawing. Dried and ground samples were left at room temperature for a minimum of 48 hours to reach the ambient humidity levels. Samples were scanned using a Foss NIRS DS2500 feed analyzer (Foss NIR Systems, Denmark) which recorded data every 0.5 nm from 400 to 2500 nm. Samples were scanned twice in a 7 cm diameter cup containing approximately 30 g of sample each time repacking the sample cup between scans. Each scan is the average of the spectra obtained from 32 spots within the sample cup and the duplicate scan values from the repacking were averaged.

Calibration model development and validation

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 the chemometric calculations. Principal component analysis (PCA) was used in the first instance to study the spectral distribution of the sample set and discover possible groups of samples. Partial least squares regression (PLSR) was used to develop the calibration models for the carbohydrates and lignin. Common spectral pretreatments were tested, including normalization, standard normal variate (SNV) (Barnes et al., 1989), multiplicative scatter correction (MSC) (MacDougall et al., 1985) and 1st and 2nd order derivatives (Savitzky and Golay, 1964) using different window widths (number of consecutive data points used to fit a polynomial function for smoothing and derivative estimation). Different wavelength ranges were evaluated for modelling. A five-fold venetian blind cross validation (CV) was used to choose the optimal pretreatments, the optimal wavelength range and the optimal number of latent variables for each model except for α-galactosides. Due to the low number of reference values leave-one-out cross-validation (LOOCV) instead of venetian blinds was used for these purposes and as a validation method. For lignin and the rest of CHO the sample set was divided into a calibration set (75 % of the samples) and a validation set (25 % of the samples) using the Duplex algorithm applied to the pretreated spectra, that retains the distribution of the spectral variation both in the calibration and the test set (Snee, 1977). It should be noted that the calibration and validation sets varied from one model to another because sample selection depended on the number of reference samples available and the chosen pretreatment. One round of outlier removal was performed where the cutoff values for the spectral outliers were high leverage (Hotelling T^2 reduced > 3) or a high percentage of residual spectral variance (Q residuals reduced > 3). A large difference between the predicted and the reference value (Studentized residuals (t) > 3) was considered indicative of reference outliers (Marten et al., 1989).

The performance of the models was evaluated from the coefficient of determination of the regression of predicted versus reference values (R^2_p) and the root mean square error of prediction (RMSEP). To compare the calibrations developed for the different constituents with each other and with those found in the literature, the coefficient of variation of prediction (CV_p) was used. CV_p allows considering the differences in the means of the data sets between different constituents and different studies (Garrido-Varo et al., 2016).

Results

Near-infrared analysis

The raw mean Vis-NIR spectrum for each of the four groups of feedstuffs is shown in Fig. 2. Despite the offset, significant differences between groups were found in the visible region (400-800 nm) and in some NIR regions of the spectrum. Some spectral bands can be linked to specific structures according to Osborne and Fearn (1988). As expected, protein-rich feedstuffs exhibited higher intensity in protein-related regions such as the N—H stretching first overtone (1510 nm) and the three prominent peaks of protein in the N—H combination region at 1980, 2050, and 2180 nm, corresponding to the asymmetrical stretching + amide II, symmetrical stretching + amide II and 2 × amide *I* + amide III, respectively. Cereals, and to a lesser extent cereal co-products and fiber-rich feedstuffs had well defined peaks in regions related to starch, such as the O—H stretching second overtone (990 nm), the O—H stretching first overtone (1440-1450 nm) and the combination band 2 × O—H deformation + 2 × *C*—O stretching (2100 nm). Fiber-rich feedstuffs showed some peaks related with cellulose more clearly than the rest of feedstuff groups, such as the peak that encompasses the C—H

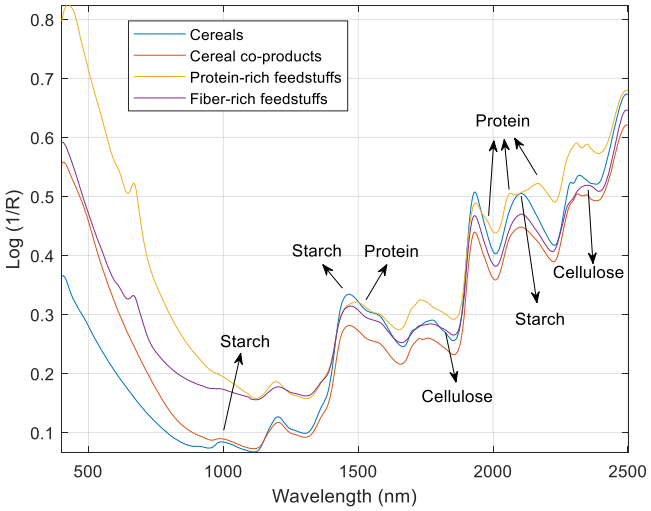

Fig. 2. Mean NIR raw spectrum of the samples grouped by type of feedstuff with band assignment for starch, protein and cellulose.

stretching first overtone (1780 nm) and the combination band O—H stretching + 2 × C—O stretching (1820 nm) and the peak that encompasses the combination band C—H stretching + C—H deformation (2336 nm) and the C—H deformation second overtone (2352 nm).

