FEED-A-GENE

Adapting the feed, the animal and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems

Deliverable D1.6

Predictive equations linking feed composition to the nutritive value to be applied with NIR technology

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Classified, as referred to in Commission Decision 2001/844/EC - Cl	

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1. Summary

Objectives

The objective of task 1.6 was to evaluate the suitability of near-infrared spectroscopy (NIRS) for the determination of the nutritive value of individual components and mixed diets for pigs. The project utilises samples from a database collected over more than 40 years of animal experimentation to develop NIRS calibrations to predict macronutrients, amino acids, and the digestibility of nutrients and energy and metabolisable energy. The deliverable is based on activities in WP1 task 1.6.

Rationale:

The nutritive value of a feed varies between and within feeds due to factors like genetics, agronomics, harvest, storage, and processing. In animal nutrition, adjustment of variation in the nutritive value is commonly done by analysing the nutrient fractions and using this information together with table values for their digestibility. However, it is known that the digestibility of nutrients may vary considerably from one feed to another and from one sample to another resulting in rather inaccurate measures of the nutritive value of the actual batches. Since it is not possible to perform *in vivo* evaluations of the nutritive value of individual batches because of time and cost constraints, there is a need for quick and reliable methods to determine the nutritive value of single feedstuffs for use in feed formulation and for control of complete feeds. Near-infrared spectroscopy (NIRS) has been widely used to evaluate the nutritional quality of agricultural products for several decades. While there are many examples on the use of NIRS to predict the chemical composition, less is known about its applicability to predict the nutritive value. In task 1.6, nutritionally evaluated samples collected for more than 40 years have been used to develop calibration models for macronutrients (i.e., ash, fat, protein, available carbohydrates, starch crude fibre, acid detergent fibre, and neutral detergent fibre) amino acids composition, and digestibility of energy and nutrient fractions and metabolisable energy. To have datasets with sufficient samples, feed types were grouped into 'like' sample types. All samples were also examined as one group (total) to determine if it was possible to have one calibration for all pig feeds

Teams involved:

AU

Species and production systems considered:

Primarily pigs, all countries in Europe, and the feed industry.

2. Abbreviations used

A-CHO	available carbohydrates (starch plus sugars)
ADF	acid detergent fibre
CAL	calibration
CV	coefficient of variation
dAPro	apparent protein digestibility
dCF	crude fibre digestibility
dCP	crude protein digestibility
d”DF”	calculated dietary fibre digestibility
dDM	dry matter digestibility
dE	energy digestibility
dFAT	fat digestibility
d”hemi”	calculated hemicellulose digestibility
DM	dry matter
dNFE	nitrogen free extract digestibility
dOM	organic matter digestibility
dTPro	true protein digestibility
EDOM	enzyme digestible organic matter
eME	estimated metabolisable energy
GE	gross energy
ME50	metabolisable energy corrected for 50% protein retention
mPLS	modified partial least squares
NDF	neutral detergent fibre
NFE	nitrogen free extract
NIRS	near-infrared spectroscopy
RER	Range error ratio
RPD	ratio of performance deviation
SD	standard deviation
SE	standard error
SEC	standard error of calibration
SECV	standard error of cross validation
SEP	standard error of prediction (corrected for bias)
VAL	validation
VR	variance ratio

3. Introduction

The importance of accurate information about feed quality is of utmost importance not only because feed accounts for two-thirds or more of the cost of livestock production but also because accurate feed quality information is pivotal for optimizing performance and minimizing the environmental footprint from livestock production (Millet et al., 2018; Wang and Zijlstra, 2018).

The nutritive values of a feed vary between and within feeds due to factors like genetics, agronomics, harvest, storage and processing. Adjusting for variation in the nutritive value are commonly done by analysing the nutrient fractions and using this information together with table values for the digestibility of the individual nutrients (Henry et al., 1988; Noblet and Perez, 1993). However, it is known that the nutrient digestibility may vary considerably from one feed to another and between samples resulting in rather inaccurate measures of the nutritive value of the actual batches (Just et al., 1983). Since it is not possibly to perform *in vivo* evaluations of the nutritive value because of time and cost constraints, there is a need for quick and reliable methods to determine the nutritive value of single feedstuffs for use in feed formulation and for control of complete feeds.

Near-infrared spectroscopy (NIRS) has been widely used to evaluate the nutritional quality of agricultural products for several decades. It is based on the principle that infrared spectra contain quantitative information of functional groups. According to Beer's law, the absorbance of spectra is proportional to the concentration of an analyte (Griffiths, 2002). Additionally, infrared spectra also reflect structural information concerning the chemical components, as absorbance at a given wavelength is induced by molecular vibrations of certain functional groups that may reflect to specific nutrients (Shurvell, 2002; Weyer and Lo, 2002). NIRS can therefore predict physical and chemical properties of feedstuffs such as the contents of crude protein, amino acids, acid detergent fibre, neutral detergent fibre, and starch (Williams and Cordeiro, 1979; Barton, 1991; L. et al., 2013). When calibrated with digestibility or digestible nutrient reference data, NIRS can also be used to predict the digestibility of nutrients such as that of dry matter (dDM) of forages (Norris et al., 1976), CP (dCP) of silage (Swift, 2003) and digestible energy (DE) of cereal grains (van Barneveld et al., 1999; McCann et al., 2006; Zijlstra et al., 2011). The limitation of using NIRS for the prediction of the chemical composition and particularly the digestibility of the nutrient fractions is the size of the database that is to make calibrations (Wang and Zijlstra, 2018). While this is relatively simple for the chemical components, it is much more difficult for the digestibility of the nutrients and publications so far have been based on limited reference data.

4. Materials and methods

4.1 Samples

A total of 857 feedstuffs and diets have been scanned by NIRS. Of these, 47 (i.e., pure starch and sugars, roughage, and animal products) were discarded because they were outliers when performing a principal component analysis. The remaining 810 samples were used to a variable extent and depending on the data available for building the individual models.

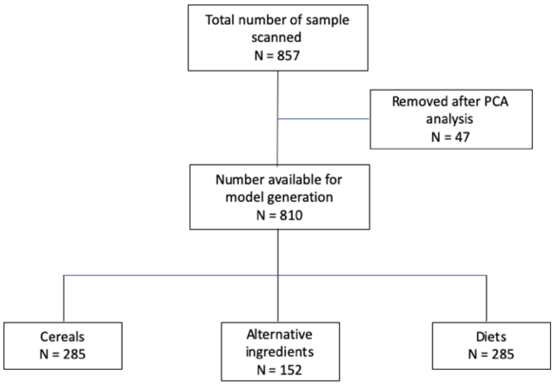


Figure 1. Overview of samples scanned.

Both conventional and unconventional feedstuffs were assessed that have been evaluated for nutritive value in the Danish Feed Evaluation system (Just, 1982; Just, 1983). The feedstuffs, representing a diverse range of feed types, have been collected since 1975 and stored at -20°C. Additionally, common and extreme feed mixture samples used in experiments with pigs were also included. Samples were classified into groups of similar types including; cereals, supplemental ingredients and feed mixtures (diets). The cereal group included oats, maize, wheat, barley, rice and triticale. The supplemental ingredient group included cereal co-products: corn gluten feed, malt sprouts, maize middlings, maize bran, wheat middlings, wheat bran, rice middlings, rice bran, barley protein, and barley groats; protein concentrates, soybean meal, linseed meal, peas, faba beans cottonseed meal, cottonseed cake, coconut cake, cottonseed cake, palm cake, lupin, potato protein, sunflower seed, rapeseed meal and rapeseed cake; and miscellaneous: tapioca, citrus pulp, apple pomace, maize silage, lucerne, guarmel, and grass meal. The feed mixtures group included commercial diets and balanced diets formulated for use in different experiments.

4.2 NIRS analysis

Feed samples were stored at -20ºC in airtight containers until needed. Samples were dried at 60ºC in an air forced oven for 48 hours and then milled to a 1 mm particle size in an ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany). Dried and ground feed samples were left to equilibrate to ambient moisture levels at room temperature for a minimum of 48 hours before scanning. Ground samples were packed in a sample cup with quartz window and scanned using a Foss NIRS DS2500 feed analyser (FOSS Analytical A/S, Silver Springs, MD, USA). Each scan was the average of 32 scans from various positions on the sample cup using the wavelength range from 400 to 2,500 nm with data recorded every 0.5 nm. Each sample was scanned in duplicate from two separate samplings and duplicate spectra were averaged.

4.3 Spectral pre-treatment and calibration development

Calibrations for macronutrients, amino acids, digestibility and metabolisable energy were developed with WinISI version 4.9.0 (FOSS Analytical A/S, Silver Springs, MD, USA). Sample spectra were mathematically pre-processed and the spectral range reduced before model development. The spectra were pre-processed using the standard-normal-variate (SNV) method along with detrending (D) (Barnes et al., 1989) to minimise baseline offset and reduce scatter. A Savitzky-Golay derivative with second-order polynomial with a gap of 8 and 4 points of smoothing was then applied (math treatment 2,8,4,1). The spectral range was reduced to remove spectra in the visible light region to include wavelengths between 780 nm and 2,400 nm with data points every 0.5 nm resulting in 1,698 data points per scan. Calibration models were built with the modified partial least squares method (mPLS). No further outlier removal was needed for the total and supplemental ingredients datasets as these included a very diverse range of samples and outliers were identified in the pre-screening procedure. To ensure samples were assigned to the correct groups, one round of outlier removal was performed on the cereals and feed mixtures datasets with a conservative critical T value of 3 as cut-off. In addition to validation with an external data set, cross validation was also performed and used to determine the number of factors to include in the model. Cross validation was performed by dividing the calibration samples that were ranked on their values into groups of 8 and building successive models with one group left out. Each group is then evaluated using the model developed on the other samples. The number of factors to be included in the models were chosen to include as much information as possible without overfitting by assessing when the standard error of cross validation (SECV) reached its lowest value. mPLS calibration models were built using the entire feedstuffs together (total) as well as for the three groups of samples types' cereal, supplemental ingredients, and feed mixtures. To assess whether using the entire quite dissimilar sample types together could make a stronger model, the equations developed with the total dataset were then evaluated with four validation sets: total, cereal, supplemental ingredients and feed mixtures. The models developed for the three groups (cereals, supplemental ingredients and feed mixtures) were evaluated with their corresponding validation set.

