Evaluation of analytical and statistical approaches for predicting in vitro nitrogen solubility and in vivo pre-caecal crude protein digestibility of cereal grains in growing pigs

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Abstract
Different analytical (enzyme system and near-infrared spectroscopy (NIRS)) and statistical (single and multiple regressions) approaches were used to predict in vivo standardized pre-caecal digestibility (PCD) of crude protein (CP) and amino acids (AA) in cereal grains for growing pigs as well as in vitro nitrogen (N) solubility. Furthermore, different chemical and physical characteristics were categorized (e.g. crude nutrients, AA, minerals, fibre components or combinations of these) and used for generating prediction equations. There were strong linear relationships ($p < .05$) between in vivo PCD of CP and essential AA and in vitro N solubility when grain species was considered as covariate in the model. Predicting in vivo PCD values using various chemical and physical characteristics produced inconsistent results among different grain species and AA and could therefore not be used for predicting PCD. It is possible to predict in vitro N solubility from chemical and physical characteristics for some grain species. However, the relationships between some of these categories and the in vitro N solubility were not consistent and not always causative or physiologically explainable. The $R^2$ of NIRS for predicting in vitro N solubility was at a relatively high level (up to $R^2 = 0.80$). This level of $R^2$ indicates that a classification of the grain samples in, for example, high, medium and low in vitro N solubility levels is possible, but it does not allow for a quantitative prediction of the in vitro N solubility. In conclusion, the present database can be used for establishing a ranking of different cereal grain species for PCD of CP and essential AA values. However, it was not possible to create clear prediction equations for in vivo or in vitro digestibility values. Therefore, greater variation within grain species, for example due to different growing and harvesting conditions, is warranted for predicting PCD values of individual grain samples.

Keywords
amino acids, cereal grains, crude protein, growing pigs, in vitro nitrogen solubility, standardized pre-caecal digestibility
1 | INTRODUCTION

Due to high starch content, cereal grains are primarily used as an energy source in diets for pigs. However, at high dietary inclusion level, cereal grains such as barley, rye, triticale and wheat can supply considerable amounts of crude protein (CP) and amino acids (AA) to the pig (Cervantes-Pahm, Liu, & Stein, 2014). The feed protein evaluation system for pigs in Germany is based on the standardized pre-caecal digestibility (PCD) of CP and AA (Gesellschaft für Ernährungsphysiologie, 2005). According to Rosenfelder et al. (2015), Spindler et al. (2016), Rosenfelder-Kuon, Strang, Spindler, Eklund, and Mosenthin (2017), and Strang, Eklund, Rosenfelder, Htoo, and Mosenthin (2017), physical and chemical characteristics such as protein source and type, starch characteristics, fat source and type, non-starch polysaccharides (NSP) and anti-nutritional factors vary between and within grain species may influence these digestibility values. Experiments for determination of in vivo standardized pre-caecal CP and AA digestibility values are time-consuming and expensive and labour-intensive and require the use of surgically modified pigs for collection of ileal digesta. Thus, an evaluation system that could provide a rapid and accurate estimate of PCD of CP and AA in individual batches of cereal grains used for feed manufacturing is needed (Jezierny, Mosenthin, Sauer, & Eklund, 2010). As an alternative to in vivo pre-caecal digestibility measurements, the so-called modified two-step enzyme system has been proposed by Boisen and Fernández (1995), which simulates the digestive processes in the stomach and small intestine of the pig through the use of respective enzymes, pH values and digesta retention times. However, it needs to be acknowledged that in vitro models do not fully reflect in vivo conditions in the pigs’ digestive tract (e.g. endogenous enzymes, microbes and absorption of nutrients is missing in vitro). Furthermore, near-infrared spectroscopy (NIRS) as a fast and easy-to-handle method has the potential for classifying a large number of grain batches by their pre-caecal digestibility. To the authors’ knowledge, no studies are available that include such a complex evaluation of cereal grains grown under identical conditions in connection with in vivo PCD and in vitro N solubility values as well as a comprehensive database on chemical and physical parameters and NIRS calibrations. Digestibility values for cereals based on predicting equations, or NIRS calibrations could be a useful tool for the feed industry to optimize diet formulation by reducing the inclusion of protein feedstuffs, thereby decreasing both feed costs and faecal nitrogen (N) losses. In vivo studies with growing pigs have been conducted previously to determine the standardized PCD in barley, rye, triticale and wheat as commonly used grains in pig diets (Rosenfelder et al., 2015; Spindler et al., 2016; Strang et al., 2017, 2016). These digestibility values were used in the present in vitro study to predict standardized pre-caecal CP, and AA digestibility of different genotypes of barley, rye, triticale and wheat determined in pigs from in vitro N solubility values. Furthermore, chemical and physical characteristics of these cereal grains were clustered in categories according to their chemical or physical properties. These categories were further used to predict in vivo PCD values. Another aim of the present study was to predict in vitro N solubility from the same categories of chemical and physical characteristics of barley, corn, oats, rye, triticale and wheat and to develop a NIRS calibration for predicting in vitro N solubility.

2 | MATERIAL AND METHODS

2.1 | Grain samples

For developing prediction equations of PCD of CP and AA from in vitro N solubility, eight different genotypes of barley, rye, triticale and wheat were used. These samples were part of a conglomerate of grain samples (21 barley, 27 corn, 14 oats, 22 rye, 21 triticale and 29 wheat samples) that were all grown under standardized field test conditions on the same site (except corn) and harvested in the same year (dataset A). A comprehensive database on physical and chemical characteristics is available for this dataset (Rodehutscord, & Rückert et al., 2016). The physical and chemical characteristics of this conglomerate were further used for evaluation of prediction equations for in vitro N solubility. Dataset A and additional 120 barley, 111 corn, 120 oats, 120 rye, 119 triticale and 120 wheat samples (dataset B) were used to develop the NIRS calibration.

2.2 | Determination of in vitro nitrogen solubility

In vitro N solubility was measured in 141 barley, 138 corn, 134 oats, 142 rye, 140 triticale and 149 wheat samples (dataset A and B) according to the method of Boisen and Fernández (1995) as described by Jezierny et al. (2010), with slight modifications outlined by Zuber, Maurer, et al. (2016). Modifications included the use of a water bath during incubation steps, and undissolved residues were removed by using fibre bags as filter.

