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# Enabling precision feeding: A novel micro-NIRS system for rapid on-site analysis of the nutritional composition of poultry feed and feed ingredients

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ABSTRACT

Poultry diets are routinely under- or over-formulated. Precision feeding aims to address this inefficiency. Miniaturized near-infrared spectrometers (micro-NIRS) may provide the necessary accurate and timely knowledge on the nutritional composition of feed ingredients and diets. In this study, we present the Pocket NIR, a novel micro-NIRS based on a unique optical arrangement of two complementary MEMS (micro-electromechanical system) Fabry-Pérot interferometers oriented toward the same focal spot. We developed 30 partial least squares regression models to demonstrate its analytical potential for rapid on-site analysis of protein, fat, fiber, water-soluble carbohydrates (WSC), moisture, and ash in poultry feed, corn, wheat, soybean, and DDGS (distillers' dried grains with solubles). A library of 1437 reference samples, 248–358 samples per material, was used for calibration and hold-out validation of these models. Cross-validation was used to select the best spectra preprocessing steps from the commonly used methods for transformation, scatter correction, smoothing, and differentiation of NIRS spectra. With exceptions, models for protein, fat, and moisture had a ratio of performance to deviation (RPD) larger than 3 on a held-out dataset. Model performances with an RPD > 2 were observed for the majority of models for fiber, WSC, and ash. The heterogeneity of the material and the variability of the nutrient parameters co-determined the models' performance. With its mobile app and cloud-based backend, the Pocket NIR could in the future assist precision feeding by offering nutritional advice and diet formulation suggestions based on its data, and considering ingredient availability, costs, traceability of supplier, and production management.

## 1. Introduction

Driven by population growth, rising incomes, and urbanization, poultry production is the world's fastest-growing livestock sector [1,2]. It is of major importance to global food security and nutrition and is expected to provide 62 % of total meat production in 2032 [2]. Poultry production and its waste products are associated with greenhouse gas emissions and contamination of air, soil, and water [3]. Feed is often reported as the largest cost factor in poultry production [4] and as the most important contributor to the environmental footprint of the final products of the poultry supply chain [5]. Optimizing feeding strategies and diet formulations is therefore important for both economic efficiency and for reducing the environmental footprint. Energy density and protein content are the most important feed characteristics for improving the (economic) feed conversion efficiency, taking into account the nutrient requirements of the animals in different growth phases [6]. The protein content of the feed is also of particular focus with respect to the environmental footprint, as it is highly correlated with nitrogen in animal excreta, which is a major source of N-containing greenhouse gas ($N_2O$) and environmental pollutants [7,8].

Due to the large variability in the quality of feed ingredients and the reliance on nutritional feed tables, diets are routinely under- or over-formulated [9]. Precision feeding aims to replace this inefficient practice by using frequent analyses of nutrient contents of the feeds to match

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the diet to the dynamic nutrient requirements of the animals. This can reduce production costs, improve productivity and animal health, and reduce the environmental footprint [9,10]. Van Kempen and Simmins [11] studied overformulation practice versus precision feeding with respect to nitrogen in poultry excreta. They found that overformulation of 7.5 % more amino acids than necessary was needed to reduce the risk of nutritionally inadequate diets to 20 %. As a result, a large excess of dietary amino acids was excreted. By contrast, precision feeding using diets formulated with nutritional contents determined by near infrared spectroscopy (NIRS) instead of using average values from tables resulted in a 13 % decrease in the ratio of nitrogen excretion to nitrogen accretion [11].

Precision feeding at the feed mill and farm levels requires accurate and timely knowledge of the nutritional value of feed ingredients and diets [9]. In the laboratory, benchtop NIRS instruments are routinely used to analyze nutritional composition of feeds and feed ingredients, replacing costly and labor-intensive conventional wet chemical analysis. Recently available miniaturized NIRS (micro-NIRS) are highly promising for precision feeding, as they are portable and may allow rapid and frequent on-site analysis of feed and feed ingredients. The mature design of benchtop NIRS instruments is predominantly based on Fourier transform spectrometers with a Michelson interferometer or a polarization interferometer [12]. Most micro-NIRS devices are based on distinct technologies that use a wide variety of working principles for both Fourier transform and dispersion-based spectrometers with dispersive optics or narrowband filters [13]. Their still rapidly evolving designs include diverse light sources, wavelength selectors, detectors, and optical materials (reviewed in [12,14]). The wavelength selectors in micro-NIRS are typically miniaturized through micro-electromechanical systems (MEMS), which are mass produced using technology similar to microchip manufacturing [12]. Micro-NIRS devices are compact and portable. They can be affordable and suitable for the consumer market. Therefore, they might contribute to democratizing the access to feed analyses methods. However, compared to benchtop instruments, they typically operate in a narrower spectral range, with lower spectral resolutions, smaller dynamic ranges, and inferior signal-to-noise ratios. This results in varying performances and underlines the need for systematic evaluation in terms of performance and analytical potential for different target applications [15]. Such evaluation of micro-NIRS devices was, for example, conducted for nutritional analysis of poultry feed [16], diverse compound feeds for various animals [17], oilseed meal as livestock feed [18], sugar cane forage [19], and grass, alfalfa, and clover as single or mixed forage [20–25].

In this study, we present the Pocket NIR, a novel micro-NIRS with a unique optical arrangement of two complementary MEMS Fabry-Pérot interferometers (MEMS-FPI) oriented toward the same focal spot. This affordable, portable device can enable on-site feed analysis for precision feeding. The goal of this study was to investigate the analytical potential and performance of this novel micro-NIRS in analyzing the nutritional composition of poultry feed and feed ingredients. We therefore developed calibrations for the prediction of crude protein, crude fat, crude fiber, water-soluble carbohydrates, moisture, and crude ash in poultry feed, corn, wheat, soybean, and distillers' dried grains with solubles (DDGS). We also compared the analytical performance of the Pocket NIR with a benchtop NIRS instrument.

## 2. Materials and methods

### 2.1. The pocket NIR

The Pocket NIR developed by aikemy GmbH is a portable, battery-powered micro-NIRS system (Fig. 1A). When fully charged, its lithium-ion battery (1000 mAh) is sufficient for approximately 5000 scans, which corresponds to the analysis of around 80 samples according to the protocol described below. Full charging via USB-C takes approximately 3.5 h. The device connects via Bluetooth LE (low energy) to a mobile app (iOS, Android) with a cloud backend. The backend provides the infrastructure for running prediction models and storing results in a database. The mobile app configures the micro-NIRS and collects spectral data, which is transmitted to the cloud-based backend for processing and prediction. The results are transmitted back to the mobile app for presentation to the user.

The micro-NIRS is equipped with two tungsten filament bulbs that emit broad spectrum (white) light. The emitted light is guided by an internal aluminum structure which reflects and directs it to a mineral glass scanning window. This configuration produces an illuminated area of approximately 6 mm in diameter on the scanning window and sample respectively. Two tunable single-pixel MEMS Fabry-Perot interferometers (MEMS-FPIs) are aligned to detect light reflected from the same spot of approximately 1 mm in diameter, located in the center of the illuminated area. They operate in complementary wavelength ranges of 1550–1850 nm and 1750–2150 nm, respectively, with an overlap from 1750 nm to 1850 nm (Fig. 2, Fig. S1 in the Supplementary Information). The spectral resolution at 1850 nm is approximately 20 nm FWHM (full width at half maximum). The signal-to-noise ratio across the spectral range averages approximately 1300:1. The sequential wavelength-by-wavelength data acquisition (1 nm interval) takes 1 s for a full scan of the 702 data points. Ten consecutive scans are automatically taken and averaged to yield one spectrum. The micro-NIRS has an aluminum housing with an O-ring to facilitate attachment to the grinder container or the sample cup (Fig. 1A). The housing has a cylindrical shape with a diameter of 7.5 cm and a height of 3 cm. The scanning window with a diameter of 14 mm is located in the center.