The variability of the samples can be observed in the PCA scores (Fig. 3) for spectra preprocessed with a first derivative (second-order polynomial and window width of 15 points) and mean centering. The cereals formed a relatively homogeneous group whereas the other feedstuffs exhibited more variability. Many protein-rich feedstuffs, some fiber-rich feedstuffs and cereal co-products were spectrally quite different from the cereals and some of these were detected as outliers when developing the models. Fig. 4 shows the spectral outliers (Hotelling T^2 reduced > 3, Q residuals reduced > 3) found for the NSP calibration as an example. Some protein-rich feedstuffs (palm cake, red clover (2)), fiber-rich feedstuffs (potato pulp and sugar beet pulp (2)) and cereal co-products (maize gluten) were identified as outliers and removed from further analysis.

Pretreatment of the spectra using derivatives and mean centering significantly improved all models. There were only minor differences in the root mean square error of cross-validation (RMSECV) when utilizing

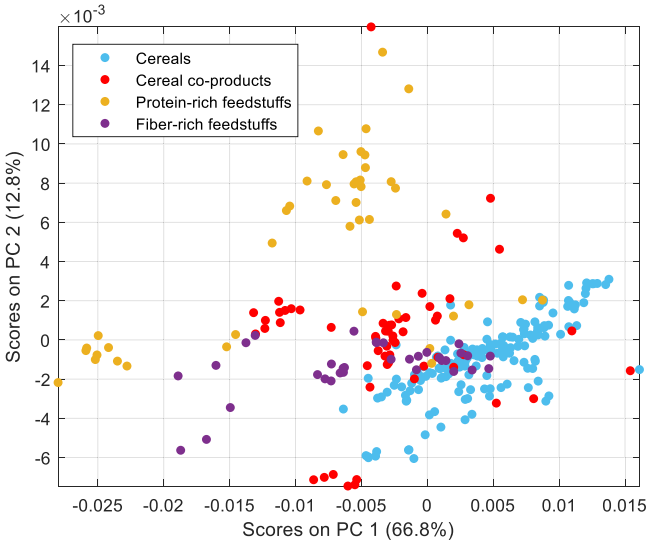

Fig. 3. Score plot of the PCA model showing the four groups of feedstuffs. Each point represents a sample. The closer the points are, the more similar the spectra of their samples.

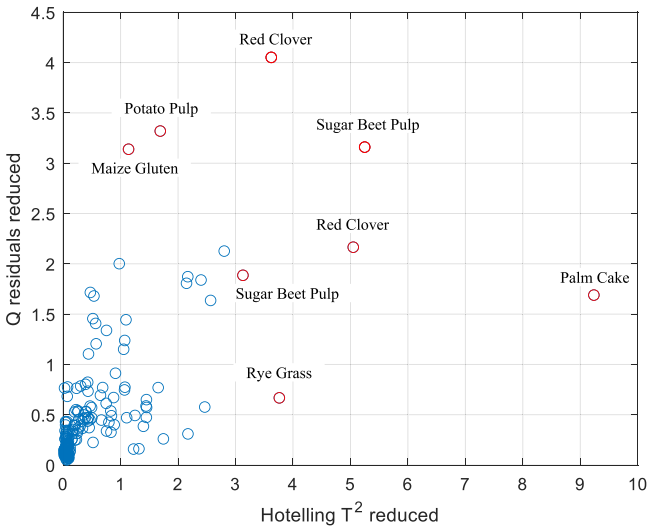

Fig. 4. Q-residuals reduced versus Hotelling T^2 reduced for total non-starch polysaccharide content (T-NSP) calibration, as example for outlier detection. Each point represents a sample. Samples with high variations within the model space (Hotelling T^2 reduced >3) and/or samples with high amount of spectral variation not explained by the model (Q-residual reduced >3) were considered outliers (red points).

the first or the second derivative, as well as when using the pretreatments SNV and MSC or none of them. The selected pretreatment is specified in the model performance tables (Tables 1 and 2). For most of the modelled constituents, a considerably large number of latent variables (ranging from 10 to 16) was necessary to accommodate all the sources of variability.