2.3 | Near-infrared spectroscopy

Samples of dataset A were finely ground (vibrating cup mill, PULVERISETTE; Fritsch GmbH, Idar-Oberstein, Germany), and spectral data were recorded in duplicates (680 to 2,500 nm; SpectraStar 2500X, Unity Scientific, Brookfield, CT, USA). Spectral data of a part of dataset B (660 samples; 50 samples were excluded from analysis as no clear allocation of sample numbers was possible) were recorded at LUFA Speyer from 400 to 2,700 nm (Foss NIR Systems 5,000, FOSS 3400 Hilleroed, Denmark) and were truncated to 1,100 to 2,498 nm. Spectral transfer between the instruments, mathematical pre-treatment of the spectra and calibration development was carried out using the Ucal™ software suite (version 3.0.0.23; Unity Scientific, Brookfield, CT, USA). To adapt spectra to the Unity spectrometer, spectral data for in vitro N solubility of 36 randomly chosen samples from dataset B were recorded on both instruments and the spectra were standardized using the transfer function of the Ucal programme.
(piecewise direct standardization (PDS), segment size: 5, number of factors: 1; \( R^2 > 0.99 \), root mean square = 0.0021, bias = 0.0017).

The total of 794 samples (134 and 660 samples of dataset A and B, respectively) was split into a calibration and a validation set prior to the calculation of calibrations. Samples were assigned to the calibration and validation set based on the reference value to achieve the whole range of values in both sets. Calibrations were calculated by partial least square regression after spectral pre-treatment by SNV–detrend. The wavelength segments (1,100–2,500 nm, 1,150–2,450 nm and 1,250–2,450 nm) and the used derivative of the spectra were varied (1st, 2nd or no derivative). The validation dataset was used as independent sample set to assess the performance of the calibrations. The performance of the calibrations was evaluated by the standard error of prediction (SEP), the coefficient of determination of the validation step \( R^2_{\text{Val}} \), and the bias and slope of the validation step.

2.4 | In vivo standardized pre-caecal digestibility data

For prediction of in vivo PCD of CP and essential AA from in vitro N solubility and/or chemical and physical characteristics of cereal grains, eight genotypes each of barley, rye, triticale and wheat were selected from the sample conglomerate of dataset A representing a great variation in CP content within grain species. The corresponding in vivo data were extracted from previous studies with growing pigs for barley (Spindler et al., 2016), rye (Strang et al., 2016), triticale (Strang et al., 2017) and wheat (Rosenfelder et al., 2015). In brief, for the determination of these PCD values, four experiments (one for each grain species) were conducted with eight (rye/triticale) or nine (barley/wheat) barrows each, fitted with simple ileal T-cannulas. The experiments were conducted as row–column designs (barley/wheat) or Latin Square designs (rye/triticale) with eight periods and eight diets each. The assay diets included 95% of the respective grain genotype as the sole dietary source of CP and AA and were supplemented with plant oil, a vitamin and mineral premix, and with titanium dioxide as an indigestible marker. Experimental periods comprised 4 (barley, rye and wheat) or 5 d (triticale) for adaptation followed by 2 d for ileal digesta collection. The daily feed intake amounted to 4% of pigs’ average BW, corresponding to about 3 times the animals’ energy requirement for maintenance (106 kcal of ME [as-fed basis]/kg of metabolic BW; NRC, 1998).

2.5 | Statistical analysis

For in vitro N solubility, homogeneity of variances and normal distribution of the data were confirmed and outliers were detected by analysis of the residuals, using the UNIVARIATE and REG procedures of SAS (SAS, 2008) respectively. The MEANS procedure was used to determine mean, min and max values as well as standard deviation and coefficient of variation (CV). Linear regression analysis between in vitro N solubility and PCD of CP and essential AA was performed using the GLM procedure of SAS. The model was \( Y = a X + b \), where \( Y \) = in vivo PCD of CP and essential AA, \( a \) = slope, \( X \) = in vitro N solubility and \( b \) = intercept. Homogeneity of the slopes and the intercepts of the linear regression were tested for the grain species. The corresponding model for analysis of covariance included grain species as a covariate. Multiple regression analysis was performed using a stepwise approach implemented in the REG procedure of SAS. Selection criterion was the \( P \)-value of an \( F \) test. Significance was determined at \( p < .05 \). The analysis aimed to predict PCD of CP or essential AA determined in pigs or in vitro N solubility from chemical and physical characteristics of different grain genotypes. For this, the analysed variables were pooled according to their characteristics. Prediction equations for the in vivo PCD of CP and each essential AA or in vitro N solubility were calculated using each of the pools. Variables were classified as significant predictors at \( p \leq .10 \) in the multiple regression analysis. Calculated equations were assessed based on the adjusted \( R^2 \).

3 | RESULTS AND DISCUSSION

3.1 | Variability of in vitro nitrogen solubility in different grain species

In vitro N solubility of all grain samples is presented in Table 1, and mean values ranged from 75.9% for rye to 86.3% for wheat. Variability in in vitro N solubility was lowest in wheat, with values ranging from 81.3% to 90.3% (CV: 2.3%) and greatest in rye, with values ranging from 68.6% to 83.4% (CV: 4.2%). The lowest in vitro N solubility value could be observed for rye and amounted to 68.6%.

| Barley | Corn | Oats | Rye | Triticale | Wheat |
|--------|------|------|-----|-----------|-------|
| Mean   | 80.8 | 82.1 | 80.7| 75.9      | 84.4  |
| Min    | 73.7 | 74.3 | 75.3| 68.6      | 79.1  |
| Max    | 85.6 | 89.0 | 85.6| 83.4      | 89.3  |
| SD     | 2.3  | 3.1  | 2.2 | 3.2       | 2.2   |
| CV     | 2.8  | 3.8  | 2.7 | 4.2       | 2.6   |

Abbreviations: SD, standard deviation; CV, coefficient of variation.