The operation procedure of the Pocket NIR for the analysis of poultry feed and feed ingredients is as follows. Samples are analyzed as ground solids or powders. Samples in other forms, such as whole grains or


**Fig. 1.** The Pocket NIR with accessories. (A) Pocket NIR with glass window for scanning of the sample, O-ring for connection to the grinder container or sample cup, USB-C connector for charging, and LED indicator for on/off and battery status. Bluetooth logo indicates communication to the mobile app via Bluetooth LE. (B) Battery-driven grinder for sample preparation. (C) Pocket NIR connected to the grinder container. On the left, the situation with the Pocket NIR on top and the sample in the grinder container, i.e., the situation when displacing the sample by shaking. On the right, the situation with the Pocket NIR on the bottom and the sample on the Pocket NIR scanning window, i.e., the situation during a measurement. See the description of the measurement procedure in the main text.

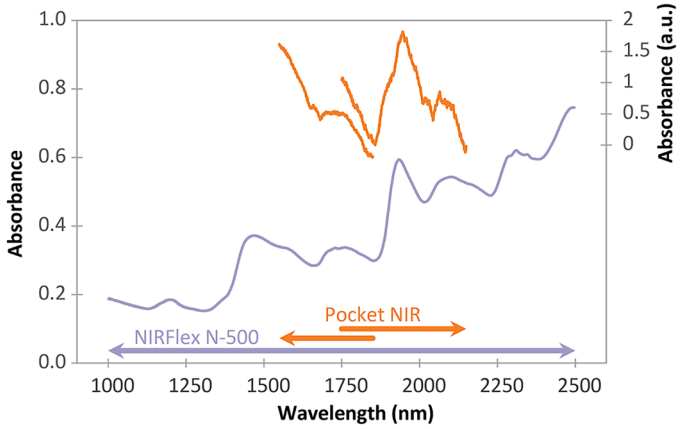


**Fig. 2.** NIR absorbance spectra of a poultry feed sample collected with the Pocket NIR and the NIRFlex N-500 as average of 10 and 32 scans, respectively. Absorbance was calculated as log(1/R) transformation of the reflectance.

pellets, are ground to a particle size of smaller than 0.3 mm with the battery-powered grinder (two to three times 20 s grinding) provided as an accessory (Fig. 1B). The Pocket NIR is directly attached to the grinder container (or a sample cup if no grinding is needed). The whole setup is turned upside down, allowing the sample in the grinder to fall onto the scanning window (Fig. 1C). A spectrum is acquired for the first 'spot'. The setup is then turned and shaken to displace the sample before acquiring a second spectrum at a different spot. The process is repeated to acquire in total six spectra from different spots. The mobile app guides the user step by step through this procedure.

### 2.2. Development of prediction models

#### 2.2.1. Reference samples library

A sample library with five different sample materials was collected, including poultry feed (compound feed for different types of poultry as grains, flakes, marshes, crumbles, pellets, and flour), corn, wheat (intact grains, flakes, marsh, crumbles, and flour), soybean (intact beans, soybean cake, and soybean meal), and DDGS. The latter is a byproduct of ethanol production, with the largest quantities coming from the biofuel industry. The primary source is corn, but wheat, barley, rye, and sorghum, or combinations of these grains, are sometimes also used [26]. Our DDGS sample collection included corn and wheat DDGS.

The sample library was created by collecting "original" samples from stakeholders and research institutions and by purchasing from various suppliers. The origin of the samples was Europe and the USA. Care was taken to collect samples with as much diversity as possible, looking for samples rich in energy and protein, as well as samples with low nutrient content. Once the nutritional composition of the original samples was analyzed, binary mixtures ("mixed" samples) of the two original samples were prepared. The purpose of these mixed samples was to minimize sample gaps in terms of the nutrient parameters, in order to achieve as even a distribution of reference values as possible.

Mixed samples were prepared according to the following procedure: Original samples (ground to <1 mm) were homogenized for 3 min with a 3D shaker-mixer (Turbula® T2F, WAB-Group, Switzerland) before weighing the required mass. The resulting mixed samples were also homogenized for 3 min. In this way, for every 2 original samples adjacent in protein content, mixed samples were prepared on a 50/50 mass ratio basis. Whenever possible, any remaining gaps in protein, fat, fiber, WSC, and ash were then filled with 20/80, 25/75, 30/70, and 40/60 mass ratio mixes.

The total number of samples in the sample library per material was between 248 and 358, of which 104–233 were original samples, and 125–144 were mixed samples (Table 1). For poultry feed, corn, and wheat, the ranges in nutritional composition were similar to those in the Agroscope database of the official controlling of feeds in Switzerland, indicating good coverage of typical values (Table S1 in the Supplementary Information; soybean and DDGS are not available in the Agroscope database).

#### 2.2.2. Reference chemical analyses

The nutritional composition of the original samples, i.e., dry matter, crude ash (ash), crude protein (protein), crude fat (fat), crude fiber (fiber), and water soluble carbohydrates (WSC), was analyzed by the accredited analytical laboratory of Agroscope, Posieux, according to Swiss official methods for feed analysis. All non-powder samples were ground with a rotary cutting mill (880804, Brabender, Duisburg, Germany) to pass a 1 mm sieve. Protein was determined via the Dumas method (LECO TruMac), ISO 16634, with protein calculated as nitrogen × 6.25. Fat was determined via a modification of ISO 6492 by pressurized solvent extraction after acid hydrolysis (Büchi, Speed Extractor 916, using petrol ether). Fiber was determined according to ISO 6865 with a Fibretherm, Gerhard FT-12. The WSC was determined in glucose equivalents via a potassium ferricyanide reducing sugar test after $H_2SO_4$ treatment (San System Skalar, glucose calibration curve). Dry matter and ash were determined, according to ISO 6496 and 5984, respectively, via thermogravimetry by heating the sample to a constant mass (Precisa Instruments AG) at 103 °C for moisture and then at 550 °C for ash. Moisture content was calculated by subtracting the dry matter weight from the initial as-fed weight. The uncertainties of the laboratory for all reference chemical analyses used in this study are given in Table S2 in the Supplementary Information. The nutritional composition of the mixed samples was calculated from the nutritional composition of the two original samples according to the mass fractions in the mix.

#### 2.2.3. Chemometrics

Partial least squares regression (PLSR) models for the Pocket NIR were developed in Python v.3.10 with the machine-learning framework scikit-learn v.1.2.2 [27]. Spectra pretreatment methods and sample set splitting strategies were implemented as scikit-learn compatible custom components. Scikit-learn 'pipelines' [28] were used throughout the model development as well as for subsequent model deployment.

For each of the five materials, PLSR models were developed to predict protein, fat, fiber, WSC, moisture, and ash content (30 models in total). Models were developed using spectra from six spots per sample,

**Table 1**
Feed and feed ingredient sample library with ranges in nutrient content. The majority, but not all, of the samples were analyzed for the full set of nutrient parameters, i.e., the number of samples (n) does not always reflect the number of samples available for the development of individual prediction models (see Results section).

| Material | n | min–max in g $kg^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Protein | Fat | Fiber | WSC | Moisture | Ash |
| Poultry feed | 358 (233) | 71–414 | 17–92 | 17–77 | 18–90 | 63–136 | 10–350 |
| Corn | 275 (158) | 51–113 | 22–93 | 6–33 | 14–35 | 71–155 | 7–27 |
| Wheat | 263 (126) | 98–159 | 17–26 | 16–31 | 18–42 | 53–140 | 12–20 |
| Soybean | 293 (157) | 352–875 | 6–249 | 2–94 | 17–123 | 34–131 | 32–88 |
| DDGS | 248 (104) | 179–403 | 38–134 | 27–82 | 14–81 | 70–128 | 41–145 |

$n$ = total number of samples (number of original samples in parentheses).

and model performance was accordingly assessed with predicted values as the average of the individual predictions of 6 spots. Using a variant of the so-called venetian blinds sampling (Fig. S2A in the Supplementary Information), the dataset was split into a training set used for calibration (~2/3 of available samples) and a test set used for validation (~1/3 of available samples). When splitting into training and test set, the sample was treated as an entity, ensuring that the spectra of the 6 spots of a sample were exclusively assigned to the either the training or the test set. The samples with the two smallest and two largest values of the nutrient parameter of interest were assigned to the training set, i.e., the training set covers the range of the test set. The test set was exclusively used to evaluate the generalization performance. We calculated the root mean square error (RMSE), the relative root mean square error (%RMSE = RMSE divided by the mean of reference values × 100), and the ratio of performance to deviation (RPD = standard deviation of reference values divided by RMSE; formulas in Supplementary Information). Additionally, we visually inspected reference (y-axis) versus predicted (x-axis) scatter plots with linear regression lines [29].