4.4 Equation evaluation

The regression coefficient (R^2), standard error of cross validation (SECV), standard error of prediction corrected for bias (SEP), and ratio of performance deviation (RPD) were used to evaluate calibration performance (Sapienza et al., 2008; 2018b). The R^2 describes the fit when the reference values are plotted against the predicted values. The higher the R^2, value the better the fit and 1 equals a perfect fit. The R^2 values were determined for the calibration and the external validation samples. The SECV shows how well the calibration model predicts the reference values when some samples are selectively removed. Lower SECV values indicate higher precision in the models' accuracy. The SEP evaluates the performance of the model on a set of independent samples. This is the most important indication of how precise the calibration model will predict new samples. The international standard (ISO 12099:2017) recommends that there should be at least 20 samples in a validation set. The RPD was calculated by dividing the standard deviation (SD) by the SEP. The RPD gives an indication on whether the SEP values are low enough in comparison to the variation seen in the population used to make the model. RDP values greater than 2 are preferred. Relative SEP was also calculated by dividing the SEP by the mean of the laboratory values for the measured amino acid and multiplying by 100. Coefficient of variation (CV) was calculated for the laboratory values by dividing the SD by the mean and multiplying by 100. The CV is an independent measure of the variation that enables the different amino acids with different means to be compared.

5. Results

The description of the concentration of macronutrients, digestibility of energy and nutrients and metabolisable energy for all feedstuffs and diets together is shown in Table 1 and for the separate groups of cereals, alternative ingredients, and diets in Annexes 1-3. There were around 200 samples with values in each of the constituents for the cereals and diets groups and roughly half that number for the alternative ingredients group. However, the alternative ingredients group had the largest range in values for most constituents due to the diverse samples in this group.

Table 1. Descriptive statistics of composition for the total calibration and validation samples sets.

	Calibration					Validation				
Constituent	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Ash	593	4.3	164.4	44.8	21.2	149	13.5	101.4	43.4	20.7
Protein	607	27.7	708.2	180.8	86.2	150	88.6	506.3	173.6	76.5
Fat	573	3.9	250.2	45.4	30.1	140	18.0	209.3	48.6	35.5
Crude fibre	524	2.7	318.1	60.4	44.0	130	13.9	302.4	60.9	43.0
A-CHO	464	30.6	911.4	543.9	172.5	118	72.2	743.2	539.1	170.7
Starch	489	2.8	916.3	481.3	183.3	125	5.2	720.0	485.9	181.8
NDF	427	9.3	725.0	159.3	83.1	110	46.6	449.6	162.9	69.2
ADF	430	1.8	377.3	76.7	55.2	112	17.7	358.9	77.9	55.9
NFE	525	182.8	897.2	672.4	129.7	129	267.7	836.6	674.9	134.2
GE	584	3,875.7	5,319.1	4,491.4	178.3	146	4,022.2	5,284.0	4,499.2	198.8
dDM	552	21	97.7	80.8	9.1	136	48.2	93.9	80.3	8.6
dOM	545	25	98.7	82.8	9.2	136	49.6	96	82.2	8.4
dE	549	23	98.2	80.4	9.2	135	49.2	95.7	79.7	8
dAPro	520	21.9	95.9	77.4	9.1	130	58	96.7	76.8	7
dTPro	456	11.7	100	82.3	9.1	114	14.9	92.9	81.6	8.4
dFAT	472	14.5	93.8	51.4	14.3	122	19.3	94.5	53.4	15.8
dCF	419	0.3	100	29.9	17.1	100	4.1	78.9	27.1	14.3
dNFE	441	30.3	99.4	88.9	8.2	115	46	98.3	87.7	9.2
d"DF"	395	6.5	100	43.8	16.6	102	3.9	85.7	41.3	14.6
d"hemi"	393	7.1	100	49.8	16.8	102	12.6	92.9	48.5	15.2
ME50	528	1117.5	4733.5	3490	399.2	130	2150.5	4687.9	3474.8	385.2
EDOM	125	16.9	96.2	80.8	13.8	33	53.6	92.9	79.1	12

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); NDF: Neutral detergent fibre (g/kg); ADF: Acid detergent fibre (g/kg); Starch (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal); dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

5.1 Calibration models for macronutrients

The statistics for the best calibration model developed for each constituent is presented in Table 2 for all feedstuffs and diets and for the groups of cereals, alternative ingredients and diets in Annexes 4-6. The models for most chemical constituents (i.e., protein, fat, crude fibre, available carbohydrates, starch, NDF, ADF, and NFE) had a good fit between the predicted and measured values (R^2_{pred}=0.9-0.99) for the total and the alternative ingredient groups, an adequate fit for the diet group (R^2_{pred}=0.75-0.95). However, some models were poorer at predicting ADF and starch in the cereals group (R^2_{pred} 0.49, 0.46 respectively), and ash was poorly predicted especially in the cereals and diet groups (R^2_{pred} 0.33 and 0.34 respectively; Annexes 4 and 6). Gross energy was only moderately well modelled with the total and cereal group (R^2_{pred} 0.84 and 0.46 respectively) but adequate predictions were obtained with the diet and alternative ingredients datasets (R^2_{pred} 0.89 and 0.9 respectively).

Table 2. Summary statistics for calibration and validation models for macronutrients on total feedstuffs.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
Ash	16	8.16	0.85	10.53	0.75	15.2	0.93	2.97	-0.28	10.31	0.76	2.07
Protein	13	12.84	0.98	15.73	0.97	43.26	0.97	4.46	-0.62	16.87	0.95	5.11
Fat	8	8.71	0.92	9.47	0.9	26.01	1	0.84	0.59	8.65	0.94	3.48
Crude fibre	12	7.15	0.97	8.56	0.96	36.86	0.99	0.26	-0.31	9.5	0.95	4.65
A-CHO	8	25.73	0.98	30.45	0.97	28.92	0.97	10.87	-6.22	31.89	0.97	5.33
Starch	8	40.18	0.95	44.66	0.94	20.45	0.97	12.04	-1.21	48.62	0.93	3.78
NDF	11	17.76	0.95	23.16	0.92	30.9	0.91	13.67	-0.6	23.25	0.9	3.59
ADF	11	9.66	0.97	11.87	0.95	31.64	1.05	-2.75	0.9	12.4	0.95	4.46
NFE	10	16.61	0.98	19.22	0.98	37.17	0.98	16.49	0.12	21.04	0.98	6.19
GE	9	78.48	0.81	87.46	0.76	16.5	1.03	-118.14	-0.76	78.94	0.84	2.27

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); Starch (g/kg); NDF: Neutral detergent fibre (g/kg); ADF: acid detergent fibre (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal).

4.2 Calibration models for digestibility and metabolisable energy

The statistics for the best calibration model developed for each of the biological constituents (i.e., dDM, dOM, dE, dAPro, dTPro, dFAT, dNFE, d"DF", d"hemi", and ME50) are presented in Table 3 for all feedstuffs and diets and for the groups of cereals, alternative ingredients, and diets in Annexes 7-9. The calibration for the biologically constituents were high for dDM, dOM, dE, dNFE, and EDOM, intermediate although relatively high for dAPro, dTPro, dFAT and ME50, but lower for the digestibility of the fibre fractions dCF, d"DF", and d"hemi".

The lower R^2 of the validation set and the lower overall R^2 of the calibration and validation of the biological than of the chemical constituents are partly due to the fact that the predictions cannot be better than what is possible based on precision by which the data in the database is obtained. This is illustrated by the comparison of the experimentally determined variability of the biologically available constituents (dDM, dOM, dE, dAPro, dTPro, dFAT, dNFE, d"DF", d"hemi", ME50, and EDOM) with the variability or error of the NIRS calibrations (Figure 3). Overall, the experimental variability, represented here as the average standard error (SE) as a percentage of the mean value, is similar to the relative SE (as either SECV or SEP) from the NIRS calibrations. The NIRS relative SE was always a little higher but was proportional to the relative experimental SE. Overall, the low relative SECV or SEP (1.2-7.5%) was obtained for dDM, dOM, dE, dAPro, dTPro, dNFE and ME50. The relative SECV or SEP for dFAT, d"DF", and d"hemi", was 13-28% and dCF had the highest relative SECV or SEP (20.4-47%).