The predictive performance of the NIR models for carbohydrates is summarized in Table 1, with sample details available in Supplementary Tables S2–S9. Sugar predictions were strong ($R_p^2 = 0.91$, $CV_p = 23$ %). The starch model, based mainly on cereals and cereal co-products, covered a wide concentration range (5–90 %) and showed high accuracy (Fig. 5A). Among oligosaccharides, verbascose calibration was poor ($Rcv^2 = 0.71$, CVcv = 50 %) due to the limited number of samples (n = 16). Raffinose and stachyose calibrations performed well in calibration ($Rc^2 > 0.98$), but their cross-validation results ($Rcv^2 < 0.90$, high RMSECV) indicated limited robustness. Fructans were predicted with low RMSEP and high R_p^2. As shown in Fig. 5B, cereals generally contained <2 g/100 g DM of fructans, with rye forming a distinct group >2 g/100 g. Total and insoluble NSP predictions were satisfactory (R_p^2 = 0.91 and 0.94; CV_p = 15 % and 17 %, respectively; Fig. 5C). Calibrations for total contents were generally slightly better than those for insoluble fractions, mainly due to lower CV_p values. Key NSP residues—arabinose, xylose, and uronic acids—were predicted with high accuracy ($R_p^2 >$ 0.95). In contrast, glucose showed inconsistent predictions, with better results for total ($R_p^2 = 0.81$, $CV_p = 36$ %) than for insoluble content (R_p^2 = 0.62, CV_p = 51 %). Galactose predictions were accurate for total content ($R_p^2 = 0.95$, $CV_p = 26$ %), while mannose and rhamnose models showed moderate to good R_p^2 (≈0.75–0.90) but higher prediction errors (CV_p up to 40 %). Models for soluble NSP residues (not shown) generally performed poorly ($R_p^2 < 0.80$, $CV_p > 30$ %), except for uronic acids, which had a good coefficient of determination ($R_p^2 = 0.95$) but high error ($CV_p = 42$ %). β-glucan predictions (Fig. 5D) were comparable to NSP ($R_p^2 = 0.92$, $CV_p = 25$ %). A separate calibration for low-content samples (0–1 % DM) did not improve prediction accuracy. Cellulose ($R_p^2 = 0.99$, $CV_p = 15$ %), total NSP ($R_p^2 = 0.96$, $CV_p = 17$ %), insoluble NSP ($R_p^2 = 0.97$, $CV_p = 15$ %), and lignin ($R_p^2 = 0.93$, $CV_p = 29$ %) were well predicted. Dietary fiber (DF) also showed good performance ($R_p^2 >$ 0.90), though slightly less precise than NSP ($CV_p = 21$ % vs. 15 %).

Discussion

The variability in calibration performance across carbohydrate fractions can be attributed to the intrinsic properties of the analytes, the limitations of NIRS and the precision of the reference techniques of analysis from which the reference values were obtained. In general, total contents were predicted more accurately than their insoluble counterparts. Although the structural characteristics of water-extractable and non-extractable NCP residues are similar, they are not identical, which may influence their spectral behavior and, consequently, prediction accuracy. Soluble fractions, in particular, exhibited higher prediction errors, partly due to the compounding of analytical variability from both total and insoluble measurements, a limitation also observed in conventional wet chemistry (Table S12). For some components like mannose and insoluble rhamnose, poor model performance is likely a

Table 1
Statistics of the calibration models developed for the carbohydrate fraction. Dataset for sugars included cereals, cereal co-products and protein-rich feedstuffs. Dataset for starch contained cereals, cereal co-products, peas, pea hull and potato pulp samples. The dataset for α-galactosides (raffinose, stachyose, verbascose) included only protein-rich feedstuffs. Fructans and β-glucan dataset included cereals and cereal co-products. The rest of calibrations were developed with the entire dataset.