The training dataset was exclusively used for model development. We use the term 'model' to refer to all mathematical steps from the raw spectra to the prediction, i.e., all pretreatment steps and the PLSR (Fig. 3A). In our model, the spectra of the two MEMS are pre-processed individually and then concatenated and standardized to zero mean and unit variance ('standard scaling') before being used in PLSR. This allows the best pre-processing steps and settings to be selected individually for the two MEMS (Fig. 3A). The choice of pretreatment steps, their settings, and the number of components used in the PLSR were all treated as tunable hyperparameters. As an optional first step, log(1/R) transformation of the reflectance spectra to an apparent absorption spectra was considered. As a second step, pre-processing always included scatter correction and a smoothing procedure to obtain the smoothed spectra or a derivative thereof. We considered scatter correction before and after smoothing, conducted either by standard normal variate (SNV; [30]) or multiplicative scatter correction (MSC; [31]) using the mean training spectrum as the reference spectrum. Smoothing with/without first or second order derivative was conducted with Savizki-Golay [32] or the gap-segment derivative [33] method.

Hyperparameter tuning was done in several steps (Fig. 3B), each

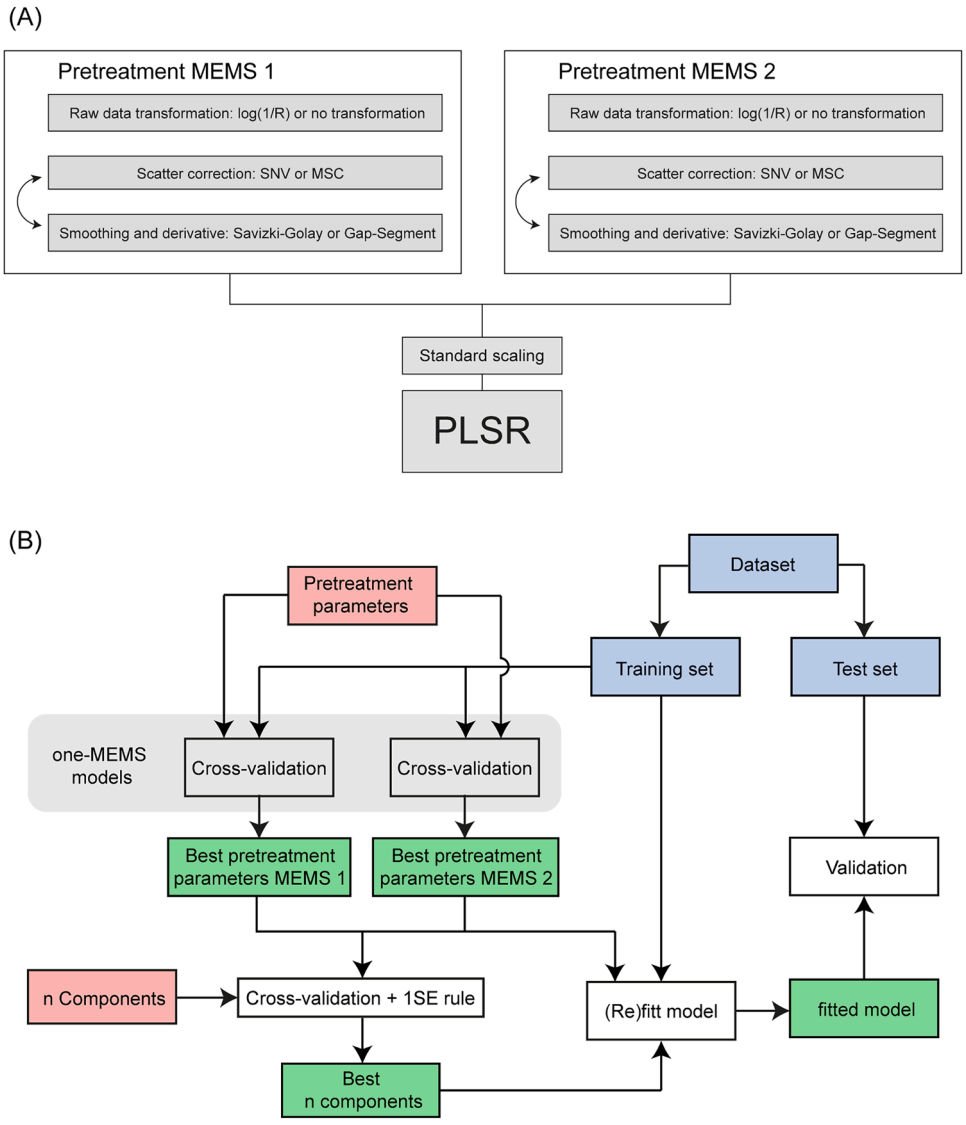


**Fig. 3.** (A) Schematic representation of the prediction models. Options for the individual pre-processing steps and options in the ordering of these steps (double-sided arrow) are shown. (B) Schematic representation of the hyperparameter tuning and model validation strategy. Blue: datasets; red: hyperparameter combinations up for testing; green: best combination of hyperparameters and fitted model. Gray shaded area with "one-MEMS model" indicates that these steps are conducted with a model as in panel A but with one MEMS only. For clarity, we do not show that to select the best hyperparameters (after CV with one-MEMS model), we also consider the other nutrient parameter models of the same material, eventually sharing the same hyperparameters across models for the same material (see main text).

using a grid search with 3-fold venetian blinds cross-validation (CV, Fig. S2B in the Supplementary Information) and RMSE as the performance metric.

The pretreatment hyperparameters were tuned separately for the two MEMS, with the model as in Fig. 3A but with one MEMS only. However, we selected the same pre-treatment for all six nutrient parameter models for a given material in order to increase the robustness of the models at the expense of a minor sacrifice in performance. First, for each of the six nutrient parameters of a material, CV performance of the different combinations of pretreatment hyperparameters was determined. The number of components in PLSR was allowed to vary to allow each of the pretreatment hyperparameter combinations to show its best CV performance. For each nutrient parameter, we then ranked all tested pretreatment hyperparameter combinations and selected the settings with the best median rank across the nutrient parameters to be used for all nutrient parameters (of the same material).

Unlike the pretreatment hyperparameters, the number of components in the final PLSR was selected individually for all 30 models. We followed the 'one-standard error' rule [34] and chose the most parsimonious model, that is, the model with the least number of PLS components, whose performance fell within 1 standard error of the CV performance of the best model (i.e., within mean ± standard error as calculated from the RMSE values of the three CV folds). Finally, with all hyperparameters tuned, the model was refitted to the full training set before subjecting it to validation on the test set. The tuned hyperparameters are given in Fig. S3 in the Supplementary Information.

### 2.3. Comparison with a benchtop instrument

We compared the performance of the Pocket NIR to the performance of a benchtop Fourier transform NIR spectrometer with a polarization interferometer (NIRFlex N-500, Büchi, Flawil, Switzerland). The benchtop NIR spectrometer was equipped with a rotating sample cup 10 cm in diameter, which allowed a large part of the cup perimeter to be scanned. Spectra were recorded in the region 10,000 $cm^{-1}$ to 4000 $cm^{-1}$ (1000 to 2500 nm) in diffuse reflectance mode, with a spectral resolution of 8 $cm^{-1}$, and a sample point interval of 4 $cm^{-1}$, resulting in 1501 data points per spectrum. Three replicate spectra were obtained, each representing an average of 32 scans. Samples were analyzed at room temperature (~22 °C) not more than two months after the analysis with the Pocket NIR.

PLSR prediction models for the benchtop instrument were built with the Büchi NIRCal 1.5.6000 software by an experienced scientist in Agroscope's analytical laboratory (S. Ampuero Kragten), following the software's typical workflow for selecting suitable spectra pre-processing and PLSR settings. Prediction models for soybean and DDGS were built from the ground up, while preexisting in-house models were updated with additional samples in the case of corn, wheat, and poultry feed. Similar to the Pocket NIR, ~1/3 of our sample library was held out for validation (test set), while the other ~2/3 were used for building or updating the prediction models (training set). The sample was treated as an entity, ensuring that the three replicate spectra were assigned exclusively to either the training or the test set.