TABLE 1 In vivo standardized pre-caecal digestibility data

In different grain species
3.2 | Predicting standardized pre-caecal crude protein and essential amino acid digestibility from in vitro nitrogen solubility and/or chemical and physical characteristics of different cereal grains

In Table 2, in vitro N solubility values and corresponding in vivo PCD of CP and essential AA of the genotypes used in the pig experiments are summarized. In vitro N solubility ranged from 80.7% to 83.6% in barley, 75.1 to 80.9% in rye, 82.5 to 86.1% in triticale and 83.5 to 85.9% in wheat, whereas in vivo PCD of CP determined in growing pigs amounted to 69 to 74% in barley, 70 to 74% in rye, 81 to 85% in triticale and 83 to 87% in wheat (Rosenfelder et al., 2015; Spindler et al., 2016; Strang et al., 2017, 2016). Similarly, for laying hens, greater digestibility values were obtained for wheat than for rye (Rodehutscord, & Krieg et al., 2016). Linear regression analysis of PCD of CP from in vitro N solubility for each cereal grain species resulted in $R^2 = -0.16$, $R^2 = 0.35$, $R^2 < 0.01$ and $R^2 = 0.33$ for barley, rye, triticale and wheat respectively (data not shown).

**TABLE 2** In vitro nitrogen solubility and standardized pre-caecal digestibility of crude protein and essential amino acids determined in growing pigs used for prediction equations

| Grain species | Genotype | In vitro nitrogen solubility (%) | Standardized pre-caecal digestibility (%) | Difference$^b$ |
|---------------|----------|---------------------------------|------------------------------------------|--------------|
| Barley        | Yool     | 83.6                            | 71 79 77 74 76 64 76 75 71 68 76          | 12.6         |
|               | Travira  | 81.3                            | 74 80 79 78 79 65 79 77 72 72 79          | 7.3          |
|               | Lomerit  | 81.5                            | 73 78 78 77 77 61 77 76 71 69 77          | 8.5          |
|               | Campanile| 80.7                            | 69 77 76 72 75 62 74 72 68 68 75          | 11.7         |
|               | Canberra | 82.3                            | 72 79 78 77 77 63 78 77 70 69 78          | 10.3         |
|               | Anisette | 82.2                            | 74 82 79 77 78 66 78 78 72 72 78          | 8.2          |
|               | Metaxa   | 81.4                            | 74 81 79 76 78 65 78 79 72 72 78          | 7.4          |
|               | Fridericus | 80.7                        | 73 79 79 75 78 64 77 79 72 72 77          | 7.7          |
| Rye           | Conduct  | 80.8                            | 74 79 77 74 76 65 78 80 66 67 74          | 6.8          |
|               | Visello  | 75.6                            | 73 78 75 72 74 64 75 78 64 65 72          | 2.6          |
|               | Helltop  | 80.9                            | 73 78 76 74 75 63 76 77 65 66 73          | 7.9          |
|               | Bellami  | 75.7                            | 70 75 74 70 73 61 75 76 62 63 71          | 5.7          |
|               | Palazzo  | 76.1                            | 72 76 73 72 73 62 74 77 64 65 72          | 4.1          |
|               | Dukato   | 79.7                            | 73 76 74 73 74 63 75 78 63 65 71          | 6.7          |
|               | Guttino  | 75.1                            | 72 76 74 71 73 60 75 78 64 65 71          | 3.1          |
|               | Dankowski| 80.5                            | 73 76 73 71 73 60 75 78 62 63 71          | 7.5          |
| Triticale     | Grenado  | 84.0                            | 81 84 83 81 83 72 84 82 73 80 80          | 3.0          |
|               | Tarzan   | 86.1                            | 85 88 86 85 86 76 87 86 77 82 84          | 1.1          |
|               | HYT Prime| 84.1                            | 83 85 83 83 84 74 85 85 75 81 81          | 1.1          |
|               | Massimo  | 84.6                            | 84 86 85 85 85 75 86 86 77 83 83          | 0.6          |
|               | Cultivo  | 82.5                            | 84 86 84 84 85 73 85 84 75 80 82          | -1.5         |
|               | SW Talentro | 84.0                        | 83 86 85 83 84 75 85 84 74 80 82          | 1.0          |
|               | Cando    | 83.6                            | 83 85 85 83 85 73 85 84 75 81 82          | 0.6          |
|               | Agostino | 83.3                            | 83 83 83 83 84 73 84 84 75 79 81          | 0.3          |
| Wheat         | Skalmeje | 84.7                            | 84 85 86 86 86 71 85 86 80 81 85          | 0.7          |
|               | Tommi    | 84.5                            | 84 84 86 86 86 69 84 87 78 80 84          | 0.5          |
|               | Tobak    | 85.1                            | 84 86 87 86 86 69 85 87 78 83 85          | 1.1          |
|               | Event    | 85.4                            | 87 88 88 88 88 73 87 89 82 85 88          | -1.6         |
|               | Mulan    | 83.5                            | 83 85 86 84 85 69 85 85 78 81 84          | 0.5          |
|               | Tabasco  | 84.8                            | 83 86 86 86 85 71 86 86 78 81 85          | 1.8          |
|               | Adler    | 85.9                            | 85 86 87 88 87 73 88 87 81 83 86          | 0.9          |
|               | KWS Erasmus | 84.9                        | 85 85 86 87 86 74 86 79 82 85          | -0.1         |

$^a$Values for standardized pre-caecal digestibility obtained for barley from Spindler et al. (2016), for rye from Strang et al. (2016), for triticale from Strang et al. (2017) and for wheat from Rosenfelder et al. (2015).

$^b$Differences between in vitro nitrogen solubility and in vivo standardized pre-caecal digestibility of crude protein in cereal grains (percentage units).
### Table 3: Analysis of covariance between standardized pre-caecal crude protein and essential amino acid digestibility determined in growing pigs and in vitro nitrogen solubility in cereal grains