	N_{cal}	N_{val}	Mean (g/100 g of dry matter)	SD (g/100 g, as is)	Range (g/100 of dry matter)	Preteatment	LV	R_c^2	RMSEC (g/100 g of dry matter)	R_p^2	RMSEP (g/100 g of dry matter)	CV_p (%)
Sugars	135	49	2.7	2.1	0.5-8.9	1d	11	0.96	0.37	0.91	0.62	23
Starch	109	41	46	25	5.3-92	MSC 1d	16	1.00	1.61	0.98	3.03	7
Raffinose	31	-	1.0	1.2	0.2-5.4	2d	10	0.98	0.17	0.82*	0.46*	46*
Stachyose	41	-	1.4	1.5	0.0-5.0	SNV 2d	11	1.00	0.07	0.90*	0.48*	34*
Verbascose	16	-	1.3	1.4	0.0-4.2	MSC 2d	2	0.80	0.59	0.71*	0.72*	55*
Fructans	89	34	1.3	0.9	0.0-3.5	1d	13	0.98	0.15	0.94	0.23	18
β-glucan	108	37	1.6	1.4	0.0-5.3	MSC 1d	16	0.98	0.2	0.92	0.4	25
T-NCP	184	64	17	11	1-51	SNV 2d	16	0.99	0.95	0.96	2.3	14
I-NCP	186	69	12	8	0.1-36	SNV 1d	16	0.98	1.1	0.94	2.3	19
Cell	201	74	6.6	9.2	0.0-53	SNV 1d	15	0.99	0.80	0.99	1.0	15
T-NSP	194	75	23	18	5.6-85	SNV 1d	16	0.99	1.9	0.96	3.9	17
I-NSP	204	75	18	15	0.4-73	SNV 1d	16	0.99	1.4	0.97	2.7	15
Lignin	189	74	3.8	3.8	0.1-15	2d	10	0.95	0.73	0.93	1.1	29
DF	201	74	28	20	4.2-87	SNV 2d	12	0.98	2.81	0.92	6.00	21

Number of samples used for calibration (N_{cal}) and validation (N_{val}), mean, standard deviation (SD) and range of calibration, pretreatment used, number of latent variables (LV), coefficient of determination of calibration (R_c^2) and prediction (R_p^2), root mean square error of calibration (RMSEC) and prediction (RMSEP) and variation coefficient of prediction (CV_p).

* These values are not obtained from a test set but by leave-one-out cross-validation (LOOCV).

Table 2
Statistics of the calibration models developed for the non-celullosic polysaccharides (NCP) residues. All the calibrations were developed with the entire dataset.

		N_{cal}	N_{val}	Mean (g/100 g of dry matter)	SD (g/100 g of dry matter)	Range (g/100 g of dry matter)	Preteatment	LV	R^2_c	RMSEC (g/100 g of dry matter)	R^2_p	RMSEP (g/100 of dry matter)	CV_p (%)
Arabinose	Total	204	75	4.2	3.7	0.1-21	SNV 2d	13	0.98	0.47	0.95	0.93	22
	Insoluble	190	72	3.1	2.6	0.3-14	2d	13	0.99	0.48	0.96	1.00	32
Xylose	Total	204	79	5.7	4.7	0.1-21	2d	16	0.99	0.51	0.95	1.2	21
	Insoluble	195	77	4.9	4.5	0.1-21	2d	16	0.99	0.48	0.96	1.53	31
Mannose	Total	198	75	0.5	0.3	0.2-1.6	2d	16	0.93	0.06	0.75	0.15	30
	Insoluble	177	64	0.3	0.2	0.1-1.0	MSC 1d	11	0.80	0.07	0.77	0.09	30
Galactose	Total	192	72	1.0	1.1	0.1-5.9	MSC 1d	11	0.99	0.11	0.95	0.26	26
	Insoluble	182	66	0.7	0.8	0.0-3.5	SNV 2d	12	0.96	0.12	0.90	0.22	31
Glucose	Total	201	71	2.4	1.9	0.3-11	MSC 2d	15	0.86	0.63	0.81	0.87	36
	Insoluble	186	68	1.5	4.7	0.0-11	1d	12	0.79	0.51	0.62	0.77	51
Rhamnose	Total	159	49	0.2	0.3	0.0-1.3	SNV 1d	15	0.99	0.03	0.94	0.05	25
	Insoluble	135	44	0.1	0.1	0.0-0.6	SNV 2d	11	0.97	0.02	0.93	0.04	40
Uronic acids	Total	202	68	2.4	4.2	0.1-20	MSC 2d	15	1.00	0.20	0.98	0.57	24
	Insoluble	191	68	1.0	1.2	0.0-5.2	MSC 2d	14	0.99	0.10	0.95	0.27	27

Number of samples used for calibration (N_{cal}) and validation (N_{val}), mean, standard deviation (SD) and range of calibration, pretreatment used (SNV: standard normal variate, MSC: multiplicative scatter correction, 1d: first derivative, 2d: second derivative), number of latent variables (LV), coefficient of determination of calibration (R^2_c) and prediction (R^2_p), root mean square error of calibration (RMSEC) and prediction (RMSEP) and variation coefficient of prediction (CV_p).

result of their low concentrations (mean <0.5 g/100 g as is), which fall near the sensitivity threshold of the NIR technique. Glucose, despite being a major NCP residue, was also predicted with limited accuracy. The reason could be that glucose is representing different polysaccharides in protein- and fiber-rich feedstuffs. Nevertheless, calibration models based solely on cereals, where glucose primarily represents β-glucan, did not show a significantly better performance. Still, prediction errors below 30 % (CV_P < 30 %) could be considered acceptable given the analytical complexity of these constituents, whose reference values carry significant experimental uncertainty (CV > 10 % in some cases, as Supplementary Table 12 shows).