Table 3. Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on total feedstuffs.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
dDM	8	2.45	0.93	2.68	0.91	28.61	0.9	7.97	-0.03	3.36	0.86	2.73
dOM	10	2.15	0.95	2.45	0.93	30.14	0.89	9.59	0.19	3.13	0.87	2.94
dE	12	2.18	0.94	2.67	0.92	28.14	0.86	11.54	0.16	3.24	0.86	2.84
dAPro	15	3.46	0.85	4.62	0.74	16.03	0.61	29.9	-0.3	5.66	0.58	1.60
dTPro	13	2.88	0.9	4.03	0.8	21.91	0.87	10.9	0.24	4.34	0.75	2.10
dFAT	10	7.39	0.73	8.99	0.61	8.82	1.02	-0.13	0.73	8.63	0.7	1.66
dCF	9	9.4	0.7	11.34	0.56	8.79	1.06	-0.92	0.56	9.92	0.52	1.73
dNFE	15	1.49	0.97	2.21	0.93	31.21	1.03	-2.44	-0.03	2.81	0.91	2.94
d"DF"	6	10.12	0.63	11.18	0.54	8.36	1.29	-11.36	0.58	10.63	0.5	1.56
d"hemi"	4	11.48	0.53	12.02	0.49	7.73	1.17	-7.31	0.78	12.57	0.33	1.34
ME50	12	118.23	0.91	141.67	0.87	25.52	0.88	420.42	14.74	162.75	0.84	2.45
EDOM	10	2.55	0.97	3.92	0.92	20.25	0.98	1.51	-0.22	2.16	0.97	6.44

dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE:–nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

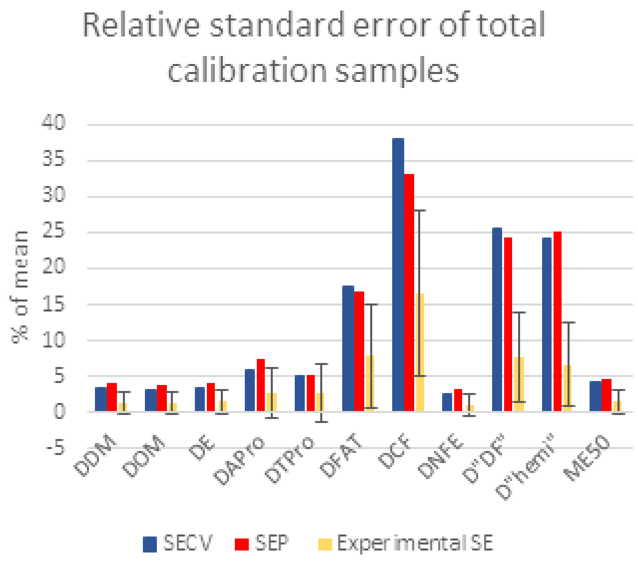


Figure 2. Relative standard error of NIRS calibrations and measured digestibility values.

SECV: standard error of cross validation of the NIRS calibration as a percentage of the mean value; SEP: standard error of prediction of the NIRS calibration as a percentage of the mean value; Experimental SE: average standard error from measured values of digestibility as a percentage of the mean value. Error bars represent ± one SD. dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility; dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM).

4.3 Prediction of digestible and metabolisable energy

Enzymatically determined organic matter (EDOM) is the currently accepted *in vitro* method to estimate DE in Denmark and used for calculating physiological energy in feedstuffs and diets. Even though there were fewer feedstuffs with EDOM measurements (n=125 total) very good predictions were obtained from the NIRS calibrations with high R^2, low SEP, high RPD, and RER values for all groups especially for the total (Table 3) and diet groups (Annex 9). In comparison, the predictions for DE directly from NIRS calibrations were less precise (but still very good) than the predictions for EDOM. However, the NIRS calibration was able to make closer estimates of the measured DE than those obtained using the EDOM method, shown in Figure 3.

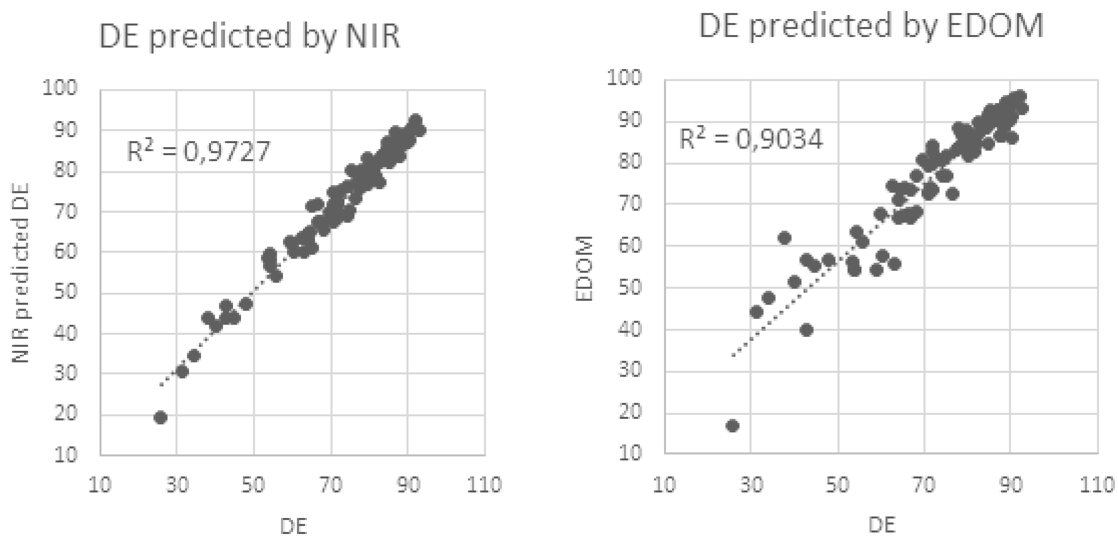


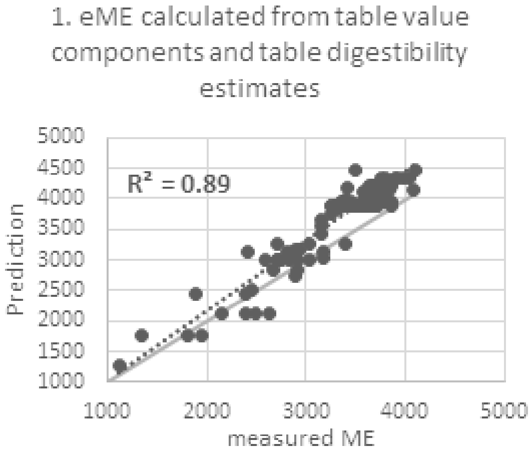
Figure 3. Relationship of digestible energy predicted by A) NIRS or B) EDOM to predict values of digestible energy.

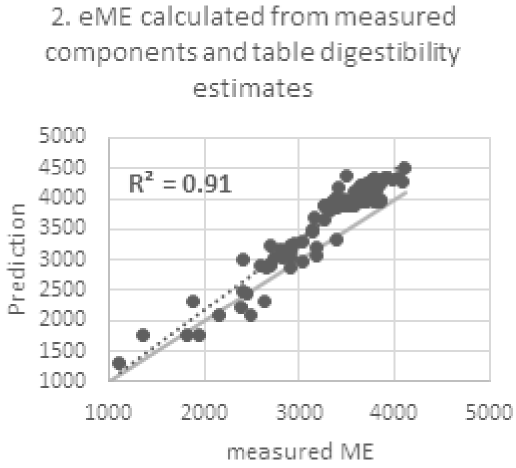
The data obtained by NIRS can be used to estimate metabolisable energy (eME) either directly from the relationship between NIRS and ME (Table 3) or by using NIRS to predict the macronutrients (Table 2) combined with predictions of the digestibility of the individual nutrient fractions (Table 3) using the following equation:

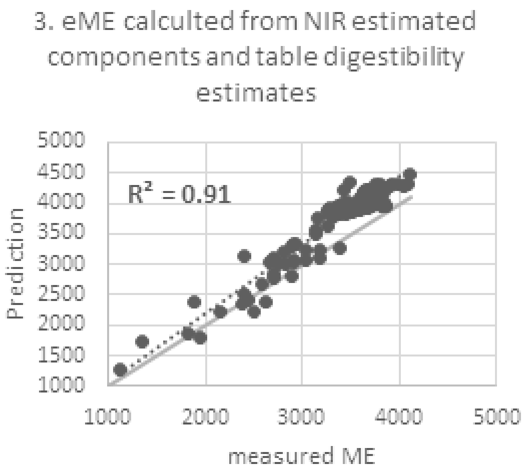
$$eME(kJ) = 21.3xX_1+37.6xX_2+19.7xX_3+19.7xX_4$$

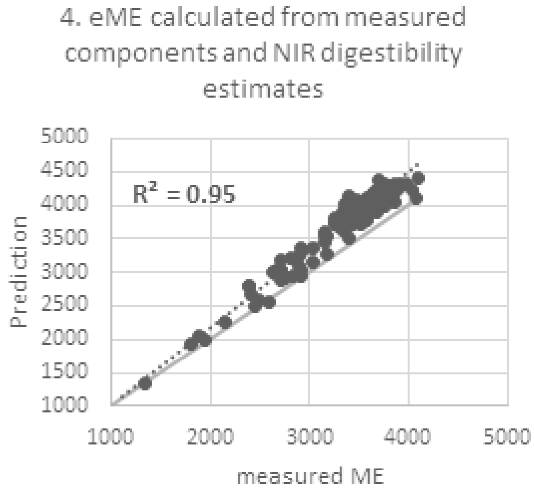
where X_1 = digestible protein, X_2 = digestible fat, X_3 = digestible crude fibre and X_4 = digestible NFE; the digestible fractions were expressed in g/kg DM.

This is illustrated and compared in Figure 4 where eME obtained by using 6 different combinations of table values, NIRS estimated values and measured values are compared. Table values (average values determined for each feedstuff) were available for cereal and alternative ingredient samples only and the dataset therefore limited to 218 samples.