## 3. Results

The performances of the Pocket NIR, as determined by RMSE, % RMSE, and RPD, are listed in Table 2. Reference versus prediction plots are shown in Figs. 4–9. Descriptive statistics of the reference values of the training and test sets are listed in Table S3 in the Supplementary Information. With respect to the reference values, the corresponding training and test sets showed very similar ranges, means, and standard deviations (Table S3). The performance determined on the test set was, with a few exceptions, close to the corresponding performance on the

**Table 2**
Performance of the Pocket NIR in predicting nutrient contents of poultry feed and feed ingredients, with performance of a benchtop instrument (last two columns) for comparison. Subscripts C and V indicate calibration and validation, respectively. RMSE in g $kg^{-1}$.

| | | Calibration (training set) | | | | Validation (test set) | | | | N-500* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $n_C$ | $RMSE_C$ | $\%RMSE_C$ | $RPD_C$ | $n_V$ | $RMSE_V$ | $\%RMSE_V$ | $RPD_V$ | $RMSE_V$ | $RPD_V$ |
| Protein | Poultry feed | 226 | 15.1 | 8.0 | 3.1 | 114 | 15.7 | 8.3 | 2.7 | 5.06 | 5.8 |
| | Corn | 182 | 4.2 | 5.4 | 3.5 | 90 | 4.0 | 5.2 | 3.6 | 1.61 | 8.9 |
| | Wheat | 176 | 3.3 | 2.9 | 3.7 | 87 | 3.6 | 3.2 | 3.3 | 2.02 | 6.4 |
| | Soybean | 186 | 14.4 | 3.0 | 6.2 | 92 | 13.5 | 2.8 | 6.3 | 7.73 | 10.1 |
| | DDGS | 163 | 6.1 | 2.2 | 6.0 | 80 | 6.7 | 2.4 | 5.3 | 3.32 | 10.7 |
| Fat | Poultry feed | 236 | 3.9 | 7.2 | 3.7 | 116 | 4.3 | 8.0 | 3.3 | 2.87 | 5.3 |
| | Corn | 176 | 4.7 | 11.1 | 2.1 | 87 | 4.1 | 9.8 | 2.3 | 3.28 | 2.8 |
| | Wheat | 175 | 1.0 | 4.6 | 1.6 | 86 | 1.0 | 4.6 | 1.6 | 0.90 | 1.7 |
| | Soybean | 187 | 7.0 | 13.6 | 8.7 | 92 | 7.0 | 14.0 | 8.4 | 3.26 | 17.4 |
| | DDGS | 165 | 3.6 | 4.4 | 3.3 | 82 | 3.2 | 3.9 | 3.3 | 1.90 | 5.5 |
| Fiber | Poultry feed | 234 | 6.9 | 19.0 | 1.4 | 116 | 6.8 | 18.8 | 1.4 | 3.32 | 2.4 |
| | Corn | 184 | 2.6 | 14.9 | 1.9 | 90 | 3.3 | 18.7 | 1.5 | 2.09 | 2.5 |
| | Wheat | 176 | 2.2 | 10.2 | 1.3 | 86 | 2.3 | 10.8 | 1.2 | 2.45 | 1.2 |
| | Soybean | 191 | 8.1 | 21.7 | 2.2 | 94 | 8.3 | 22.2 | 2.1 | 4.26 | 4.2 |
| | DDGS | 164 | 2.9 | 4.6 | 3.1 | 81 | 3.2 | 5.0 | 2.7 | 2.18 | 3.7 |
| WSC | Poultry feed | 136 | 4.9 | 10.4 | 2.6 | 68 | 6.0 | 13.0 | 2.0 | 2.65 | 3.8 |
| | Corn | 183 | 2.7 | 11.9 | 1.6 | 90 | 2.6 | 11.8 | 1.5 | 1.57 | 2.6 |
| | Wheat | 171 | 3.3 | 10.7 | 1.2 | 82 | 3.3 | 10.7 | 1.1 | 2.18 | 1.7 |
| | Soybean | 177 | 6.0 | 6.6 | 3.2 | 91 | 6.7 | 7.2 | 2.8 | 3.74 | 4.7 |
| | DDGS | 160 | 3.3 | 9.4 | 5.2 | 78 | 3.5 | 10.0 | 4.8 | 2.87 | 5.9 |
| Moisture | Poultry feed | 234 | 6.4 | 6.1 | 1.9 | 116 | 6.3 | 6.1 | 1.8 | 5.10 | 2.2 |
| | Corn | 178 | 5.2 | 4.4 | 3.2 | 89 | 4.3 | 3.6 | 3.8 | 3.35 | 5.0 |
| | Wheat | 168 | 3.2 | 2.7 | 4.8 | 84 | 4.1 | 3.5 | 3.5 | 4.42 | 3.1 |
| | Soybean | 194 | 6.1 | 6.2 | 3.5 | 96 | 6.6 | 6.7 | 3.2 | 5.05 | 4.2 |
| | DDGS | 166 | 2.9 | 3.0 | 3.8 | 81 | 3.0 | 3.0 | 3.7 | 2.42 | 4.6 |
| Ash | Poultry feed | 230 | 30.3 | 33.2 | 1.9 | 115 | 35.0 | 38.5 | 1.5 | 9.67 | 5.1 |
| | Corn | 183 | 1.6 | 12.8 | 2.2 | 91 | 1.4 | 11.5 | 2.3 | 0.85 | 4.5 |
| | Wheat | 175 | 1.0 | 6.3 | 1.5 | 88 | 1.1 | 7.1 | 1.3 | 0.94 | 1.5 |
| | Soybean | 192 | 3.9 | 6.6 | 2.0 | 94 | 3.7 | 6.3 | 2.0 | 2.14 | 3.2 |
| | DDGS | 164 | 3.9 | 7.0 | 4.9 | 82 | 3.7 | 6.7 | 4.8 | 2.23 | 7.8 |

$n$ = number of data points.
* NIRFlex N-500 benchtop instrument.

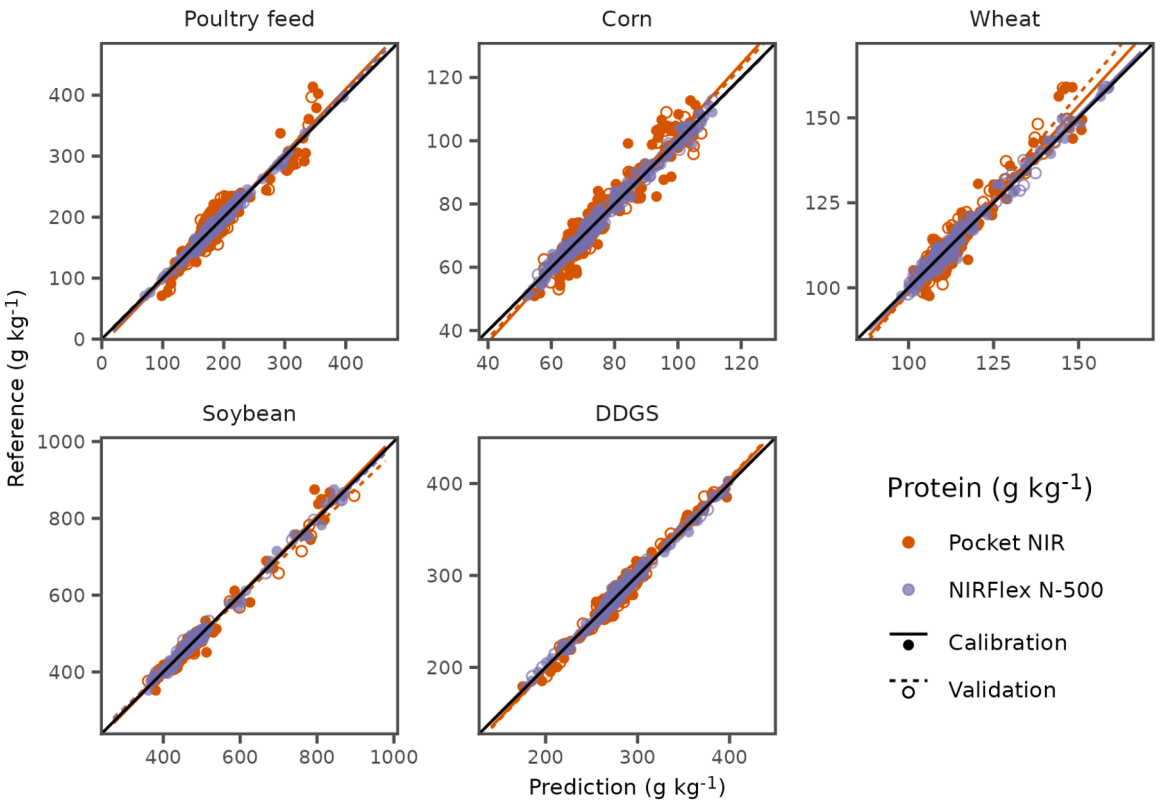


**Fig. 4.** Reference versus prediction scatter plot for the determination of protein content of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