|       | Barley | Rye | Triticale | Wheat |
|-------|--------|-----|-----------|--------|
|       | n = 8  | n = 8 | n = 8     | n = 8  |
| Slope ± SE | p-value<sup>a</sup> | Intercept ± SE | p-value<sup>b</sup> | Intercept ± SE | p-value<sup>c</sup> | Intercept ± SE | p-value<sup>d</sup> | Intercept ± SE | p-value<sup>e</sup> | R²     |
| CP    | 0.33 ± 0.16 | .053 | 45.8 ± 13.2 | .002 | 47.0 ± 12.6 | .001 | 55.8 ± 13.6 | <.001 | 56.7 ± 13.7 | <.001 | .96   |
| Arg   | 0.36 ± 0.16 | .036 | 49.6 ± 13.5 | .001 | 48.4 ± 12.9 | <.001 | 54.8 ± 13.8 | <.001 | 54.8 ± 14.0 | <.001 | .91   |
| His   | 0.26 ± 0.13 | .061 | 56.8 ± 10.9 | <.001 | 54.1 ± 10.4 | <.001 | 62.3 ± 11.2 | <.001 | 64.4 ± 11.3 | <.001 | .96   |
| Ile   | 0.44 ± 0.17 | .017 | 39.7 ± 14.1 | .009 | 37.7 ± 13.5 | .010 | 46.3 ± 14.5 | .004 | 48.9 ± 14.7 | .003 | .95   |
| Leu   | 0.27 ± 0.13 | .040 | 55.0 ± 10.3 | <.001 | 52.6 ± 9.8  | <.001 | 61.6 ± 10.6 | <.001 | 63.0 ± 10.7 | <.001 | .97   |
| Lys   | 0.41 ± 0.20 | .053 | 30.1 ± 16.6 | <.011 | 30.1 ± 15.9 | .068 | 39.3 ± 17.1 | .029 | 36.2 ± 17.2 | .045 | .91   |
| Met   | 0.37 ± 0.14 | .016 | 47.2 ± 11.7 | <.001 | 46.8 ± 11.1 | <.001 | 54.3 ± 12.0 | <.001 | 54.7 ± 12.1 | <.001 | .95   |
| Phe   | 0.27 ± 0.19 | .162 | 54.3 ± 15.5 | .002 | 56.5 ± 14.8 | <.001 | 61.5 ± 16.0 | <.001 | 63.5 ± 16.1 | <.001 | .90   |
| Thr   | 0.28 ± 0.17 | .122 | 48.5 ± 14.1 | .002 | 42.3 ± 13.4 | .004 | 52.0 ± 14.5 | .001 | 55.9 ± 14.6 | <.001 | .95   |
| Trp   | 0.23 ± 0.19 | .244 | 51.7 ± 15.5 | .003 | 47.2 ± 14.8 | .004 | 61.7 ± 16.0 | <.001 | 62.8 ± 16.1 | <.001 | .96   |
| Val   | 0.29 ± 0.14 | .051 | 53.2 ± 11.8 | <.001 | 48.9 ± 11.3 | <.001 | 57.1 ± 12.1 | <.001 | 60.3 ± 12.2 | <.001 | .96   |

<sup>a</sup>P-values of the estimates for the slopes of the regression equations.

<sup>b</sup>P-values of the estimates for the intercepts of the regression equations for barley.

<sup>c</sup>P-values of the estimates for the intercepts of the regression equations for rye.

<sup>d</sup>P-values of the estimates for the intercepts of the regression equations for triticale.

<sup>e</sup>P-values of the estimates for the intercepts of the regression equations for wheat.
### TABLE 4  Description of pools of variables used for prediction of standardized pre-caecal crude protein and essential amino acid digestibility of barley, rye, triticale and wheat and in vitro nitrogen solubility of barley, oats, corn, rye, triticale, wheat and a sum of these grain species from chemical and physical characteristics

| Pool | Variable |
|------|----------|
| 1    | Physical properties, proximate nutrients and gross energy (g/kg DM, unless otherwise stated): thousand seed weight (g), test weight (kg/HL), falling number (s), ash, crude protein, crude fibre, crude fat, starch, neutral detergent fibre, acid detergent fibre, acid detergent lignin, gross energy (MJ/kg DM) |
| 2    | Amino acids (g/kg DM): Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val |
| 3    | Amino acids (g/16 g N): Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val |
| 4    | Minerals (mg/kg DM, unless otherwise stated): calcium, iron, magnesium, manganese, potassium, sodium, phosphorus, zinc, copper, inositol phosphate (g/100 g DM) |
| 5    | Non-starch polysaccharides (g/kg DM): cellulose (insoluble and total), arabinoxylans (insoluble, soluble and total), mixed-linkage (1 → 3; 1 → 4)-β-glucans (insoluble, soluble and total), arabinose (insoluble and total), xylose (insoluble and total), mannose (insoluble and total), galactose (insoluble and total), glucose (insoluble and total), uronic acids (insoluble and total), total non-starch polysaccharides (insoluble, soluble and total), dietary fibre (insoluble, soluble and total) |
| 6    | Combination of all variables listed above |
|      | Including in vitro nitrogen solubility as variable |

*Only for prediction of standardized pre-caecal crude protein and essential amino acid digestibility.

### TABLE 5  Adjusted coefficients of determination (adjusted $R^2$) for predicting standardized pre-caecal crude protein and essential amino acid digestibility of barley from different pools of variables with or without considering in vitro nitrogen solubility