1. eME calculated from table value components and table digestibility estimates
R² = 0.89
Prediction
measured ME
1000
1500
2000
2500
3000
3500
4000
4500
5000


2. eME calculated from measured components and table digestibility estimates
R² = 0.91
Prediction
measured ME
1000
1500
2000
2500
3000
3500
4000
4500
5000


3. eME calculted from NIR estimated components and table digestibility estimates
R² = 0.91
Prediction
measured ME
1000
1500
2000
2500
3000
3500
4000
4500
5000


4. eME calculated from measured components and NIR digestibility estimates
R² = 0.95
Prediction
measured ME
1000
1500
2000
2500
3000
3500
4000
4500
5000

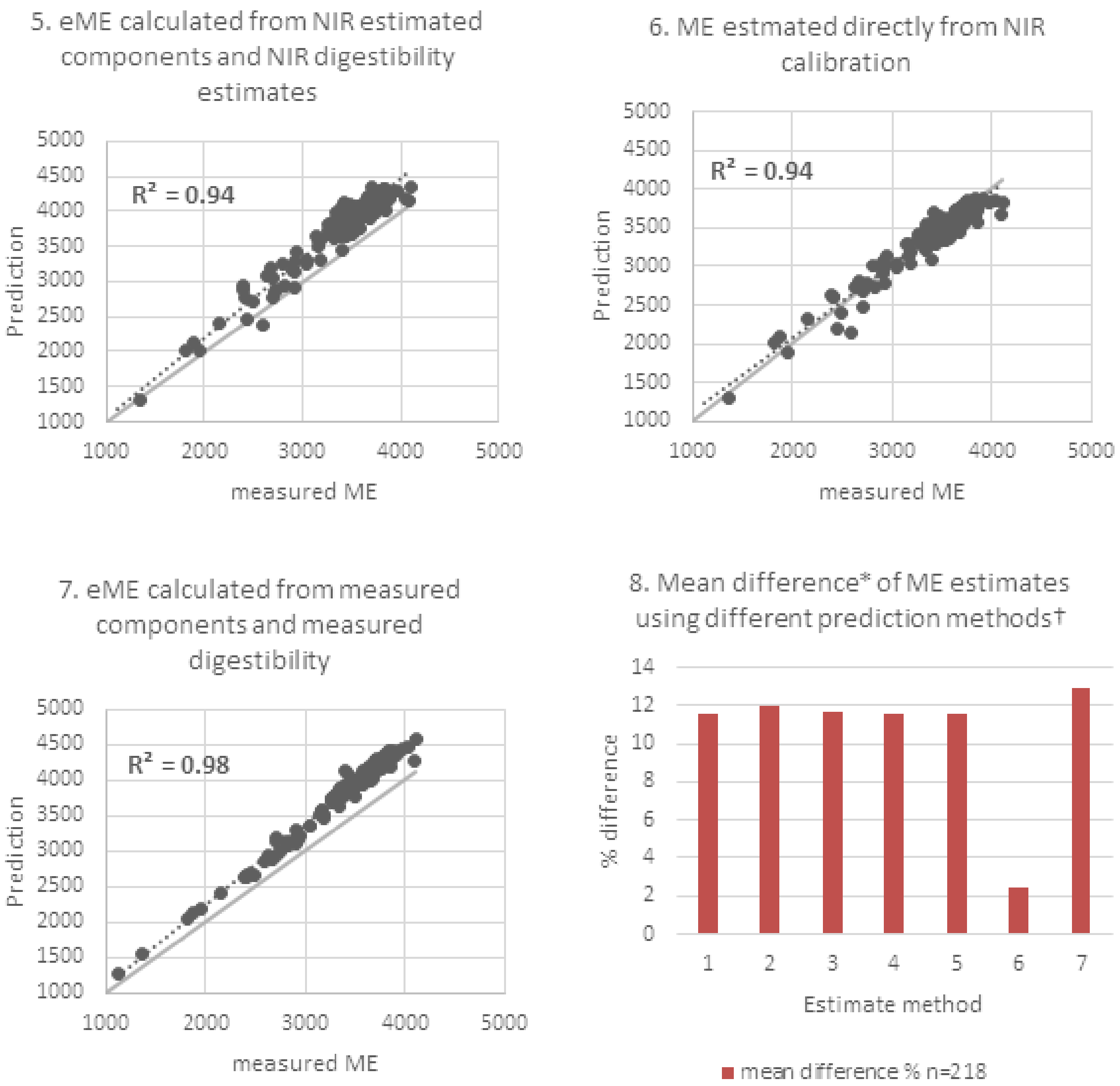


Figure 4. Comparison of estimated metabolisable energy (eME) predicted by an equation (part 1-5 and 7) or directly with NIRS (part 6) with measured values of ME for 218 cereal and alternative ingredient samples. The grey solid line represents the perfect relationship between predicted and measured values. Part 8 shows the mean difference of the predicted values from the measured values.
*Mean difference =Mean of [(Lab_n – eME_n)/Lab_n*100] and Mean of [(Lab_n – NIR_n)/Lab_n*100]
† prediction methods 1-7 depicted in this figure.

The eME using table values for constituent values and table digestibility values had the lowest R^2 (0.89) of all the combinations. The R^2 was improved equally (0.91) if either the measured constituent values or the NIRS estimated constituent values were used the estimate ME instead of the table values. The R^2 was further improved (0.95 and 0.94) if the NIRS estimated digestibility values were used in combination with either the measured constituent values or the NIRS estimated constituent values, respectively. Directly estimating ME with NIRS calibration had an R^2 equal to the eME using both NIRS estimated values and NIRS estimated digestibility. The best relationship (R^2 = 0.98) was found using the measured values and the measured digestibilities. However, all the predictions (eME) using the formula were higher than the measured values of ME so when the mean difference of the ME estimates was compared (part 8 Figure 4.) the direct estimates from NIRS calibrations were much closer to the measured values of ME (2.5% vs 11.5-12.9%).

5.2 Amino acids

A correct supply of dietary protein and amino acids is important for optimal growth and protein accretion in pigs (Moughan, 2018). The importance of a balanced protein and amino acid supply to pigs is not only related to animal performance but has also a strong impact on the environment through nitrogen leaching to the aquatic environment and drinking water, and nitrogen fallout from evaporation from pig housing and slurry storage facilities (Millet et al., 2018). Optimizing the amino acid composition is usually done by using table values, which are average values from each feed type, but crops will vary in their amino acid content due to growing conditions, harvest year, and processing (Just, 1983). However, measurement of the amino acid profiles by chemical methods is too time consuming and expensive to use routinely. Using NIRS to predict amino acids composition in cereals and feedstuffs has been widely accepted as an alternative to wet chemistry methods (Chen et al., 2013).

Calibration equations were developed using 607 samples of all sample types, the remainder (n=150) were used to test the model in the validation set. The statistics describing the calibration model and validation of the total samples are shown in Table 4. The equations for 18 amino acids and crude protein showed high coefficients of determination for calibration (R^2_{CAL}=0.91-0.99) and validation (R^2_{VAL}=0.87-0.97). It is normal that the R^2 values found by validation are a little lower than those obtained by calibration if the range is lower (Fearn, 2014). The standard errors of cross validation (SECV) and prediction (SEP) were low and in good agreement with each other. Relative SEP (SEP/meanx*100) puts the SEP in context with the mean value of the amino acid being estimated, with larger SEP values expected for higher measured values.

Relative SEP values are also easier to compare between studies that may have used different units to report amino acid values. The relative SEP ranges between 8.8 and 20.5% with a mean of 12.1%. Arginine and methionine have higher relative SEP of 15% and tryptophan has the highest of 20.5%. Overall the relative SEP values are higher than that found in calibration derived from single sample types; wheat or corn (3-6.7%) (Fontaine et al., 2001), soybean/soybean meal (1.75-4.38%) (Fontaine et al., 2001), brown rice flour (~3-15%) (Zhang et al., 2011), soybean (FOSS instrument, PLS model 2-16%) (Kovalenko et al., 2006). However, our calibrations were made with many different samples and the amino acids were not all analysed in the same laboratory which could be expected to increase the variability in the measurements and thereby the SEP.

Because NIRS calibrations are based on references, the accuracy of the predictions will not be higher than the accuracy of the amino acid measurements. Fontaine et al. (2001) reported that the reproducibility of their reference method was ~2-5% for amino acids. The ISO standard for the determination of amino acids in animal feedstuff (2005) has measured the repeatability and the reproducibility of the method on several sample types and the observed variation was dependent on the amino acid and the type of sample analysed. Repeatability is derived from the observed difference when the same sample is measured twice using the same method, in the same laboratory with the same equipment and by the same operator. Reproducibility is derived from the observed difference when the same sample is measured twice in different labs with different equipment and with different operators. Thus reproducibility is a more appropriate measure to compare samples in this study as the amino acid analysis were performed over the course of many years in different laboratories with different operators. The reproducibility of the method for determining 16 amino acids in various chicken feed and corn ranges between 5.98-23.33%. Therefore, the relative SEP values obtained from the total calibrations are reasonable considering the error expected from the measured values. Reproducibility for broiler finisher feed from the ISO standard is shown in Figure 5 in comparison to the relative SEP of each calibration.

Table 4. Summary statistics for calibration and validation of the amino acid composition of total (individual ingredient and mixtures) pig feedstuffs (g/kg of dry matter).