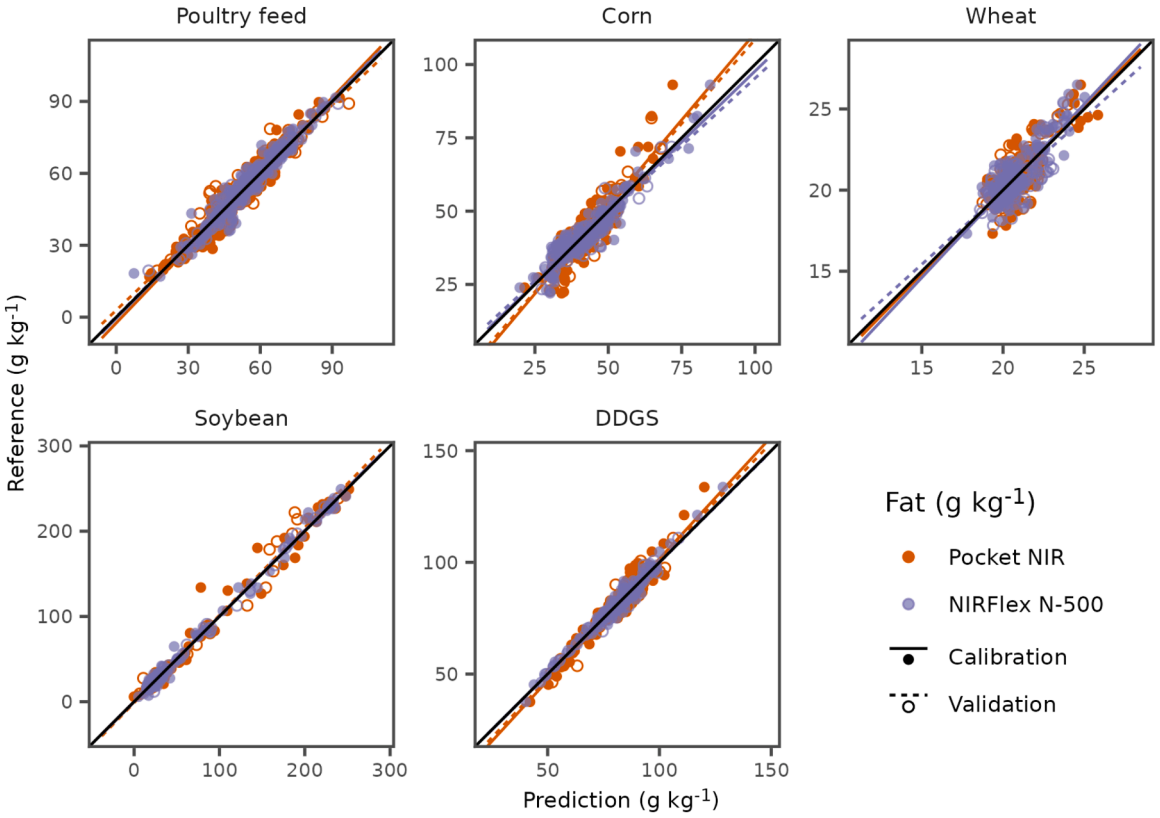


**Fig. 5.** Reference versus prediction scatter plot for the determination of fat content of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

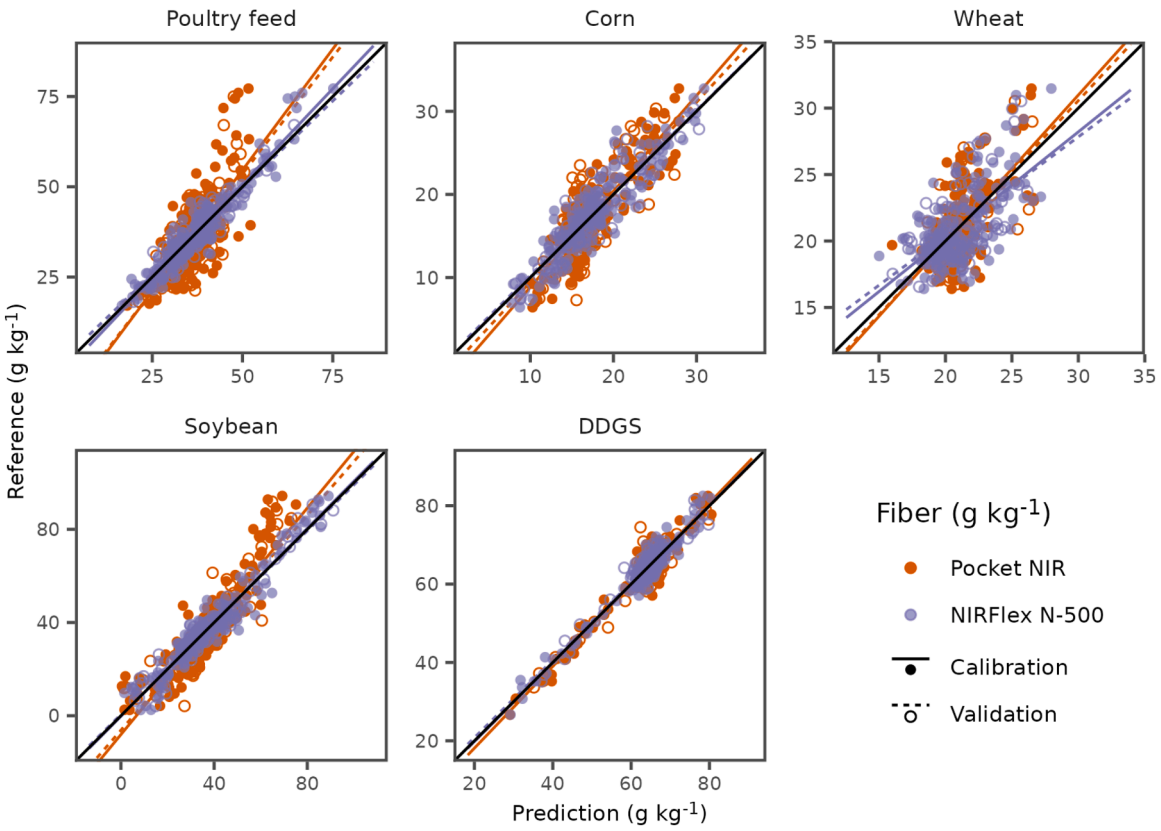


**Fig. 6.** Reference versus prediction scatter plot for the determination of fiber content of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