| Pool | Variables | CP | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Trp | Val |
|------|-----------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1    | Linear    | 0  | 0.42| 0.48| 0.37| 0.55| 1.00| 0.32| 1.00| 0.49| 0.73| 1.00|
|      | Linear + quadratic | 0.29| 0.42| 0.48| 0.37| 1.00| 1.00| 0.33| 1.00| 0.51| 0.73| 1.00|
| 1a   | Linear    | 0  | 0.42| 0.48| 0.37| 0.55| 1.00| 0.32| 1.00| 0.49| 0.73| 1.00|
|      | Linear + quadratic | 0.29| 0.42| 0.48| 0.37| 1.00| 1.00| 0.33| 1.00| 0.51| 0.73| 1.00|
| 2    | Linear    | 0  | 0   | 0   | 0   | 0   | 0   | 0.53| 0   | 0.51| 0   | 0.51|
|      | Linear + quadratic | 0.32| 0   | 0   | 0   | 0   | 0.53| 0   | 0.51| 0   | 0.51| 0   |
| 2a   | Linear    | 0  | 0   | 0   | 0   | 0   | 0   | 0.53| 0   | 0.51| 0   | 0.51|
|      | Linear + quadratic | 0.32| 0   | 0   | 0   | 0.53| 0   | 0.51| 0   | 0.51| 0   | 0.51|
| 3    | Linear    | 0.70| 0   | 0.73| 0.79| 0.32| 0   | 0.86| 0.92| 0.82| 0.33| 0.81|
|      | Linear + quadratic | 0.72| 0.73| 0.79| 0.32| 0.86| 1.00| 0.93| 0.60| 0.89| 0.89| 0.89|
| 3a   | Linear    | 0.70| 0   | 0.95| 1.00| 0.32| 0   | 0.98| 1.00| 0.82| 0.33| 0.81|
|      | Linear + quadratic | 0.72| 0.95| 1.00| 0.32| 0.98| 1.00| 0.82| 0.33| 0.81| 0.89| 0.89|
| 4    | Linear    | 0.42| 1.00| 0.69| 0.33| 0.79| 0.71| 0.72| 0.59| 0.76| 0.85| 0   |
|      | Linear + quadratic | 0.44| 0.98| 0.69| 0.35| 0.85| 0.74| 0.88| 0.78| 0.77| 0.85| 0   |
| 4a   | Linear    | 0.42| 1.00| 0.69| 0.33| 0.79| 0.71| 0.72| 0.59| 0.76| 0.85| 0   |
|      | Linear + quadratic | 0.44| 1.00| 0.69| 0.35| 0.85| 0.74| 0.88| 0.78| 0.77| 0.85| 0   |
| 5    | Linear    | 0.61| 0.82| 1.00| 1.00| 0.67| 0.93| 1.00| 0.54| 0.79| 0.46| 0.96|
|      | Linear + quadratic | 1.00| 0.82| 1.00| 1.00| 0.67| 0.93| 1.00| 0.54| 0.79| 0.46| 0.96|
| 5a   | Linear    | 0.61| 0.82| 1.00| 1.00| 0.84| 0.99| 1.00| 0.54| 0.88| 0.46| 0.96|
|      | Linear + quadratic | 1.00| 0.82| 1.00| 1.00| 0.84| 0.99| 1.00| 0.54| 0.88| 0.46| 0.96|
| 6    | Linear    | 0.61| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00|
|      | Linear + quadratic | 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00|
| 6a   | Linear    | 0.61| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 0.95| 0.85| 1.00|
|      | Linear + quadratic | 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 1.00| 0.95| 0.85| 1.00|
shown). Therefore, in vitro N solubility did not provide direct estimates of in vivo PCD of CP in the assayed grains. Only for triticale and wheat, poor linear relationships between in vivo PCD values of essential AA and in vitro N solubility ($R^2 = 0.48$ and $0.50$ for Lys and Met in triticale, respectively, and $R^2 = 0.84$, $0.46$ and $0.46$ for Ile, Leu and Met in wheat respectively; data not shown) were found. In most cases, in vitro N solubility values were greater than the corresponding PCD values, which have also been reported by Jezierny et al. (2010) for grain legumes. These authors concluded that in vitro methods lack interactions with the digestive tract of the animal including digesta transit and microbial activity in digesta. Moreover, part of the feed proteins may be digested in the ileum but not absorbed by the intestinal wall because of detrimental physical conditions, such as high viscosity and increased osmolarity in ileal digesta. In vivo, the digested but not absorbed part of N is recognized as undigested protein, while in vitro all solubilized N is regarded as being digested (Cone & Poel, 1993). In the present study, differences in in vitro N solubility and corresponding PCD of CP increased with decreasing in vivo PCD of CP values ($R^2 = 0.77$). Differences between in vitro N solubility and in vivo PCD of CP amounted to 7.3 to 12.6%-units, 2.6 to 7.9%-units, −1.5 to 3.0%-units and −1.6 to 1.8%-units for barley, rye, triticale and wheat respectively. This may, at least in part, be explained by various fibre fractions present in cereal grains. The fibre concentration (e.g. NSP) was higher in barley and rye when compared to triticale and wheat (Rodehutscord. & Rückert et al., 2016). This may have reduced digestibility in vivo due to an increase in digesta viscosity (Bedford & Schulze, 1998), but with much smaller effects on in vitro digestibility.

In a next step, grain species was used as a covariate in a linear model. The homogeneity of the slopes and the intercepts between barley, rye, triticale and wheat in the linear regression model between in vivo (PCD of CP and essential AA) and in vitro (N solubility) were tested. The slopes of the linear relationship between in vivo PCD of CP and AA determined in pigs, and in vitro N solubility did not differ between grain species ($p > .05$), except for Arg, Ile, Leu and Met, whereas all intercepts differed ($p < .05$; Table 3). However, it remains open, why the slopes for the AA Arg, Ile, Leu and Met differed. With grain species as a covariate, there were strong linear relationships ($p < .05$) between in vivo PCD of CP and essential AA and in vitro N solubility. The coefficients of linear determination ranged between $R^2 = 0.90$ and $R^2 = 0.97$ for Phe and Leu respectively. These observations are in line with previous reports in grain legumes (faba beans, field peas and lupins), where

| Pool | Variables | CP | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Trp | Val |
|------|-----------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1    | Linear    | 0  | 0   | 0.31| 0.42| 1.00| 0.88| 0.87| 0   | 0.34| 0.40| 0   |
|      | Linear + quadratic | 0  | 0   | 0.31| 0.42| 1.00| 0.88| 0.79| 0   | 0.34| 0.40| 0   |
| 1a   | Linear    | 0.35| 0   | 0.31| 0.42| 1.00| 0.88| 0.87| 0   | 0.34| 0.87| 0   |
|      | Linear + quadratic | 0.35| 0   | 0.31| 0.42| 1.00| 0.88| 0.79| 0   | 0.34| 0.87| 0   |
| 2    | Linear    | 0.30| 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0.91| 0   | 0   |
|      | Linear + quadratic | 0.81| 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0.93| 0   | 0   |
| 2a   | Linear    | 0.35| 0   | 0   | 1.00| 0.62| 0   | 0   | 0   | 0.91| 0   | 0   |
|      | Linear + quadratic | 0.35| 0   | 0   | 1.00| 0.69| 0   | 0   | 0   | 0.93| 0   | 0   |
| 3    | Linear    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0.77| 0.61| 0   | 0   |
|      | Linear + quadratic | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0.77| 0.61| 0   | 0   |
| 3a   | Linear    | 0.89| 0   | 0   | 1.00| 0.70| 0   | 0   | 0   | 0.77| 0.61| 0   |
|      | Linear + quadratic | 0.89| 0   | 0   | 0.99| 1.00| 0   | 0   | 0   | 0.77| 0.61| 0   |
| 4    | Linear    | 0.45| 0.96| 0.42| 0   | 0.34| 0.74| 0   | 0.53| 0   | 0.32| 0.32|
|      | Linear + quadratic | 0.45| 0.99| 0.68| 0   | 0.34| 0.73| 0.29| 0.56| 0   | 0   | 0.61|
| 4a   | Linear    | 0.45| 0.96| 0.42| 0.99| 0.34| 0.74| 0   | 0.53| 0   | 0   | 0.32|
|      | Linear + quadratic | 0.45| 0.99| 0.68| 0.99| 0.36| 0.74| 0.29| 0.56| 0   | 0   | 0.61|
| 5    | Linear    | 0.81| 0.64| 0.61| 1.00| 0.98| 0.36| 0   | 0   | 0.64| 0.47| 0.76|
|      | Linear + quadratic | 0.94| 0.64| 0.65| 1.00| 1.00| 0.38| 0.94| 0   | 0.64| 0.47| 0.76|
| 5a   | Linear    | 0.89| 0.64| 0.61| 1.00| 0.98| 0.36| 0   | 0   | 0.64| 0.47| 0.76|
|      | Linear + quadratic | 0.94| 0.64| 0.65| 1.00| 1.00| 0.38| 0.94| 0   | 0.64| 0.47| 0.76|
| 6    | Linear    | 1.00| 0.96| 1.00| 1.00| 1.00| 0.84| 1.00| 1.00| 1.00| 0.87| 0.87|
|      | Linear + quadratic | 1.00| 1.00| 1.00| 1.00| 1.00| 0.84| 1.00| 1.00| 1.00| 0.87| 0.87|
| 6a   | Linear    | 1.00| 0.96| 1.00| 1.00| 1.00| 0.84| 1.00| 1.00| 1.00| 0.87| 0.87|
|      | Linear + quadratic | 1.00| 1.00| 1.00| 1.00| 1.00| 0.84| 1.00| 1.00| 1.00| 0.87| 0.87|