	Calibration statistics							Validation statistics				
Constituent	N_{CAL}	$Range_{CAL}$	Mean	SD	Factors	R^2_{CAL}	SECV	N_{Val}	$Range_{VAL}$	R^2_{VAL}	SEP	RPD
Ala	387	1.1 - 30	7.3	4.7	13	0.98	1.01	94	4 - 23.5	0.96	0.84	5.58
Arg	387	1.2 - 52.7	10.5	8.8	15	0.99	1.89	94	2.8 - 46.6	0.97	1.57	5.62
Asp	387	1.8 - 79.4	13.2	12.4	14	0.99	1.85	94	5.6 - 39.2	0.97	1.69	7.3
Cys	395	0.3 - 11.1	3.3	1.6	8	0.95	0.44	94	1.1 - 11.8	0.94	0.44	3.58
Glu	387	3.4 - 129.8	37.4	18.3	10	0.97	4.22	94	12.8 - 98.9	0.96	3.3	5.56
Gly	387	1 - 29.2	7.5	5	5	0.97	0.93	94	3.6 - 27.6	0.96	0.96	5.21
His	387	0.4 - 19.3	4	2.7	7	0.97	0.54	94	2 - 14.3	0.97	0.44	6.26
Ile	387	0.8 - 34.6	6.8	4.9	8	0.98	0.84	94	3.2 - 22.6	0.97	0.71	6.94
Leu	387	1.4 - 55.4	12.1	7.7	8	0.98	1.43	94	5.4 - 38.9	0.95	1.42	5.43
Lys	395	0.9 - 44.4	7.9	6.7	14	0.98	1.3	94	2.8 - 28.3	0.96	1.05	6.39
Met	395	0.3 - 9.7	2.8	1.6	13	0.97	0.41	94	1.3 - 9.7	0.94	0.42	3.84
Phe	387	0.6 - 36.6	8.1	4.8	5	0.97	0.95	94	4.1 - 22.1	0.96	0.85	5.69
Pro	387	1 - 36.1	13.5	5.2	10	0.91	1.91	94	6.3 - 32.5	0.92	1.36	3.8
Ser	387	0.9 - 38.3	8	5.2	7	0.98	0.94	94	4 - 23.5	0.97	0.78	6.65
Thr	395	0.8 - 27.9	6.1	4.1	16	0.99	0.71	94	3.2 - 21.8	0.96	0.68	6.09
Trp	163	0.2 - 9.1	2.2	1.6	2	0.93	0.45	37	0.8 - 6	0.87	0.45	3.62
Tyr	369	0.5 - 26.5	5.5	3.9	8	0.98	0.72	92	2.6 - 16.5	0.96	0.58	6.7
Val	387	1 - 36.2	8.5	5.2	8	0.98	0.84	95	1.1 - 28.3	0.94	1.13	4.59
CP	607	27.7 - 708.2	180.8	86.2	13	0.98	15.73	150	88.6 - 506.3	0.95	16.87	5.11

Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; CP: crude protein.

The ratio of the standard deviation (SD) of the amino acid in the calibration population to the SEP gives the ratio of performance deviation (RPD), which is a way to estimate how useful a calibration is (Fearn, 2002). Ratio values were all high (>3) indicating that the models are adequate for screening for improved selection and 14 amino acids (all excluding cysteine, methionine, proline and tryptophan) as well as crude protein had values >5 indicating that the model is very precise (Sapienza et al., 2008). Together the results indicate the values obtained by NIRS calibrations are meaningful for the total dataset.

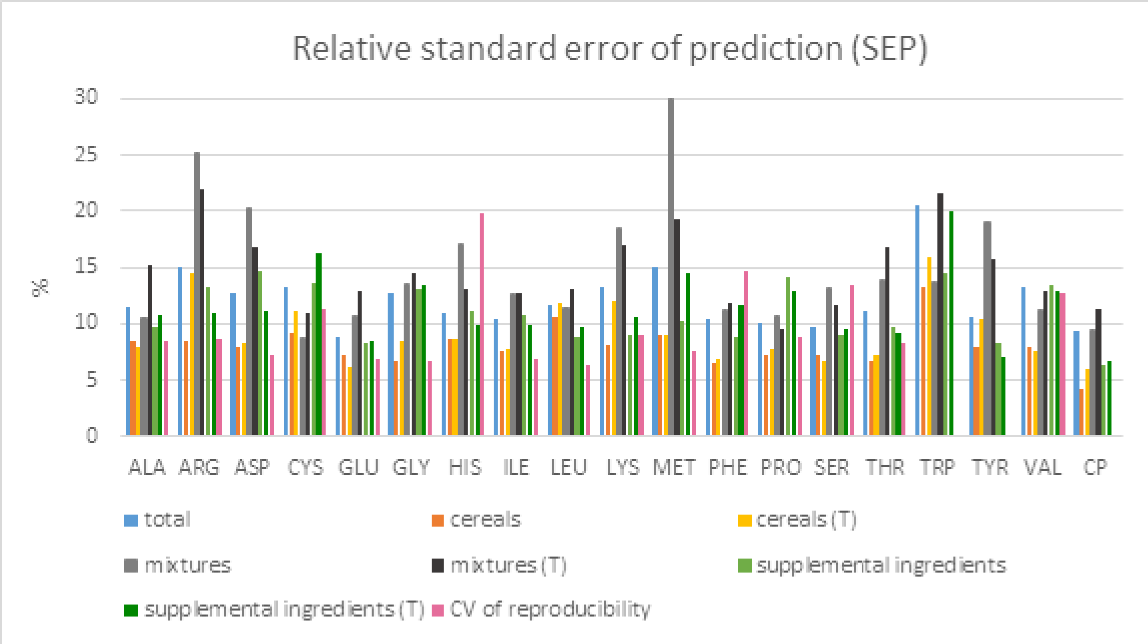


Figure 5. Relative standard error of prediction (SEP/mean of lab values*100) of NIRS calibration equations developed with either the total dataset or the three subgroups; cereals mixtures and alternative ingredients. Subgroups followed by (T) are from the calibration equation developed on the total dataset predicting the independent validation sets for the subgroups. The CV of reproducibility is the coefficient of variation (%) for between laboratory standard deviation from 36-46 single determinations of amino acids in broiler finisher feed reported in the method standard ISO 13903:2005 – Determination of amino acid content.

6. Conclusions

A total of 857 feedstuffs (cereals and alternative ingredients) and diets have been scanned by NIRS of which 810 samples were used and split 80:20. 80% was used for developing calibration models and 20% for validating the models. Models were developed and validated for the total dataset, cereals, alternative ingredients, and mixed diets. In most cases, the models for the total dataset were better than for the groups consisting of cereals, alternative ingredients, and mixed diets.

The models for most chemical constituents (i.e., protein, fat, crude fibre, available carbohydrates, starch, NDF, ADF, and NFE) had a good fit between the predicted and measured values with R^2 of prediction in the order of 0.9-0.99 for the total and the alternative ingredient groups, an adequate fit with the diet group (R^2_{pred} 0.75-0.95). However, some models were poorer at predicting with the cereals group (e.g., ADF and starch; R^2_{pred} 0.49 and 0.46, respectively) and ash was poorly predicted. Gross energy was only moderately well modelled with the total and cereal group (R^2_{pred} 0.84 and 0.46, respectively) but adequate predictions were obtained with the diet and alternative ingredients datasets (R^2_{pred} 0.89 and 0.9, respectively).

The calibration models for the biologically constituents were high for dDM, dOM, dE, dNFE, and EDOM, intermediate for dAPro, dTPro, dFAT, and ME50 but lower for the digestibility of the fibre fractions (i.e., dCF, d“DF” and d“hemi”). The variation in the R^2 of the validation set followed in general that of the calibration set but, as expected, at a lower level.

The data obtained by NIRS were used to estimate metabolisable energy (eME) either directly from the relationship between NIRS and ME or by using NIRS to predict the macronutrients combined with predictions of the digestibility of the individual nutrient fractions. Both methods resulted in better predictions than table values for the individual feedstuffs or chemically analysed data for macronutrients combined with table values.

Precise and accurate estimates of amino acids and crude protein can be made on both raw ingredients and diet mixtures of plant-based pig feeds with one calibration model. Increased precision for some amino acids could be obtained by using groups that are more specific but as these groups also contained a mixture of sample types, a fair comparison of specific versus general could not be made. Overall, the models developed on the total samples offer greater robustness, and can be used on a wide range of measured values and sample types, and with good accuracy.

7. Annexes

Annex 1: Descriptive statistics of composition for the cereal calibration and validation samples sets.

Annex 2: Descriptive statistics of composition for the alternative ingredients calibration and validation samples sets.

Annex 3: Descriptive statistics of composition for the pig diet calibration and validation samples sets.

Annex 4: Summary statistics for calibration and validation models for macronutrients on cereals.

Annex 5: Summary statistics for calibration and validation models for macronutrients on alternative ingredients.

Annex 6: Summary statistics for calibration and validation models for macronutrients on pig diets.

Annex 7: Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on cereals.

Annex 8: Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on alternative ingredients.

Annex 9: Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on pig diets.

Annex 10: Summary statistics for calibration and validation of the amino acid composition of cereals (g/kg of dry matter).

Annex 11: Summary statistics for calibration and validation of the amino acid composition of supplemental feed ingredients (g/kg of dry matter).

Annex 12: Summary statistics for calibration and validation of the amino acid composition of pig feed mixtures (g/kg of dry matter).

Annex 13: Summary statistics for equations built on the all sample types with independent validation samples representing the total samples, cereals, alternative ingredients or feed mixtures (g/kg of dry matter).

Annex 14: Scientific output

Annex 1. Descriptive statistics of composition for the cereal calibration and validation samples sets.