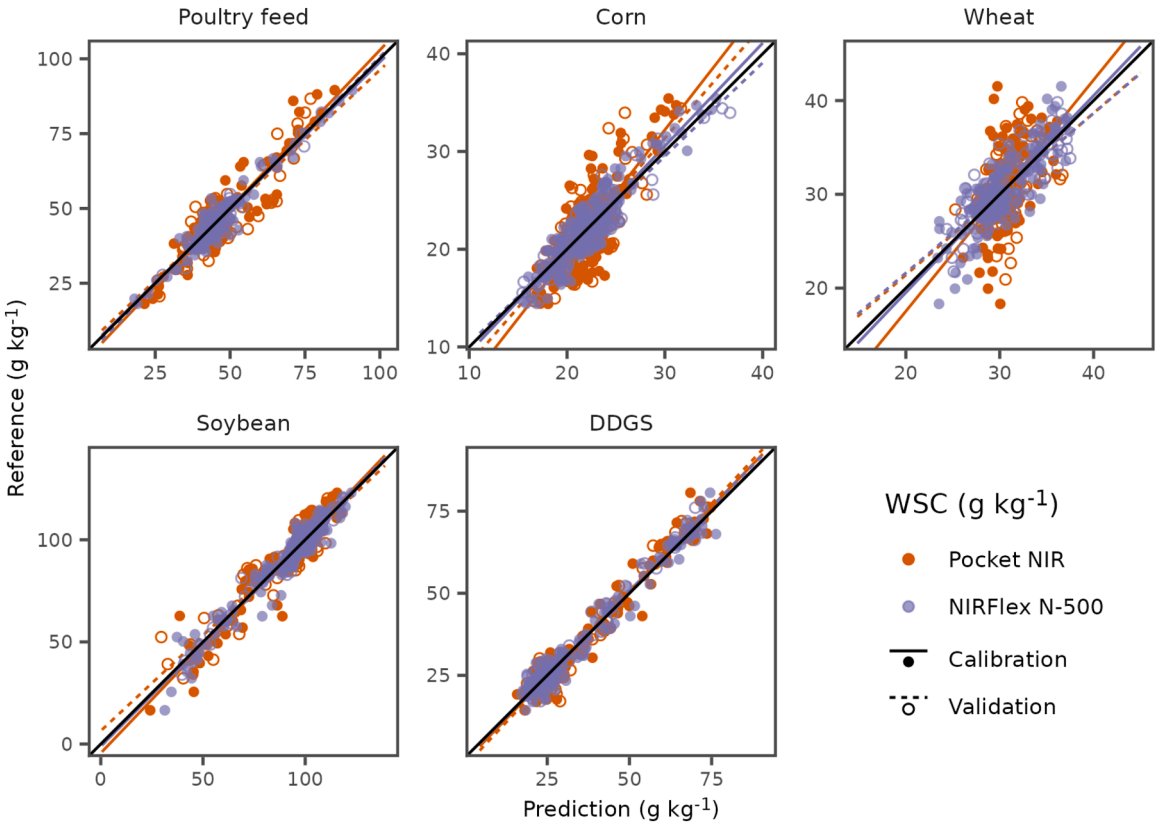


**Fig. 7.** Reference versus prediction scatter plot for the determination of WSC contents of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

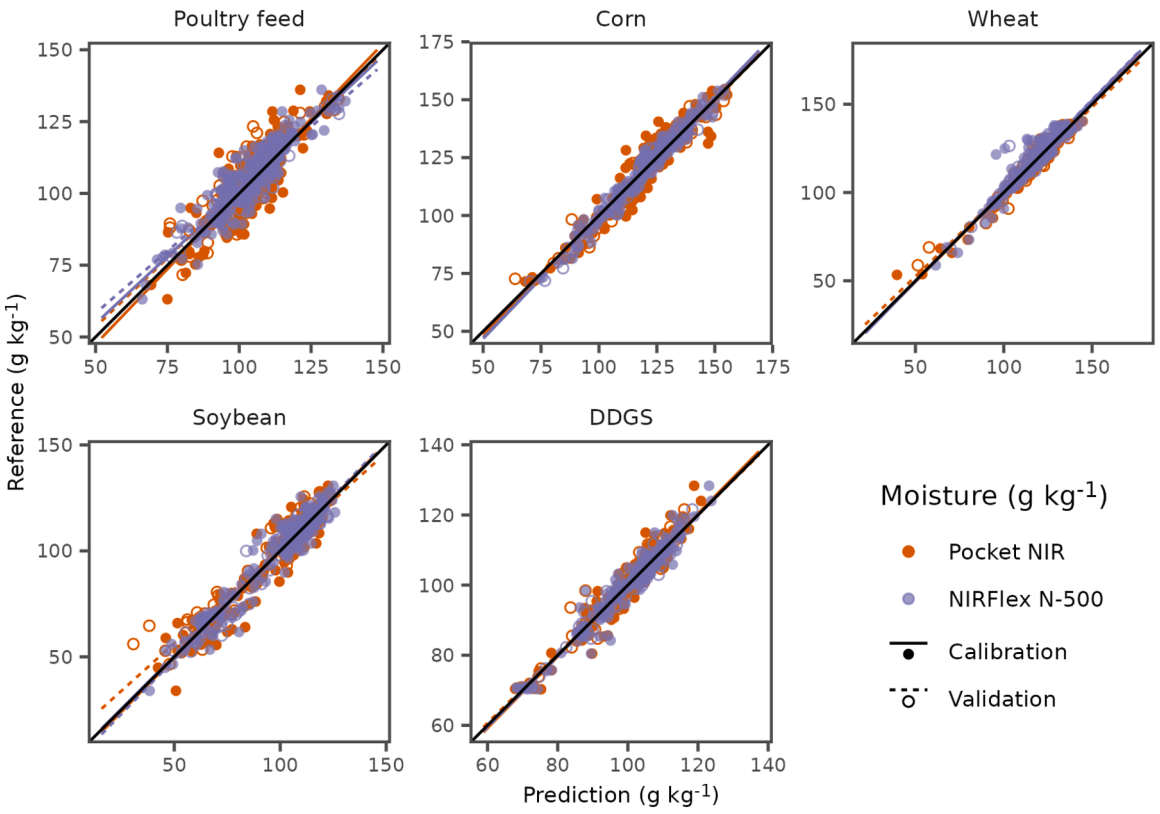


**Fig. 8.** Reference versus prediction scatter plot for the determination of moisture content of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

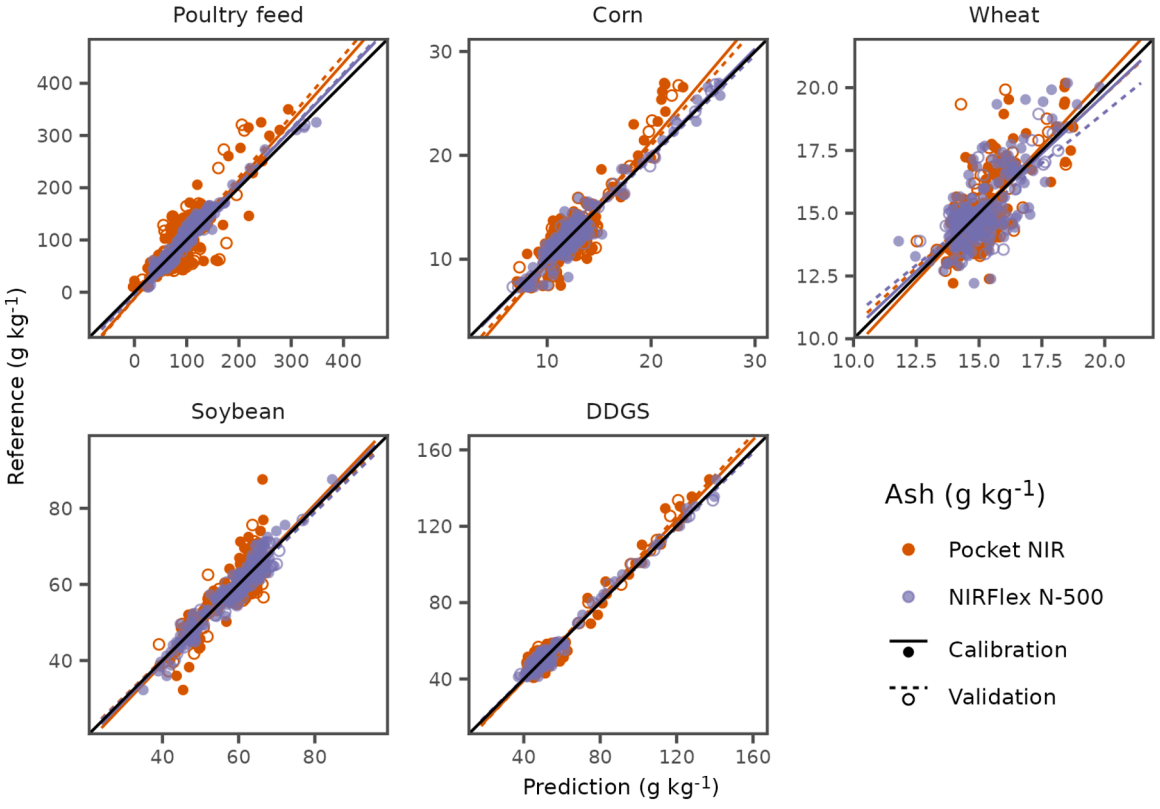


**Fig. 9.** Reference versus prediction scatter plot for the determination of ash content of poultry feed and feed ingredients with the Pocket NIR and a benchtop instrument (NIRFlex N-500). Colored lines are linear regression lines fitted to the data shown.

training set, indicating the absence of substantial overfitting (Table 2). The $RPD_V$ values ranged from 1.1 to 8.4 for the different parameters and materials, with a median of 2.7. The benchtop instrument outperformed the Pocket NIR in terms of $RMSE_V$ and $RPD_V$ (Table 2, last two columns) for all parameters and materials, except for moisture and fiber in wheat, for which both instruments showed comparable performance. Trends in the $RPD_V$ and $\%RMSE_V$ values for the Pocket NIR were mirrored in those for the benchtop instrument; across all parameters and materials, $RPD_V$ values between the two instruments were correlated with a Spearman's $\rho$ of 0.86 and $\%RMSE_V$ values were correlated with $\rho$ of 0.89 (Fig. S4 in the Supplementary Information).