Earlier research revealed the capacity of NIRS to predict certain CHO in specific groups of feedstuffs. Our results for sugars and starch were similar to those obtained by Losada et al. (2009) who used a dataset that included different cereal and cereal co-products. As far as we know no NIRS calibrations for fructans have been reported and only Hollung et al. (2005) predicted α-galactosides in soybean meal, where only raffinose showed a good correlation between the reference and the predicted values. Gomes et al. (2020), instead of developing global calibrations like us, divided their dataset into cereals, protein- and fiber-rich feedstuffs and developed three sets of specific calibrations (R^2_{cv}>0.86) for most of the total and insoluble NCP residues. Prediction errors were not reported in this work. Nieto-Ortega et al. (2022) used a dataset that contained cereals and cereal co-products and accurately predicted (R^2_{cv}>0.90 and CV_{cv}<25 %) starch and most of the total and insoluble NCP residues, cellulose and NSP. Like in our investigation, they obtained the lowest precision for mannose (R^2_{cv}=0.54, CV_{cv}>100), which is also the sugar residue present in generally the lowest concentration. Blakeney and Flinn (2005) employed a cereals sample set and predicted cellulose, β-glucan, arabinose, xylose, glucose and total, insoluble and soluble fractions of NSP. Like in our research, the models for the soluble NSP fraction were not as good (R^2_{cv}<0.70, CV_{cv}>30) as for the total and insoluble fractions (R^2_{cv}>0.80, CV_{cv}<25). In none of these works, they developed models for rhamnose, uronic acids or NCP as the sum of all residues, which to the best of our knowledge for the first time is described in this work. Archibald and Kays (2000) predicted well DF in cereals for human consumption. However, this calibration and our's might not be comparable. They used another reference method of analysis and did not account for OS and RS content in the DF calculation. For the moment, there are no calibrations reported for DF in feedstuffs used in monogastric animal diets. Our study is also the first to take the NIR calibrations a step further by evaluating the predictive power of NIR calibrations on an independent dataset.

The use of NIRS can help the community to access information about the CHO and lignin content of feedstuffs in a much cheaper and faster way than by chemical analysis. Formulating diets that closely align with the targeted nutritional composition can lower feed costs, enhance animal performance, and minimize environmental impact. Bodin and Aubret (2005) estimated that a saving of 2 €/T in feed could be assumed when broiler fed diets are formulated using NIRS values instead of table values. Soto et al. (2013) and Neto et al. (2017) showed significative improvements on broiler's body weight with NIRS-based formulation and Van Kempen and Simmins (1997) calculated a drop of 13 % in the ratio of nitrogen excretion to nitrogen accretion when poultry diets are formulated based on digestible AAs predicted by NIRS against when they are predicted based on protein content.

Conclusions

NIR calibration models proved to be useful to predict the CHO that are easily digested by monogastric animals (sugars and starch) and most of those which are part of DF, from a variety of feedstuffs used in animal feeding. We accurately predicted DF, TF, lignin, T-NSP, I-NSP, cellulose, T-NCP, I-NCP, β-glucan, fructans and the total and insoluble content of the NCP residues: arabinose, xylose, galactose, rhamnose and uronic acids. The models for mannose, glucose, especially insoluble glucose and α-galactosides, especially verbascose were not good enough to considered them for routine analysis. The successful models can lead to a deeper understanding of the CHO fraction present in feedstuffs, enabling a more efficient formulation of monogastric diets and the development of nutritional strategies such as the use of carbohydrases, resulting in a positive impact on host metabolism.

Ethics approval

Not applicable.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004770.