	Calibration					Validation				
Constituent	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Ash	212	11.9	65.2	23.8	7.9	59	13.5	69.7	24.3	10.1
Protein	211	90.6	176.7	127.8	17.8	59	88.6	161.1	118.5	18.4
Fat	200	21.4	107.9	33.1	9.2	55	21.4	98.9	35.1	11.7
Crude fibre	200	8.2	105.9	44.1	16	55	13.9	100.9	43	14.1
A-CHO	199	464.9	792.1	654.5	44	54	506.4	743.2	659.8	42.5
Starch	191	429.3	778.2	613.7	59.5	54	515.8	720	629.7	45.7
NDF	193	30.5	293.4	136.7	37	52	46.6	184	133.6	30.3
ADF	193	12.9	148.6	55.4	19.2	53	17.7	88.8	51.3	13.4
NFE	200	624.5	832.4	771	32.4	55	664.5	836.6	777.8	30.9
GE	209	4013.7	4731	4453.1	80.2	58	4022.2	4819.7	4437.7	99.8
dDM	201	67.1	93.7	82.4	3.9	54	69.3	91.6	82.3	4
dOM	197	68	95.6	84.4	3.9	54	71	92.5	84.3	3.9
dE	201	68.1	94.4	81.8	4	54	70.2	90.8	81.6	4
dAPro	201	58.2	89.3	75.6	5.4	54	61.7	84.6	74.2	6
dTPro	201	67.5	96.5	82.8	4.91	54	69.6	92.9	81.8	5.5
dFAT	193	15.2	79.5	43.1	10.1	53	27.6	77.2	44.2	10.7
dCF	170	3.4	69.8	22.4	10.8	42	4.1	50.5	17.2	11.6
dNFE	193	72.8	97.9	91.2	2.8	53	79	97.4	91.3	2.7
d"DF"	192	6.5	74	37.6	13.0	51	3.9	63.4	35.5	13.5
d"hemi"	191	7.1	77.6	43.3	13.3	50	12.6	72.8	41.9	14.7
ME50	201	3099	4035.5	3561.7	174.7	54	3143.5	4109	3560.8	200.1
EDOM	38	68.2	93	86.9	5.6	9	81.8	91.7	87.5	3.6

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); NDF: Neutral detergent fibre (g/kg); ADF: Acid detergent fibre (g/kg); Starch (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal); dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 2. Descriptive statistics of composition for the alternative ingredients calibration and validation samples sets.

	Calibration					Validation				
Constituent	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Ash	93	4.3	164.4	57.5	29.4	22	17.2	92.7	53.5	25.5
Protein	101	27.7	708.2	269.3	155.3	23	103.1	506.3	261.2	131.2
Fat	93	3.9	201.7	46.5	33.7	20	24	155.6	57.7	35.9
Crude fibre	90	3	318.1	107.7	80.3	20	30.7	302.4	118.6	72.9
A-CHO	88	30.6	911.4	331.4	255.4	19	72.2	646.4	267.4	211
Starch	80	2.8	916.3	256.2	271.3	19	5.2	622.5	210.6	225.2
NDF	93	9.3	725	227.2	150.6	18	108.8	449.6	246.6	100.8
ADF	79	1.8	377.3	138.9	95.2	18	47.7	358.9	157.3	90.3
NFE	87	182.8	897.2	523.4	193.4	21	265.7	765.8	475.2	183.7
GE	85	3810	5228	4587.8	266.6	23	4397.3	5284	4670.6	228
dDM	88	21	96	71.4	17.2	22	48.2	92	70.3	13.2
dOM	86	25	97.7	73.7	17.8	21	49.6	92.1	71.7	12.8
dE	85	23	96.8	71.6	18	21	49.2	89.8	70.7	12.3
dAPro	85	21.9	91.9	71.8	17	21	58	89.3	76.1	7.8
dTPro	86	11.7	100	75.6	17.3	21	14.9	88.4	76.2	15.5
dFAT	77	14.5	86.9	53.5	17.2	21	19.3	94.5	62.3	19.7
dCF	80	8	100	43	22.1	19	8.8	78.9	40.8	19.3
dNFE	85	30.3	99.4	81.4	15.3	21	46	98.1	75.7	15.2
d"DF"	80	12.2	100	54.9	20.3	19	28.9	87.3	49.3	18.9
d"hemi"	81	12.2	100	61.4	20	19	27.7	93.9	54.5	19.3
ME50	84	1117.5	4177	3154.7	723.7	21	2150.5	4083.6	3146	562.5
EDOM	65	16.9	96.2	74.2	17.3	20	56.8	92.6	75.3	11.9

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); NDF: Neutral detergent fibre (g/kg); ADF: Acid detergent fibre (g/kg); Starch (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal); dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 3. Descriptive statistics of composition for the pig diet calibration and validation samples sets.

	Calibration					Validation				
Constituent	N	Min	Max	Mean	SD	N	Min	Max	Mean	SD
Ash	250	23.8	90.8	56.6	9.5	59	24.5	101.4	56.7	11
Protein	284	102.6	299.3	189.2	40	69	108.3	271.3	192.1	37.4
Fat	283	19.6	250.2	54	35.5	68	18	209.3	57.3	44.5
Crude fibre	277	10.5	169.5	57.5	27.1	65	19.4	164.3	57.8	27.7
A-CHO	230	292.2	711.8	522.5	90.3	55	293.3	699.7	508.9	79.8
Starch	152	228.2	622.4	452.3	85.5	41	258.5	694.9	437.2	88.1
NDF	276	67.5	406	156.2	55.5	65	85.2	406	163.3	58.1
ADF	150	27.7	188.4	73.3	32.2	40	29.5	186.8	77.5	34.6
NFE	230	427.6	778.8	645.2	67.1	56	476.7	759	641.4	61.9
GE	175	4140.3	5319.1	4483.9	188.1	45	4192	5211.8	4493.5	220.8
dDM	230	49	97.7	82.2	6.3	55	61.5	93.9	82.3	6.9
dOM	266	50	98.7	84	6.2	64	63	96	84	6.6
dE	267	49.1	98.2	81.7	6.2	63	60.1	95.7	81.4	6.6
dAPro	236	62.5	95.9	80.5	6	58	60.5	96.7	79.9	6.4
dTPro	170	67.9	99.7	84.6	4.7	42	77.7	92.6	84.3	4.3
dFAT	204	34.1	93.8	58.2	12.7	51	33.7	92.8	59.5	13
dCF	170	0.3	79	31	14.5	42	11.5	62.1	30.9	12.2
dNFE	165	59.2	96.5	89.5	5.1	44	69	98.3	89.5	4.9
d"DF"	124	6.8	82.4	45.2	13.5	35	21.6	76.6	48.3	12.4
d"hemi"	122	7.7	83.3	51.5	13.9	36	24.8	92.9	56	13.8
ME50	247	2093	4733.5	3525	332.8	58	2579.7	4687.9	3521.7	375
EDOM	24	66.8	93.1	85.9	6.4	5	53.6	92.9	81.8	16.4

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); NDF: Neutral detergent fibre (g/kg); ADF: Acid detergent fibre (g/kg); Starch (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal); dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 4. Summary statistics for calibration and validation models for macronutrients on cereals.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
Ash	6	5.71	0.48	7.73	0.04	6.9	1.33	-7.22	0.53	8.4	0.33	0.95
Protein	9	3.91	0.95	5.47	0.91	15.75	1.01	-2.51	-1.81	5.47	0.91	3.26
Fat	12	1.64	0.97	3.59	0.85	24.07	1.07	-2.27	0.16	3.96	0.89	2.35
Crude fibre	7	4.47	0.92	5.67	0.87	17.23	1.12	-5.62	-0.4	6.01	0.83	2.68
A-CHO	8	17.93	0.83	25.03	0.68	13.07	1.07	-52.15	-4.17	22.52	0.72	1.94
Starch	7	29.66	0.75	39.97	0.55	8.73	0.87	85.42	1.6	33.91	0.46	1.77
NDF	7	12.35	0.89	16.13	0.81	16.3	1.1	-12.56	0.78	14.95	0.76	2.49
ADF	6	7.23	0.86	8.61	0.8	15.76	0.8	8.36	-2.43	9.89	0.49	1.9
NFE	7	8.34	0.93	12.9	0.84	16.11	1.06	-43.7	0.99	9.16	0.92	3.55
GE	6	59.23	0.45	75.18	0.12	9.54	0.99	20.7	-7.13	61.22	0.46	1.31

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); Starch (g/kg); NDF: Neutral detergent fibre (g/kg); ADF: acid detergent fibre (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal).

Annex 5. Summary statistics for calibration and validation models for macronutrients on alternative ingredients.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
Ash	5	14.45	0.76	18.42	0.6	8.69	0.81	3.92	-7.45	12.5	0.8	2.06
Protein	7	12.19	0.99	18.46	0.99	36.86	0.96	13.81	2.84	16.93	0.99	9.17
Fat	8	4.96	0.98	8.42	0.94	23.49	0.95	0.25	-3.07	5.07	0.98	5.80
Crude fibre	7	10.95	0.98	14.27	0.97	22.07	1.01	-1.17	-0.13	17.25	0.94	4.78
A-CHO	8	20.73	0.99	31.53	0.98	27.94	1	4.94	5.07	21	0.99	12.13
Starch	8	23.95	0.99	40.67	0.98	22.46	0.98	-0.2	-4.98	29.62	0.98	9.27
NDF	8	25.13	0.97	43.52	0.92	16.44	0.94	0.57	-14.21	43.2	0.82	3.40
ADF	8	14.36	0.98	22.94	0.94	16.37	1.09	-7	6.18	20.89	0.95	4.49
NFE	9	11.68	1	20.91	0.99	34.16	0.99	8.82	5.07	21.58	0.99	8.93
GE	3	81.29	0.9	110.62	0.83	12.82	0.89	517.54	15.87	75.4	0.9	3.47

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); Starch (g/kg); NDF: Neutral detergent fibre (g/kg); ADF: acid detergent fibre (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal).