In similar studies on the use of NIRS for the nutritional analysis of forages [35–37], calibration performance was classified as "excellent" for RPD > 2.5, "good" for RPD at 2–2.5, "moderate" for RPD at 1.5–2, and "poor" for RPD < 1.5. However, depending on the field or sample type, different classifications have been used in previous studies, with more stringent classifications typically used for physically simpler sample types and less stringent classifications (such as the one mentioned) for physically more complex sample types. In any case, RPD depends on the range of values in the set of samples, different data structures can give rise to the same RPD, and single individual samples of high and low values can inflate RPD [38]. Therefore, we refrain from strict RPD classification and also consider the reference versus prediction plots.

$RPD_V$ values for the prediction of protein in the different materials ranged from 2.72 to 6.28, increasing in the order poultry feed < wheat < corn < DDGS < soybean (Table 2, Fig. 4). $RMSE_V$ values were around 15 g $kg^{-1}$ for poultry feed and soybean and < 7 g $kg^{-1}$ for corn, wheat, and DDGS. In relative terms, expressed as $\%RMSE_V$, these errors were ≤ 5.2 %, except for poultry feed, which had 8.3 %. As described below also for other nutrient parameters, the nutritional contents of poultry feed seem more challenging to predict compared to the contents of individual pure ingredients. Additionally, considering the reference versus prediction plots, with the even distribution of reference values, the slope of the reference versus prediction regression line close to 1, and the narrow spread of predictions around this line, we consider the performance of the Pocket NIR in predicting protein very satisfactory.

$RPD_V$ values for the prediction of fat in the different materials increased in the order wheat < corn < poultry feed < DDGS < soybean (Table 2, Fig. 5), the latter three having an $RPD_V \geq 3.3$, whereas wheat and corn had lower $RPD_V$ of 2.3 and 1.6, respectively. $RMSE_V$ values were ≤ 7 g $kg^{-1}$, and $\%RMSE_V$ were ≤ 14 %. Predicting the fat content of soybean showed an $RPD_V$ of 8.4, the largest, despite comparably high error in terms of $RMSE_V$ (7 g $kg^{-1}$), reflecting the large variability of the reference values in the dataset (relative standard deviation %SD of 118 % for both test and training dataset, Table S3). The low $RPD_V$ in predicting the fat content of wheat is related to the generally low levels (mean of 20.8 g $kg^{-1}$) and the lack of variability of fat in wheat in our sample sets (Table S3), particularly when viewed in the context of the uncertainty of the laboratory for the corresponding wet chemical reference method (Table S2). For corn fat content prediction, the variability in the reference values in terms of SD was larger than for wheat, resulting in a larger $RPD_V$ despite its $RMSE_V$ being larger. However, the distribution of the values revealed a few high values separated from most of the rest of the data (Fig. 5). In such cases, it might be justified to consider $RPD_V$ to be unjustifiably inflated by these values.

Regarding the prediction of fiber, the $RPD_V$ was 2.7 for DDGS, 2.1 for soybean, and ≤1.5 for poultry feed, corn, and wheat (Table 2, Fig. 6). Predicting DDGS fiber content showed an $RMSE_V$ of 3.2 g $kg^{-1}$, and the % $RMSE_V$ was the lowest, at 5 %. For the fiber content of wheat, the % $RMSE_V$ was 10.8 %, while that of poultry feed, corn, and soybean was as high as around 20 %. For corn, the ratio of $RMSE_V$ to $RMSE_C$ was 1.26, considerably larger than 1, which might indicate an undesirable degree of overfitting. However, the test set containing harder-to-predict samples than the training set is always a plausible explanation for this observation. Overall, fiber appeared to be the most difficult nutrient parameter to determine with the Pocket NIR, which was also true with the benchtop instrument.

Concerning WSC, the $RPD_V$ was 4.8 for DDGS, 2.0 and 2.8 for poultry feed and soybean, respectively, and < 1.5 for corn and wheat (Table 2, Fig. 7). $RMSE_V$ values were between 2.6 and 6.7 g $kg^{-1}$, and $\%RMSE_V$ values were ≤13 %. For the WSC of poultry feed, the ratio of $RMSE_V$ to $RMSE_C$ was 1.25, significantly greater than 1, which could indicate an undesirable degree of overfitting.

Regarding predicting moisture, the $RPD_V$ was between 3.2 and 3.8 for corn, wheat, soybean, and DDGS (Table 2, Fig. 8), whereas it was considerably lower for poultry feed, at 1.8. $RMSE_V$ values were < 6.6 g $kg^{-1}$, and %RMSE values were < 6.7 %. Except for poultry feed, we consider these calibrations to be very good for a micro-NIRS, with RPD > 3 and slope of the reference versus prediction regression line close to 1 (Fig. 8). After protein, moisture seems to be the property that is most consistently well-predicted across the different materials.

Regarding ash content, the $RPD_V$ was 4.8 for DDGS, 2.0 and 2.3 for soybean and corn, respectively (Table 2, Fig. 9), and ≤ 1.5 for poultry feed and wheat. With the exception of poultry feed, $RMSE_V$ values were between 1.1 and 3.7 g $kg^{-1}$, while $\%RMSE_V$ values were ≤ 11.5 %. For the ash content of poultry feed, the error was considerably larger, with $RMSE_V$ of 35.0 g $kg^{-1}$ and $\%RMSE_V$ of 38.5 %.

## 4. Discussion

We presented the Pocket NIR, a micro-NIRS with a unique configuration with 2 MEMS-FPIs. Using a library of poultry feed and feed ingredient samples analyzed by wet chemical reference methods, we established prediction models for the Pocket NIR using PLSR and spectra pre-treatment methods selected through extensive hyperparameter tuning. Test set validation using a hold-out dataset was conducted to determine generalization performance. Ranking for each material the nutrient parameters according to the achieved performance in terms of $RPD_V$ (rank 1 being best) and subsequently calculating the mean of these ranks across the materials, we observed the sequence: protein (mean rank 1.8), moisture (2.6), fat (2.8), WSC (4.0), ash (4.2), and fiber (5.6). Apparently, for protein, moisture, and fat, we were more successful in developing performant prediction models. The $RPD_V$ was consistently > 3.2 for these nutrient parameters, with the exception of poultry feed for protein and moisture ($RPD_V$ 2.7 and 1.8) and corn and wheat for fat ($RPD_V$ 2.3 and 1.6; Table 2). Considering also the visual inspection of reference versus prediction plots in our assessment, we consider these models to be very good for a micro-NIR, clearly demonstrating the promising analytical potential of the Pocket NIR for nutritional analysis of feeds and feed ingredients (Fig. 4–9). In predicting ash, WSC, and fiber content, the models' performances were more variable, with 8 of 15 models exhibiting $RPD_V > 2$ and the best performance achieved for DDGS, which had $RPD_V$ 2.7, 4.8, and 4.8, respectively. Many of the lower $RPD_V$ values were related to the limited variability of the nutrient parameters in the respective material in our dataset. Possibly more samples extending and filling the range of observed values could help improve these prediction models.