Availability of data and materials

The data used and the models developed were not deposited in an

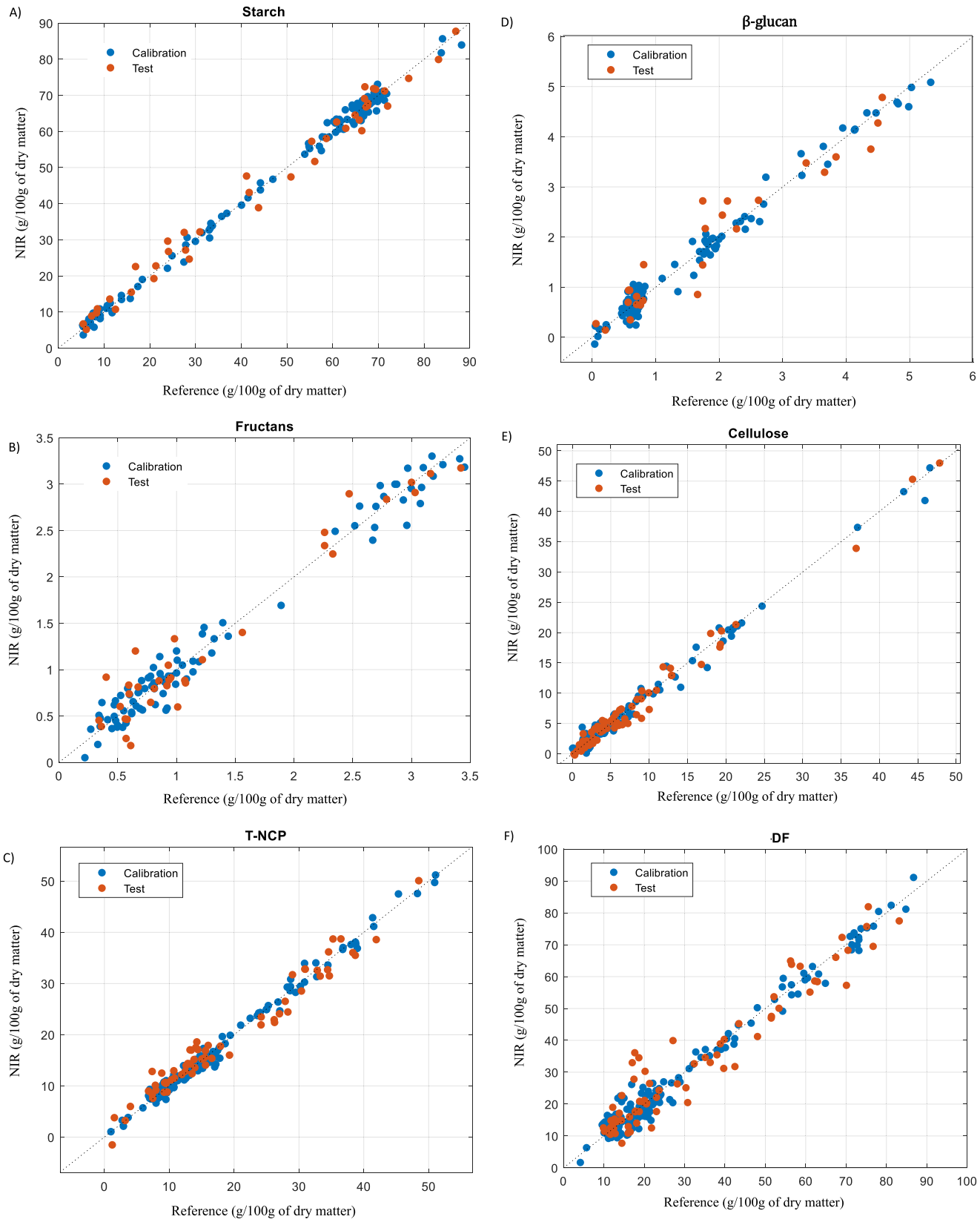

Fig. 5. Predicted vs measured values for selected calibrations developed for carbohydrates: A) Starch, B) Fructans, C) β-glucans, D) Total non-cellulosic polysaccharides (T-NCP), E) Cellulose and F) Dietary fiber (DF). The points, representing the samples, should be placed as close as possible to the dotted line which is the ideal line of slope 1 and intercept 0.

official repository. They are available upon request.

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.

Acknowledgments

A. Cruz-Conesa acknowledges Universitat Rovira i Virgili (Tarragona, Spain) and the Institute of Agrifood Research and Technology (Constantí, Spain) for providing a Marti Franqués Research Fellowship (2019PMF-PIPF-62) and the Department of Animal and Veterinary Sciences at Aarhus University (Foulum, Denmark) for providing the dataset used in this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105394.

References

Archibald, D.D., Kays, S.E., 2000. Determination of total dietary fiber of intact cereal food products by near-infrared reflectance. J. Agric. Food Chem. 48, 4477–4486. https://doi.org/10.1021/jf000206j.

Bach Knudsen, K.E., 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poul. Sci. 93, 2380–2393. https://doi.org/10.3382/ps.2014-03902.