Annex 6. Summary statistics for calibration and validation models for macronutrients on pig diets.

	Calibration					Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
Ash	13	5.63	0.65	7.72	0.33	8.68	0.7	17.87	0.79	9.4	0.34	1.01
Protein	7	11.51	0.92	12.82	0.9	15.35	0.91	15.14	-1.52	17.89	0.78	2.24
Fat	14	6.01	0.97	9.76	0.92	23.62	0.95	4.5	1.52	10.16	0.95	3.48
Crude fibre	12	5.34	0.96	7.7	0.92	20.64	0.94	3.86	0.18	7.1	0.94	3.84
A-CHO	8	23.07	0.93	30.34	0.89	13.83	0.89	49.37	-5.83	32.26	0.85	2.78
Starch	7	33.38	0.85	36.77	0.82	10.72	0.96	8.77	-9.54	44.2	0.75	1.95
NDF	9	13.62	0.94	19.7	0.87	17.18	0.88	22.37	3.75	22.84	0.86	2.43
ADF	10	6.42	0.96	9.72	0.91	16.54	1	3.72	3.65	10.71	0.9	2.88
NFE	13	11.78	0.97	17.05	0.94	20.6	0.87	85.38	-0.57	28	0.82	2.42
GE	11	55	0.91	77.4	0.83	15.23	0.97	133.11	4.48	73.38	0.89	2.58

Ash (g/kg); Protein: crude protein (g/kg); Fat (g/kg); Crude fibre (g/kg); A-CHO: available carbohydrates (starch plus sugars, g/kg); Starch (g/kg); NDF: Neutral detergent fibre (g/kg); ADF: acid detergent fibre (g/kg); NFE: Nitrogen free extract (g/kg); GE: gross energy (kcal).

Annex 7. Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on cereals.

	Calibration					Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
dDM	4	1.49	0.86	1.64	0.82	16.19	1.27	-22.26	-0.11	1.96	0.79	2.02
dOM	5	1.34	0.88	1.5	0.85	18.46	1.21	-17.56	-0.17	1.96	0.77	2.02
DE	4	1.58	0.85	1.72	0.81	9.60	1.23	-18.52	-0.15	2.06	0.76	1.96
dAPro	3	2.91	0.71	3.24	0.64	9.14	1.03	-2.11	-0.06	4.2	0.52	1.30
dTPro	2	2.96	0.64	3.17	0.58	10.12	1.01	-0.84	0.08	4.36	0.36	1.14
dFAT	6	5.78	0.67	7.05	0.51	7.61	0.9	4.32	-0.14	7.67	0.5	1.33
dCF	2	8.24	0.42	8.72	0.35	22.74	0.48	8.2	-3.91	10.6	0.11	0.97
dNFE	6	0.88	0.9	1.1	0.84	7.10	1.12	-10.92	-0.15	1.39	0.74	2.00
d"DF"	3	8.87	0.54	9.52	0.46	6.66	1.17	-7.41	-1.26	9.87	0.48	1.32
d"hemi"	3	9.98	0.43	10.59	0.36	15.25	1.07	-2.95	0.07	11.33	0.32	1.18
ME50	5	80.32	0.79	87.58	0.75	10.69	1.36	-1279.23	-5.17	113.25	0.73	1.56
EDOM	6	0.81	0.98	1.63	0.91	15.17	1.11	-10.16	-0.38	1.16	0.91	4.81

dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 8. Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on alternative ingredients.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
dDM	7	3.68	0.95	5.6	0.89	13.40	0.98	2.47	1.31	4.41	0.89	3.83
dOM	7	3.45	0.96	5.1	0.92	14.27	0.93	6.44	1.57	4.26	0.89	4.01
DE	7	3.57	0.96	5.05	0.92	14.61	0.91	8.59	2.26	4.29	0.89	3.78
dAPro	3	7.78	0.79	9.55	0.68	7.33	0.64	28.33	1.71	5.73	0.67	2.90
dTPro	3	6.87	0.84	8.28	0.77	10.67	1.03	-1.7	0.44	4.55	0.92	3.88
dFAT	4	11.26	0.57	13.98	0.33	5.18	1	7.77	7.98	14.95	0.43	1.03
dCF	8	9.26	0.83	13.73	0.61	6.70	0.99	4.4	3.84	8.78	0.79	2.36
dNFE	7	3.59	0.94	5.38	0.87	12.85	1.12	-10.08	-0.67	6.41	0.83	2.43
d"DF"	3	11.92	0.65	13.04	0.58	6.73	1.2	-10	-0.24	10.85	0.69	1.92
d"hemi"	3	12.4	0.62	13.74	0.52	6.39	1.43	-25.79	-1.77	11.78	0.69	1.73
ME50	7	167.75	0.95	234.64	0.89	13.04	0.89	448.49	104.78	206.91	0.88	3.18
EDOM	5	4.08	0.94	4.78	0.92	16.57	1.14	-11.75	-1.06	4.28	0.88	4.02

dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 9. Summary statistics for calibration and validation models for digestibility of nutrients and energy and metabolisable energy on pig diets.

	Calibration						Validation					
Constituent	Factors	SEC	R^2	SECV	1-VR	RER	Slope	Intercept	Bias	SEP(C)	R^2	RPD
dDM	13	2.24	0.87	2.78	0.8	17.48	0.87	10.85	0.47	3.07	0.82	2.05
dOM	11	1.93	0.9	2.47	0.83	19.74	0.88	10.94	0.53	2.96	0.81	2.02
DE	10	2.31	0.86	2.95	0.77	16.63	0.89	9.6	0.66	3.24	0.77	1.90
dAPro	8	3.02	0.75	3.63	0.64	9.19	0.97	3.01	0.42	3.07	0.77	1.97
dTPro	5	2.81	0.65	3.4	0.48	9.37	0.87	11.64	0.63	3.03	0.52	1.55
dFAT	5	6.86	0.71	7.72	0.63	7.73	0.89	7.63	1.32	7.59	0.67	1.67
dCF	6	8.59	0.65	10.88	0.43	7.24	0.75	9.86	2.74	9.36	0.47	1.50
dNFE	11	1.19	0.94	1.87	0.85	20.00	0.88	11.68	0.62	1.85	0.88	2.52
d"DF"	5	7.98	0.65	9.38	0.51	8.06	0.84	11.06	3.92	8.02	0.6	1.53
d"hemi"	5	8.53	0.62	9.99	0.48	7.57	0.91	9.54	4.91	8.95	0.58	1.37
ME50	7	143.61	0.81	179.24	0.71	14.73	0.96	169.93	44.58	172.44	0.79	1.88
EDOM	4	1.5	0.94	3.4	0.7	7.72	0.95	4.42	0.64	2.73	0.98	2.53

dDM: dry matter digestibility (% DM); dOM: organic matter digestibility (% OM); dE: energy digestibility (%); dAPro: apparent protein digestibility (% AP); dTPro: true protein digestibility (% TP); dFAT: fat digestibility (% FAT); dCF: crude fibre digestibility (% of crude fibre); dNFE: nitrogen free extract digestibility (% NFE); d"DF": calculated dietary fibre digestibility (% "DF", dietary fibre calculated by subtracting protein, ash, A-CHO and fat from dry matter); d"hemi": calculated hemicellulose digestibility (% "hemi", hemicellulose calculated by subtracting soluble carbohydrates from nitrogen free extract); ME50: metabolisable energy corrected for 50% protein retention (kcal/kg DM); EDOM: enzyme digestible organic matter (% OM corrected for ash).

Annex 10. Summary statistics for calibration and validation of the amino acid composition of cereals (g/kg of dry matter).

	Calibration statistics							Validation statistics				
Constituent	N_{CAL}	$Range_{CAL}$	Mean	SD	Factors	R^2_{CAL}	SECV	N_{VAL}	$Range_{VAL}$	R^2_{VAL}	SEP	RPD
Ala	192	2.5 - 8.4	5	0.7	9	0.9	0.32	55	4 - 8	0.76	0.42	1.7
Arg	192	4.4 - 12.1	6.3	1	8	0.89	0.41	55	4.4 - 10.2	0.78	0.53	1.81
Asp	192	5.1 - 14.1	7.2	1.1	8	0.87	0.51	55	5.6 - 11.7	0.76	0.57	1.87
Cys	194	2 - 4.6	2.7	0.4	8	0.88	0.18	55	2 - 4.5	0.73	0.25	1.57
Glu	193	13.7 - 53.3	31.7	7.7	11	0.98	1.93	55	17.4 - 44.4	0.88	2.32	3.31
Gly	192	3.7 - 8.2	5.1	0.7	8	0.88	0.32	55	3.9 - 7.4	0.8	0.34	2.07
His	193	2 - 4	2.8	0.4	5	0.76	0.23	55	2 - 3.8	0.72	0.24	1.71
Ile	194	1.4 - 6.8	4.6	0.7	7	0.88	0.3	55	3.2 - 6	0.77	0.35	2
Leu	191	5.6 - 13.5	8.7	1.4	11	0.96	0.49	55	5.4 - 13.8	0.69	0.93	1.47
Lys	194	2.5 - 8.8	4.3	0.7	8	0.83	0.37	55	2.8 - 6.3	0.67	0.35	1.97
Met	192	1.4 - 3.1	2.1	0.3	7	0.84	0.14	55	1.3 - 2.6	0.59	0.19	1.45
Phe	194	3.8 - 9.1	6.1	1.1	9	0.93	0.39	55	4.1 - 8.5	0.87	0.4	2.64
Pro	194	5.7 - 28.7	12.7	2.8	10	0.96	0.88	55	6.3 - 18.9	0.89	0.93	3
Ser	192	3.9 - 8	5.7	0.9	10	0.94	0.34	55	4 - 7.7	0.82	0.41	2.14
Thr	194	3 - 6.4	4	0.5	10	0.92	0.25	55	3.2 - 5	0.72	0.27	2.02
Trp	64	0.8 - 2.1	1.5	0.3	3	0.65	0.18	15	0.8 - 1.9	0.52	0.2	1.29
Tyr	193	2.4 - 5.8	3.9	0.7	9	0.93	0.25	55	2.6 - 5.7	0.81	0.31	2.16
Val	195	4.4 - 9.4	6.2	0.9	7	0.87	0.42	55	4.8 - 8.2	0.73	0.49	1.89
CP	211	90.6 - 176.7	127	17.8	9	0.95	5.47	59	88.6 - 161.1	0.91	5.47	3.26

Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; CP: crude protein.