Modern formulation of poultry diet is based on the nitrogen-corrected apparent metabolizable energy (AMEn) of the feeds and the requirements of the animals in their current growing phase [39]. AMEn can be calculated from proximate nutrient analyses such as those determined with NIRS, using established multivariate empirical relationships [40]. For example, AMEn for corn and soybean can be calculated using the nutrient parameters for which we developed prediction models for the Pocket NIR in this study [41]. However, for the calculation of AMEn in wheat or other starch-rich cereal grains, knowledge of the starch content is needed [41]. The same is true for the calculation of AMEn in mixed poultry feeds, such as according to EU and Swiss regulations on feedstuffs (EC 152/2009 annex VII; FMBV annex III). Starch is a common parameter determined with benchtop and micro-NIRS (e.g., [16]). Follow-up work with the Pocket NIR will thus

develop prediction models for starch.

Besides energy, dietary protein is of high importance in poultry feed formulation. The protein prediction models for the Pocket NIR were very good, and we therefore suppose that the Pocket NIR might excel in frequent and accurate dietary protein level monitoring. This might be particularly helpful in the challenging formulation of low-protein diets, with the potential to lower nitrogen emissions and reduce soybean usage. The latter is being sought in Europe, which is dependent on soybean imports from the Americas, which have a large environmental footprint (e.g., [42]). In this context, many alternative protein sources, such as grain legumes [43], insects [44], oil-seed by-products [45], or microalgae [46] are examined. Micro-NIRS systems, such as the Pocket NIR, could assist in adjusting diet formulations when dynamically choosing alternative protein sources, depending on the requirements of the animals in the given growth phase, availability, prices, and environmental footprint. However, prediction models for potentially many alternative ingredients would have to be developed for the Pocket NIR. Instead of developing many small prediction models, one for each material, it might be preferable to develop a single model for a group of materials. Linearity between the spectral signal and the nutrient parameters likely breaks down for a multi-material dataset. Thus, PLSR would have to be replaced by non-linear calibration algorithms, such as artificial neural networks (ANN). Given training on enough data for a group of feed ingredients, such an ANN might perform competitively after updating with fewer samples of a new material as compared to a separate calibration, as it can learn from similar examples. The multi-material calibrations that FOSS offers ready-made for its benchtop instruments are an example of such ANN-based calibrations [47].

In addition to the total protein, the contents of individual amino acids are important in feed formulation. In focus are the amino acids that either cannot be synthesized by poultry at all or not at sufficient rate to meet metabolic requirements [40]. These must be taken up via dietary protein or from added feed-grade amino acids. Benchtop NIRS instruments have been successfully calibrated to predict these amino acids (e.g., [48,49]), but similar reports using micro-NIRS are missing. The National Research Council [40] published two methods to estimate digestible amino acids in poultry feed ingredients using empirical relationships to the nutrient variables we determined with the Pocket NIR in this study. The first method uses protein content, and the second method uses protein, fat, fiber, and ash content. We implemented the second method in the Pocket NIR, returning the estimates of amino acids together with the nutrient composition predicted (more directly) from the spectra. In the future, similar empirical relationships established by other authors could be added to the Pocket NIR, from which users could then choose.

Ranking for each nutrient parameter the materials according to achieved performance in terms of $RPD_V$ (rank 1 being best), and subsequently calculating the mean of these ranks across the nutrient parameters, we observed the following sequence: DDGS (mean rank 1.5), soybean (2.2), corn (2.8), poultry feed (4.0), and wheat (4.5). For DDGS, we were most successful in achieving good calibrations with high $RPD_V$. DDGS is a very homogeneous material, as it is obtained as a byproduct of dry-mill ethanol production after extensive processing (grinding, cooking, enzymatic treatment for starch hydrolysis, fermentation, distillation, and finally drying; [26]). Furthermore, nutrient contents of DDGS vary largely [26], which is also reflected in our dataset. Both the homogeneity of the material and the large variability in the nutrient parameters will have helped with more performant prediction models. Poultry feed and wheat were apparently the most challenging materials. However, for wheat, the ranges and variability were lower than those of the other materials, hampering the achievement of good prediction models with high $RPD_V$. For poultry feed, the comparatively lower performance of the prediction models may be related to the more diverse matrix across the samples (different ingredients in different ratios) and the more heterogeneous matrix of the individual samples (mixtures of ingredients) compared to the pure ingredients.

These results highlight a major challenge in micro-NIRS devices in obtaining representative measurements of heterogeneous materials. For a fast and on-site method such as the Pocket NIR, it would be desirable to analyze feed and feed ingredients in their as-fed form, including whole grains and pellets. Benchtop instruments can be used to analyze whole grains (e.g., [50]). With micro-NIRS, such samples are typically analyzed after grinding, although this additional sample preparation step limits the portability of the analytical method. One major reason for this is the smaller sample area that is scanned compared to benchtop devices. Benchtop instruments come with accessories to rotate the sample during the measurements. Different parts of a larger sample are scanned, averaging out inhomogeneities, thereby achieving a higher representativeness of a heterogeneous sample. Such accessories are challenging to build with a highly portable, rugged, and affordable design but successful examples exist, e.g., the Rotator-Kit for the ProxyScout handheld NIRS [51]. The Pocket NIR does not rely on an accessory to automatically rotate the sample. Instead, its measurement operation procedure requires the user to displace the sample manually. The procedure was defined based on a series of tests with poultry feed samples. We developed sets of preliminary calibrations (not shown in this publication) using different protocols. Reducing sample heterogeneity by grinding was found to considerably improve achievable performance and was thus deemed a prerequisite for reducing sample heterogeneity. The user is required to displace the sample several times, thereby taking spectra from different spots, which are individually used to obtain preliminary predictions that are subsequently averaged to obtain the final prediction. We found six spots to be a good compromise between obtainable accuracy and the time it takes to analyze one sample. For materials less heterogeneous than poultry feed (e.g., DDGS), we expect to have answered this trade-off more in favor of accuracy.

We claim that the Pocket NIR will allow fast on-site analysis. Whether this holds true is ultimately determined by the portability of the device, as well as by the overall procedure, from taking the sample to receiving the results. The Pocket NIR and all its accessories, including the grinder, are highly portable; they are lightweight, small size, battery-driven devices. The ‘lab’ can therefore come on-site to the sample, rather than the usual way of sample to lab. For a sample needing grinding (two to three times 20 s), we found that the measurement procedure, including sample preparation, takes about 5–10 min, which we would consider rapid for this kind of analysis.

The Pocket NIR is intended to be used with ready-made prediction models. To be affordable, these prediction models cannot be developed individually for each Pocket NIR device but should be usable with many devices. Therefore, developing an appropriate calibration transfer strategy to account for instrument-to-instrument differences is an essential next step.

## 5. Conclusion

We demonstrated the analytical potential of the Pocket NIR micro-NIRS system in analyzing poultry feed and feed ingredients. The performance of the prediction models for protein, fat, and moisture in the different materials was, with view exceptions, characterized by $RPD_V > 3$. A performance of $RPD_V > 2$ was observed for the majority of models for predicting fiber, WSC, and ash. Both the variability of the values of the nutrient parameters and the heterogeneity of the material were indicated to have co-determined the achieved performances; poultry feed, the most heterogeneous material, was the most challenging, and DDGS, a very homogeneous material with large variability in nutrient contents, was the least challenging. The trends in performance across materials and nutrient parameters observed with the Pocket NIR mirrored the trends observed with the benchtop instrument. Extending the training dataset, potentially in combination with exploring novel chemometric algorithms, will further improve the performance and robustness of the Pocket NIR. With the cloud-based backend, the Pocket NIR opens a wide variety of possibilities for future features assisting

precision feeding, such as nutritional advice and suggestions of diet formulation based on the data obtained with the device and considering information on ingredient availability, costs, traceability of suppliers, and production management.

## Ethical_statement

No humans or animals were involved in the study and no ethics approval was required.

## CRediT authorship contribution statement

**Michael Simmler:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kotaro Ishizaki:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Conceptualization. **Alessandro Mulloni:** Writing – review & editing, Software. **Rossella Abbate:** Writing – review & editing, Investigation, Data curation. **Alexander Juste:** Writing – review & editing, Investigation, Data curation. **Agata Sroka:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

K. Ishizaki and A. Sroka from aikemy GmbH have commercial interests in future products based on this research. All other authors declare that they have no competing interests that could have influenced the work presented in this publication.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.atech.2025.101220.

## Data availability

Data will be made available on request.

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