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67, 319–338. https://doi.org/10.1016/s0377-8401(97)00009-6.

Bach Knudsen, K.E., Laerke, H.N., 2018. Carbohydrates and lignin in the feed - from sugars to complex composed fibres. In: Moughan, P.J., Hendriks, W.H. (Eds.), Feed Evaluation Science. Wageningen Academic Publishers, Wageningen, Netherlands, pp. 113–144.

Bach Knudsen, K.E., Laerke, H.N., Jorgensen, H., 2023. Carbohydrates and carbohydrate utilization in swine. Sustainable Swine Nutrition (ed. Chiba, L.I.). John Wiley & Sons Inc., Hoboken, New Jersey, pp. 151–187.

Bach Knudsen, K.E., Li, B.W., 1991. Determination of oligosaccharides in protein-rich feedstuffs by gas-liquid chromatography and high-performance liquid chromatography. J. Agric. Food Chem. 39, 689–694. https://doi.org/10.1021/jf00004a013.

Badaró, A.T., Hebling e Tavares, J.P., Blasco, J., Aleixos-Borrás, N., Barbin, D.F., 2022. Near infrared techniques applied to analysis of wheat-based products: recent advances and future trends. Food Control 140, 109115. https://doi.org/10.1016/j.foodcont.2022.109115.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. Appl. Spectrosc. 43, 772–777. https://doi.org/10.1366/0003702894202201.

Blakeney, A.B., Flinn, P.C., 2005. Determination of non-starch polysaccharides in cereal grains with near-infared reflectance spectroscopy. Molec. Nutr. and Food Res. 49, 546–550. https://doi.org/10.1002/mnfr.200500038.

Bodin, J. C., Aubret, J. M. 2005. The NIRS method to support practical feed formulation. Proceedings of the 15th European Symposium on polutry nutritiion, Balatonfured, Hungary, pp. 420-427.

Cheli, F., Battaglia, D., Pinotti, L., Baldi, A., 2012. State of the art in feedstuff analysis: a technique-oriented perspective. J. Agric. Food Chem. 60, 9529–9542. https://doi.org/10.1021/jf302555b.

Choct, M., 2015. Feed non-starch polysaccharides for monogastric animals: classification and function. Anim. Prod. Sci. 55, 1360–1366. https://doi.org/10.1071/AN15276.

Cummings, J.H., Stephen, A.M., 2007. Carbohydrate terminology and classification. Eu. J. Clinic. Nutr. 61, 5–18. https://doi.org/10.1038/sj.ejcn.1602936.

Englyst, H., Wiggins, H.S., Cummings, J.H., 1982. Determination of the non-starch polysaccharides in plant foods by gas - liquid chromatography of constituent sugars as alditol acetates. Analyst 107, 307–318. https://doi.org/10.1039/AN9820700307.

García-Sánchez, F., Galvez-Sola, L., Martínez- Nicolás, J.J., Muelas-Domingo, R., Nieves, M., 2017. Using Near-Infrared Spectroscopy in Agricultural Systems. In: Kyprianidis, K.G., Skvaril, J. (Eds.), Developments in Near-Infrared Spectroscopy. Intech Open publishers, pp. 97–127. https://doi.org/10.5772/67236.

Garrido-Varo, A., Vega, S., Maroto-Molina, F., De La Haba, M.J., Pérez-Marín, D., 2016. On-site quality control of processed land animal proteins using a portable micro-electro-mechanical-systems near infrared spectrometer. J. Near Infrared Spectrosc. 24, 47–58. https://doi.org/10.1255/jnirs.1192.

Gomes, G. A., dos Santos, T. T., Piotrowski, C., García, R., 2020. Development of near infrared calibrations for determination of non-starch polysaccharides content in feedstuff. In 31st Annual Australian Poultry Science Symposium. Sidney, Australia, pp. 54–57.

Hollung, K., Øverland, M., Hrustić, M., Sekulić, P., Miladinović, J., Martens, H., Narum, B., Sahlstrøm, S., Sørensen, M., Storebakken, T., Skrede, A., 2005. Evaluation of nonstarch polysaccharides and oligosaccharide content of different soybean varieties (Glycine max) by near-infrared spectroscopy and proteomics. J. Agric. Food Chem. 53, 9112–9121. https://doi.org/10.1021/jf051438r.

Jha, R., Berrocoso, J.D., 2015. Review: dietary fiber utilization and its effects on physiological functions and gut health of swine. Animal 9, 1441–1452. https://doi.org/10.1017/S1751731115000919.