**Table 6** Adjusted coefficients of determination (adjusted $R^2$) for predicting standardized pre-caecal crude protein and essential amino acid digestibility of rye from different pools of variables with or without considering in vitro nitrogen solubility.
in vitro prediction of PCD of AA in grain legume cultivars for growing pigs depended on type of grain legume as a covariate as well (Jezierny et al., 2010).

Results of the multiple linear regression analysis for predicting in vivo PCD of CP and essential AA from a pool of physical and chemical characteristics as well as in vitro N solubility (Table 4) are presented in Table 5, 6, 7 and 8 for barley, rye, triticale and wheat, respectively. The $R^2$ of the calculated equations using the same pool of variables varied among CP and all essential AA. The inclusion of in vitro N solubility as a variable and the use of Pool 6 (including all analysed variables) increased $R^2$ in many cases. Thus, the accuracy of prediction equations appears to be strongly dependent on the number of variables included in the statistical model. The linear plus quadratic approach seems to be more useful than the linear approach alone. There is evidence that common analytical methods such as proximate nutrient analysis and determination of physical characteristics (Pool 1) may allow for in vivo prediction of PCD for some AA present in barley, rye and wheat. For practical approaches, other pools are not suitable as they contain variables such as single AA or NSP that are too expensive and time-consuming to analyse. However, as results have proven to be rather inconsistent, it is not possible to make a conclusive recommendation on the use of investigated variables for predicting in vivo PCD. This conclusion is in accordance with Zuber and Rodehutscord (2016), Zuber, Maurer, et al. (2016), and Zuber, Miedaner, Miedaner, Rosenfelder, and Rodehutscord (2016), who also failed to establish multiple regression equations with adequate accuracy for routine applications to predict in vivo AA digestibility of wheat, triticale and rye in laying hens, based on chemical and physical characteristics or in vitro N solubility. It needs to be emphasized that both in the present study and the aforementioned studies, all cereals grains were grown under identical conditions, with low variation in nutritional composition among grains.

### 3.3 Predicting in vitro nitrogen solubility from chemical and physical characteristics or by NIRS analysis

Adjusted coefficients of determination for predicting in vitro N solubility from different pools of variables are presented in Table 9. Pool 1 (physical properties, proximate nutrients, energy) may be

| Pool | Variables | CP | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Trp | Val |
|------|------------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1    | Linear     | 0.54 | 0.31 | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
|      | Linear + quadratic | 0.85 | 0.31 | 1.00 | 0.68 | 0   | 0   | 0   | 0.32 | 0   | 0   | 0   |
| 1a   | Linear     | 0.92 | 0.61 | 0   | 0   | 0.10 | 0.50 | 0   | 0.35 | 0   | 0   | 0   |
|      | Linear + quadratic | 0.66 | 0.61 | 1.00 | 1.00 | 0   | 1.00 | 0.98 | 1.00 | 0.36 | 0   | 0   |
| 2    | Linear     | 0.60 | 0.37 | 0   | 0.54 | 0.68 | 0.28 | 0   | 0.38 | 0   | 0   | 0.33 |
|      | Linear + quadratic | 0.60 | 0.39 | 0   | 0.55 | 1.00 | 0.28 | 0.30 | 0.40 | 0   | 0   | 0.34 |
| 2a   | Linear     | 0.60 | 0.76 | 0   | 0.54 | 0.91 | 1.00 | 0.99 | 0.70 | 0.35 | 0.89 |
|      | Linear + quadratic | 0.60 | 0.76 | 0   | 0.55 | 1.00 | 1.00 | 1.00 | 0.89 | 0.36 | 0.96 |
| 3    | Linear     | 0.43 | 0.31 | 0   | 0.35 | 0.94 | 0   | 0   | 0   | 0   | 0   | 0   |
|      | Linear + quadratic | 0.43 | 0.31 | 0   | 0.35 | 0.94 | 0   | 0   | 0   | 0   | 0   | 0   |
| 3a   | Linear     | 0.64 | 0.86 | 0   | 0.35 | 0.94 | 1.00 | 0.94 | 0   | 0.35 | 0   |
|      | Linear + quadratic | 0.64 | 1.00 | 0   | 0.57 | 0.94 | 1.00 | 0.94 | 0   | 0.36 | 0   |
| 4    | Linear     | 0   | 0.66 | 0   | 0   | 0.33 | 0.39 | 0   | 0   | 0.33 | 0   |
|      | Linear + quadratic | 0   | 0.68 | 0   | 0   | 0.33 | 0.44 | 0   | 0   | 0.33 | 0   |
| 4a   | Linear     | 0   | 0.66 | 0   | 0   | 0   | 0.33 | 0.82 | 0.68 | 0   | 0   | 0.35 |
|      | Linear + quadratic | 0   | 0.68 | 0   | 0   | 0.33 | 0.82 | 0.69 | 0   | 0   | 0.36 | 0   |
| 5    | Linear     | 0.96 | 0.89 | 0.34 | 1.00 | 1.00 | 0.68 | 0.79 | 0.37 | 0.51 | 0.34 | 0.54 |
|      | Linear + quadratic | 0.99 | 0.89 | 0.34 | 0.95 | 0.98 | 0.68 | 0.92 | 0.37 | 0.51 | 0.34 | 0.71 |
| 5a   | Linear     | 0.96 | 0.89 | 0.34 | 1.00 | 1.00 | 0.68 | 0.79 | 0.99 | 0.86 | 0.76 | 0.54 |
|      | Linear + quadratic | 0.99 | 0.89 | 0.34 | 0.95 | 0.98 | 0.68 | 0.92 | 0.99 | 1.00 | 0.77 | 0.71 |
| 6    | Linear     | 1.00 | 1.00 | 0.84 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.51 | 0.34 | 1.00 |
|      | Linear + quadratic | 0.98 | 1.00 | 0.85 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.51 | 0.34 | 1.00 |
| 6a   | Linear     | 1.00 | 1.00 | 0.84 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
|      | Linear + quadratic | 0.98 | 1.00 | 0.85 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
used for predicting in vitro N solubility in rye. This might be due to a greater variability in in vitro N solubility and variables in rye when compared to other grain species. The rye genotypes that were used in the present project exhibited a wider range in nutrient content than genotypes of the other grain species, maybe due to different breeding strategies. Using Pool 5 (NSP content) for