Annex 11. Summary statistics for calibration and validation of the amino acid composition of supplemental feed ingredients (g/kg of dry matter).

	Calibration statistics							Validation statistics				
Constituent	N_{CAL}	$Range_{CAL}$	Mean	SD	Factors	R^2_{CAL}	SECV	N_{VAL}	$Range_{VAL}$	R^2_{VAL}	SEP	RPD
Ala	92	1.1 - 30	12.4	6.7	7	0.98	1.58	20	4.8 - 23.5	0.94	1.2	5.63
Arg	92	1.2 - 52.7	19.9	13.3	9	0.99	3.45	20	2.8 - 46.6	0.96	2.64	5.03
Asp	92	1.8 - 79.4	26.2	18.4	7	0.99	3.28	20	6.8 - 39.2	0.92	3.84	4.8
Cys	92	0.3 - 11.1	4.6	2.7	7	0.97	0.6	20	1.1 - 11.8	0.96	0.63	4.33
Glu	92	3.4 - 129.8	50.1	30.6	8	0.98	6.26	20	12.8 - 98.9	0.98	4.12	7.43
Gly	92	1 - 29.2	12.7	7.4	6	0.98	1.54	20	3.6 - 27.6	0.95	1.67	4.41
His	92	0.4 - 19.3	6.7	4.3	6	0.98	0.83	20	2.5 - 14.3	0.96	0.75	5.71
Ile	92	0.8 - 34.6	11.5	7.7	6	0.99	1.09	20	3.7 - 22.6	0.96	1.24	6.2
Leu	92	1.4 - 55.4	19.7	12	9	0.99	1.88	20	8.2 - 38.9	0.96	1.74	6.87
Lys	92	0.9 - 44.4	14.2	10.5	7	0.98	2.33	20	2.8 - 28.3	0.97	1.27	8.29
Met	92	0.3 - 9.7	4.2	2.7	8	0.98	0.6	20	1.8 - 9.7	0.98	0.43	6.2
Phe	92	0.6 - 36.6	12.5	7.7	5	0.98	1.41	20	4.7 - 22.1	0.94	1.43	5.37
Pro	92	1 - 36.1	15.1	8.7	9	0.98	2.51	20	7.2 - 32.5	0.93	2.13	4.1
Ser	92	0.9 - 38.3	12.9	8.2	8	0.99	1.28	20	4.7 - 23.5	0.97	1.16	7.04
Thr	92	0.8 - 27.9	10.2	6.4	6	0.99	1.07	20	3.8 - 21.8	0.97	1	6.44
Trp	47	0.2 - 9.1	3.6	2.4	1	0.93	0.66	9	2 - 6	0.85	0.52	4.63
Tyr	92	0.5 - 26.5	9.1	5.9	4	0.97	1.23	20	3.4 - 16.5	0.97	0.76	7.82
Val	92	1 - 36.2	13.7	8	5	0.99	1.15	20	5.4 - 28.3	0.93	1.85	4.36
CP	101	27.7 - 708.2	269.3	155.3	7	0.99	18.46	23	103.1 - 506.3	0.99	16.93	9.17

Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; CP: crude protein.

Annex 12. Summary statistics for calibration and validation of the amino acid composition of pig feed mixtures (g/kg of dry matter).

	Calibration statistics							Validation statistics				
Constituent	N_{CAL}	$Range_{CAL}$	Mean	SD	Factors	R^2_{CAL}	SECV	N_{VAL}	$Range_{VAL}$	R^2_{VAL}	SEP	RPD
Ala	92	4.1 - 13.6	6.8	2.2	9	0.97	0.65	20	4.2 - 10.7	0.88	0.72	3.06
Arg	93	5 - 20.1	9.7	3.9	8	0.97	1.09	20	5 - 17	0.68	2.44	1.6
Asp	92	5.9 - 26.5	12.5	5.6	9	0.98	1.24	20	5.9 - 23.2	0.81	2.54	2.23
Cys	100	2.1 - 4.7	3.1	0.6	5	0.83	0.29	20	2.3 - 4.2	0.75	0.27	2.1
Glu	93	22.7 - 53.2	37.3	7.9	3	0.86	3.52	20	23.4 - 55	0.79	4	1.97
Gly	92	4 - 16.8	7	2.3	4	0.92	0.75	20	4.1 - 10.9	0.83	0.95	2.4
His	93	2.2 - 8.1	4	1.1	7	0.93	0.42	20	2.4 - 6.3	0.61	0.68	1.64
Ile	91	3.7 - 12.5	6.6	2.1	3	0.94	0.63	20	3.7 - 11.4	0.84	0.84	2.56
Leu	91	6.8 - 20.3	11.7	3.4	6	0.96	0.93	20	6.9 - 18.7	0.84	1.34	2.57
Lys	98	3.2 - 15.5	9	2.3	8	0.87	1.26	20	7.4 - 13	0.3	1.66	1.41
Met	100	1.2 - 4.5	2.8	0.7	9	0.88	0.42	20	1.6 - 3.9	0.16	0.84	0.78
Phe	90	4.7 - 14.1	7.9	2.2	5	0.95	0.62	20	4.9 - 13.3	0.84	0.88	2.54
Pro	93	6.5 - 21.6	13.5	2.9	3	0.75	1.66	20	8.5 - 20.5	0.75	1.45	2
Ser	93	4.4 - 14.7	7.9	2.3	5	0.91	0.87	20	4.6 - 13.1	0.77	1.04	2.21
Thr	101	3.3 - 10.4	6.3	1.5	2	0.6	0.97	20	3.7 - 9.5	0.43	0.88	1.64
Trp	45	1.3 - 3.3	1.9	0.6	7	0.97	0.18	14	1.3 - 3.1	0.81	0.26	2.15
Tyr	74	3.1 - 10.4	5.3	1.8	8	0.98	0.5	18	3.2 - 9.2	0.68	1.01	1.76
Val	91	5.1 - 14.2	8.1	2.3	8	0.97	0.63	20	5.2 - 13.1	0.83	0.91	2.54
CP	284	102.6 - 299.3	189.2	40	7	0.92	12.82	69	108.3 - 271.3	0.78	17.89	2.24

Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; CP: crude protein.

Annex 13. Summary statistics for equations built on the all sample types with independent validation samples representing the total samples, cereals, alternative ingredients or feed mixtures (g/kg of dry matter).

	Total			Cereals			Supplemental ingredients			Mixtures		
Constituent	N	R^2	SEP	N	R^2	SEP	N	R^2	SEP	N	R^2	SEP
Ala	94	0.96	0.84	55	0.75	0.4	20	0.94	1.34	20	0.78	1.04
Arg	94	0.97	1.57	55	0.63	0.91	20	0.98	2.18	20	0.76	2.13
Asp	94	0.97	1.69	55	0.82	0.6	20	0.95	2.92	20	0.87	2.09
Cys	94	0.94	0.44	55	0.61	0.3	20	0.94	0.75	20	0.7	0.34
Glu	94	0.96	3.3	55	0.91	1.94	20	0.98	4.26	20	0.72	4.84
Gly	94	0.96	0.96	55	0.68	0.43	20	0.94	1.7	20	0.8	1.01
His	94	0.97	0.44	55	0.71	0.24	20	0.96	0.66	20	0.75	0.52
Ile	94	0.97	0.71	55	0.76	0.36	20	0.96	1.14	20	0.85	0.84
Leu	94	0.95	1.42	55	0.56	1.03	20	0.95	1.93	20	0.8	1.54
Lys	94	0.96	1.05	55	0.62	0.52	20	0.96	1.5	20	0.24	1.53
Met	94	0.94	0.42	55	0.59	0.19	20	0.95	0.61	20	0.36	0.54
Phe	94	0.96	0.85	55	0.86	0.42	20	0.94	1.45	20	0.82	0.93
Pro	94	0.92	1.36	55	0.9	0.98	20	0.93	1.95	20	0.8	1.3
Ser	94	0.97	0.78	55	0.84	0.38	20	0.97	1.24	20	0.82	0.92
Thr	94	0.96	0.68	55	0.72	0.29	20	0.97	0.94	20	0.3	1.06
Trp	37	0.87	0.45	15	0.42	0.24	9	0.73	0.72	14	0.54	0.41
Tyr	92	0.96	0.58	55	0.65	0.41	20	0.98	0.65	18	0.79	0.83
Vcal	95	0.94	1.13	55	0.72	0.47	20	0.93	1.76	20	0.78	1.05
CP	150	0.95	16.87	59	0.85	7.71	23	0.98	18.26	69	0.72	21.47

Ala: alanine; Arg: arginine; Asp: aspartic acid; Cys: cysteine; Glu: glutamic acid; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine; Val: valine; CP: crude protein.

Annex 14 : Scientific output

The results of the deliverable are currently being considered for publication in a peer-reviewed journals.

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