Larsson, K., Bengtsson, S., 1983. Bestämning av lättilgängelig kolhydrater I växtmaterial (Determination of readily available carbohydrates in plant material). In: Chemistry Methods Report no, 22. Statens Lantbrukskemiska Laboratorium, Uppsala, Sweden.

Losada, B., García Rebollar, P., Cachaldora, P., Álvarez, C., Méndez, J., Blas, C.d., 2009. A comparison of the prediction of apparent metabolisable energy content of starchy grains and cereal by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. Spanish J. Agric. Res. 7, 813–823. https://doi.org/10.5424/sjar/2009074-1096.

MacDougall, D., Martens, H., Geladi, P., 1985. Linearization and scatter-correction for near-infrared reflectance spectra of meat. Appl. Spectrosc. 39, 491–500.

Marten, G.C., Shenk, J.S., Barton II, F.E., 1989. Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality, Agriculture Handbook No. 643. United States Department of Agriculture, Springfield.

McCleary, B.V., Glennie-Holmes, M., 1985. Enzymic quantification of (1-3), (1-4)-β-D-glucan in barley and malt. J. Inst. Brew. 91, 285–295.

McCleary, B.V., Monaghan, D.A., 2002. Measurement of resistant starch. J. AOAC Int. 85, 665–675. https://doi.org/10.1093/jaoac/85.3.665.

Navarro, D.M.D.L., Abelilla, J.J., Stein, H.H., 2019. Structures and characteristics of carbohydrates in diets fed to pigs: a review. J. Anim. Sci. Biotech. 10, 39. https://doi.org/10.1186/s40104-019-0345-6.

Neto Montanhini, R., N'Guetta, E., Gady, C., Francesch, M., Preynat, A., 2017. Combined effect of using near-infrared spectroscopy for nutritional evaluation of feed ingredients and non-starch polysaccharide carbohydrase complex on performance of broiler chickens. Anim. Sci. J. 88, 1979–1986. https://doi.org/10.1111/asj.12822.

Nieto-Ortega, B., Arroyo, J.J., Walk, C., Castañares, N., Canet, E., Smith, A., 2022. Near infrared reflectance spectroscopy as a tool to predict non-starch polysaccharide composition and starch digestibility profiles in common monogastric cereal feed ingredients. Anim. Feed Sci. Technol. 285, 115214. https://doi.org/10.1016/j.anifeedsci.2022.115214.

Osborne, B.G., Fearn, T., 1988. Near Infrared Spectroscopy in Food Analysis. Longman Scientific & Technical, England.

Petry, A.L., Patience, J.F., 2020. Xylanase supplementation in corn-based swine diets: a review with emphasis on potential mechanisms of action. J. Anim. Sci. 98, 1–12. https://doi.org/10.1093/JAS/SKAA318.

Rodehutscord, M., Rückert, C., Maurer, H.P., Schenkel, H., Schipprack, W., Bach Knudsen, K.E., Schollenberger, M., Laux, M., Eklund, M., Siegert, W., Mosenthin, R., 2016. Variation in chemical composition and physical characteristics of cereal grains from different genotypes. Arch. Anim. Nutr. 70, 87–107. https://doi.org/10.1080/1745039X.2015.1133111.

Savitzky, A., Golay, M.J.E., 1964. Smoothing and differentiation of data by simplified least squares procedures. Anal. Chem. 36, 1627–1639. https://doi.org/10.1021/ac60214a047.

Serena, A., Bach Knudsen, K.E., 2007. Chemical and physicochemical characterisation of co-products from the vegetable food and agro industries. Anim. Feed Sci. Technol. 139, 109–124. https://doi.org/10.1016/j.anifeedsci.2006.12.003.

Snee, R.D., 1977. Validation of regression models: methods and examples. Technometrics 19, 415–428. https://doi.org/10.1080/00401706.1977.10489581.

Soto, C., Avila, E., Arce, J., Rosas, F., McIntyre, D., 2013. Evaluation of different strategies for broiler feed formulation using near infrared reflectance spectroscopy as a source of information for determination of amino acids and metabolizable energy. J. Appl. Poult. Res. 22, 730–737. https://doi.org/10.3382/japr.2013-00655.

Theander, O., Aman, P., 1979. Studies on dietary fibres. Swed. J. Agric. Res. 9, 97–106.

Van Kempen, T., Simmins, H., 1997. Near-infrared reflectance spectroscopy in precision feed formulation. J. Appl. Poult. Res. 6, 471–477. https://doi.org/10.1093/japr/6.4.471.

Ward, N.E., 2021. Debranching enzymes in corn/soybean meal–based poultry feeds: a review. Poult. Sci. 100, 765–775. https://doi.org/10.1016/j.psj.2020.10.074.