### TABLE 8

| Pool  | Variables | CP   | Arg  | His  | Ile  | Leu  | Lys  | Met  | Phe  | Thr  | Trp  | Val  |
|-------|-----------|------|------|------|------|------|------|------|------|------|------|------|
| 1     | Linear    | 0.57 | 0.72 | 0.96 | 0.73 | 0.59 | 0.97 | 0.94 | 0.95 | 0.41 | 1.00 | 0.54 |
|       | Linear + quadratic | 0.57 | 0.74 | 0.96 | 0.73 | 0.60 | 1.00 | 0.95 | 0.97 | 0.99 | 1.00 | 0.59 |
| 1a    | Linear    | 0.57 | 0.72 | 0.96 | 0.84 | 0.59 | 0.97 | 0.83 | 0.95 | 0.41 | 1.00 | 1.00 |
|       | Linear + quadratic | 0.57 | 0.74 | 0.96 | 0.85 | 0.60 | 1.00 | 0.96 | 0.97 | 0.99 | 1.00 | 1.00 |
| 2     | Linear    | 0   | 0   | 0   | 0   | 0.98 | 0.81 | 0   | 0   | 0   | 0   | 0   |
|       | Linear + quadratic | 0   | 0   | 0   | 0   | 0.97 | 0.81 | 0   | 0   | 0   | 0   | 0   |
| 2a    | Linear    | 0.33 | 0   | 0.32 | 0.84 | 0.46 | 0.29 | 0.46 | 0.37 | 0.34 | 0.34 | 0.40 |
|       | Linear + quadratic | 0.33 | 0   | 0.79 | 0.85 | 0.46 | 0.31 | 0.46 | 0.37 | 0.34 | 0.69 | 0.40 |
| 3     | Linear    | 0   | 0   | 0   | 0   | 0   | 1.00 | 0   | 0   | 0   | 0   | 0   |
|       | Linear + quadratic | 0   | 0   | 0   | 0   | 0   | 1.00 | 0   | 0   | 0   | 0   | 0   |
| 3a    | Linear    | 0.33 | 0   | 0.32 | 0.84 | 0.46 | 0.29 | 0.46 | 0.37 | 0.34 | 0.34 | 0.40 |
|       | Linear + quadratic | 0.33 | 0   | 0.79 | 0.85 | 0.46 | 0.31 | 0.46 | 0.37 | 0.34 | 0.69 | 0.40 |
| 4     | Linear    | 0.72 | 0   | 0.00 | 0.94 | 0.81 | 0.99 | 0.41 | 0.99 | 0.85 | 0.41 | 0.39 |
|       | Linear + quadratic | 0.73 | 0   | 0.95 | 0.97 | 0.91 | 1.00 | 0.84 | 0.98 | 0.86 | 0.43 | 0.42 |
| 4a    | Linear    | 0.72 | 0   | 0.72 | 0.91 | 0.81 | 1.00 | 0.46 | 1.00 | 0.85 | 0.41 | 0.99 |
|       | Linear + quadratic | 0.73 | 0   | 0.99 | 0.92 | 0.91 | 1.00 | 0.46 | 1.00 | 0.86 | 0.43 | 0.42 |
| 5     | Linear    | 0   | 0.42 | 1.00 | 0   | 0   | 0   | 0   | 0   | 0.45 | 0   | 0   |
|       | Linear + quadratic | 0   | 0.77 | 0.68 | 0   | 0   | 0   | 0   | 0   | 0.45 | 0   | 0   |
| 5a    | Linear    | 0.59 | 0.88 | 1.00 | 0.93 | 0.94 | 0.97 | 0.46 | 0.91 | 1.00 | 1.00 | 0.40 |
|       | Linear + quadratic | 0.59 | 0.95 | 1.00 | 0.94 | 0.96 | 1.00 | 0.46 | 0.99 | 1.00 | 0.69 | 0.40 |
| 6     | Linear    | 1.00 | 1.00 | 1.00 | 0.93 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
|       | Linear + quadratic | 1.00 | 0.98 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 6a    | Linear    | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
|       | Linear + quadratic | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

### TABLE 9

| Pool  | Variables | Barley | Oats<sup>a</sup> | Corn<sup>b</sup> | Rye | Triticale | Wheat | All grains |
|-------|-----------|--------|-----------------|-----------------|-----|-----------|-------|------------|
| 1     | Linear    | 0.36   | 0.15            | 0               | 0.74 | 0.47     | 0.30  | 0.79       |
|       | Linear + quadratic | 0.36   | 0.15            | 0               | 0.74 | 0.64     | 0.36  | 0.79       |
| 2     | Linear    | 0.20   | 0               | 0.54            | 0.71 | 0.38     | 0     | 0.57       |
|       | Linear + quadratic | 0.21   | 0               | 0.54            | 0.92 | 0.38     | 0     | 0.65       |
| 3     | Linear    | 0.14   | 0.41            | 0.47            | 0.69 | 0.43     | 0.29  | 0.58       |
|       | Linear + quadratic | 0.29   | 0.49            | 0.48            | 0.74 | 0.72     | 0.29  | 0.59       |
| 4     | Linear    | 0.09   | 0               | 0.55            | 0.12 | 0.13     | 0     | 0.56       |
|       | Linear + quadratic | 0.10   | 0               | 0.59            | 0.12 | 0.13     | 0     | 0.61       |
| 5     | Linear    | 0.96   | -               | -               | 0.56 | 0.42     | 0.42  | 0.84       |
|       | Linear + quadratic | 0.98   | -               | -               | 0.85 | 0.43     | 0.65  | 0.85       |
| 6     | Linear    | 1.00   | 0.89            | 0.94            | 1.00 | 0.59     | 1.00  | 0.85       |
|       | Linear + quadratic | 1.00   | 0.94            | 0.98            | 1.00 | 0.60     | 1.00  | 0.95       |

<sup>a</sup>Pool 5 was excluded from analysis as no data were available.

<sup>b</sup>Falling number, acid detergent lignin, potassium and Pool 5 were excluded from analysis as no data were available.
| Settings | Calibration | Cross-Validation | Validation |
|----------|-------------|------------------|------------|
| Wavelength (nm) | Math (D,G,S) | Samples Used/rejected | Variation | SEC | SECV | Variation | SEP | R | Bias (%) DM | Slope | Intercept |
| 1,100–2,498 | 0,8,8 | 12 | 584/08 | 71.8–91.1 | 1.71 | 0.82 | 1.80 | 0.80 | 71.8–90.8 | 2.09 | 0.77 | −0.17 | 1.00 | −0.34 |
| 1,100–2,498 | 1,8,8 | 10 | 580/12 | 1.61 | 0.84 | 1.66 | 0.82 | 1.97 | 0.80 | −0.13 | 1.00 | −0.26 |
| 1,100–2,498 | 2,8,8 | 8 | 580/12 | 1.64 | 0.84 | 1.68 | 0.80 | 1.94 | 0.80 | −0.14 | 1.00 | −0.27 |
| 1,150–2,448 | 0,8,8 | 13 | 580/12 | 1.64 | 0.84 | 1.70 | 0.82 | 2.01 | 0.79 | −0.17 | 1.00 | −0.33 |
| 1,150–2,448 | 1,8,8 | 9 | 578/14 | 1.62 | 0.84 | 1.67 | 0.81 | 1.93 | 0.80 | −0.14 | 1.00 | −0.28 |
| 1,150–2,448 | 2,8,8 | 8 | 578/14 | 1.63 | 0.84 | 1.68 | 0.81 | 1.94 | 0.80 | −0.14 | 1.00 | −0.28 |
| 1,250–2,448 | 0,8,8 | 13 | 580/12 | 1.64 | 0.84 | 1.70 | 0.82 | 2.01 | 0.79 | −0.17 | 1.00 | −0.33 |
| 1,250–2,448 | 1,8,8 | 9 | 578/14 | 1.62 | 0.84 | 1.67 | 0.81 | 1.93 | 0.80 | −0.14 | 1.00 | −0.28 |
| 1,250–2,448 | 2,8,8 | 8 | 578/14 | 1.63 | 0.84 | 1.68 | 0.81 | 1.94 | 0.80 | −0.14 | 1.00 | −0.28 |

Note: Abbreviations: SEC, standard error of calibration; SECV, standard error of cross-validation; SEP, standard error of prediction.

Mathematical pre-treatment of the spectra, D = derivation order, G = derivation gap, S = derivation smooth.

Factors used for prediction.

Variation of the reference data.

Coefficient of variation.
predicting in vitro N solubility might be useful in barley; however, analysis of different NSP fractions is expensive and labour-intensive. Using all analysed variables (Pool 6) for calculating prediction equations resulted in highest $R^2$ due to the great number of variables used. However, the relationships between some of these variables and the in vitro N solubility are not always causal, and a physiological background is frequently missing. It appears that these relationships reflect statistical artefacts. Consequently, it might be more useful to analyse in vitro N solubility directly instead of analysing all of these variables summarized in Pool 6 and further predict in vitro N solubility.

A rapid and cheap alternative to chemical analysis could be the estimation of in vitro N solubility by NIRS. The $R^2_{\text{Val}}$ of the calculated NIRS calibrations varied between 0.77 and 0.80, with SEP values between 1.93% and 2.01% (Table 10). The best performance was obtained for the second derivative of the spectra from 1,250 to 2,450 nm. In previously published calibrations (Krieg et al., 2018), the wavelength segment from 1,250 to 2,450 nm was used to predict the CP content of cereal grains (barley, durum wheat, maize, rye, triticale and wheat) because it showed the best performance of the wavelength segments under investigation. Accordingly, this region is known to contain absorption bands of N bonds such as primary amids (Bokobza, 1998), and the absorption bands of protein in wheat grains from 2,148 to 2,200 nm determined by Manley (2014) are within this region. The NIRS estimates might be partly based on the absorbance of N bonds in the grains. Accordingly, the correlation between the CP content and the in vitro N solubility was significant, if calculated for all grains ($p < .001$). The correlation remained significant if calculated within every grain species ($p < .001$). This leads to the conclusion that NIRS predictions of in vitro N solubility might be based on N contents in the grain samples, due to their correlation with in vitro N solubility. This is in accordance with the results of the prediction of in vitro N solubility from chemical and physical characteristics, as if the CP content was available for selection in the multiple linear regression approach (Table 9), it was used in all equations except for oats and corn.

The $R^2$ of NIRS predictions of the in vitro N solubility was, compared to the estimation from chemical and physical characteristics, at a relatively high level for all calibrations (Table 10). However, the performance (highest $R^2 = 0.80$) allows a classification of samples into groups of, for example, high, medium and low in vitro N solubility. A quantitative interpretation of the in vitro N solubility based on the absorbance of N bonds in the spectra of protein in wheat grains from 2,148 to 2,200 nm determined by Manley (2014) is within this region. The NIRS estimates might be partly based on the absorbance of N bonds in the grains. Accordingly, the correlation between the CP content and the in vitro N solubility was significant, if calculated for all grains ($p < .001$). The correlation remained significant if calculated within every grain species ($p < .001$). This leads to the conclusion that NIRS predictions of in vitro N solubility might be based on N contents in the grain samples, due to their correlation with in vitro N solubility. This is in accordance with the results of the prediction of in vitro N solubility from chemical and physical characteristics, as if the CP content was available for selection in the multiple linear regression approach (Table 9), it was used in all equations except for oats and corn.

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