A comparison of the prediction of apparent metabolisable energy content of starchy grains and cereal by-products for poultry from its chemical components, *in vitro* analysis or near-infrared reflectance spectroscopy

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Abstract

Regression models including chemical composition, *in vitro* digestibility and near infrared reflectance spectroscopy (NIRS) were compared in order to predict the energy value of several feed ingredients for poultry. The nitrogen-corrected apparent metabolisable energy content (AMEn) in cockerels and its proportion on total gross energy (AMEn/GE) were determined in 94 batches from six starchy grains and six cereal byproducts. Two preliminary trials were also designed to adapt *in vitro* methods for prediction of *in vivo* energy values for poultry. Mean concentrations of AMEn of the ingredient studied ranged from 2,464 to 3,595 kcal kg^{-1} DM, and those of AMEn/GE from 53.7 to 80.0%. The most precise model of prediction of AMEn and AMEn/GE values was that based on NIRS equations ($R^2cv = 0.823$ and 0.861, respectively). The best single chemical predictor of these energy values was the neutral detergent fibre concentration ($R^2 = 0.616$ and 0.736, respectively). Further inclusion of ether extract and ash contents in the AMEn model and those of starch and ether extract in the AMEn/GE model allowed increasing coefficients of determination up to 0.791 and 0.839, respectively. A model including linear and quadratic effects of *in vitro* organic matter digestibility (IVOMd) provided a similar prediction of AMEn/GE values ($R^2 = 0.833$). However the prediction of AMEn from IVOMd was worse ($R^2 = 0.62$), as variations among batches of GE concentration (from 4,225 to 5,896 kcal kg^{-1} DM) were little related to *in vitro* digestibility values.

Additional key words: cockerels, energy content, energy utilization, NIRS, prediction models.

Resumen

Comparación de la predicción de la concentración en energía metabolizable aparente de granos ricos en almidón y subproductos de cereales para aves a partir de componentes químicos, análisis *in vitro* o espectroscopía de reflectancia en el infrarojo

En este trabajo se compararon varios modelos de regresión basados en parámetros de composición química, digestibilidad *in vitro* y en la espectroscopía de reflectancia en el infrarrojo cercano (NIRS) al objeto de predecir el valor energético de varios ingredientes alimenticios para aves. La concentración en energía metabolizable aparente corregida por nitrógeno (EMAn) en gallos adultos y su proporción sobre energía bruta (EMAn/EB) se determinaron en 94 partidas de seis concentrados de almidón y en seis subproductos de cereales. Dos ensayos preliminares fueron realizados para adaptar los métodos *in vitro* a los valores energéticos *in vivo* obtenidos en aves. Las concentraciones medias de EMAn de los ingredientes estudiados oscilaron entre 2.464 y 3.595 kcal kg^{-1} MS y las de EMAn/EB entre 53,7 y 80,0%. El modelo más preciso de predicción de los valores de EMAn y EMAn/EB fue el basado en ecuaciones NIRS ($R^2cv = 0,823$ y 0,861, respectivamente). El mejor predictor químico de estos valores energéticos fue la concentración en FND de los ingredientes ($R^2 = 0,616$ y 0,736, respectivamente). La inclusión adicional de los contenidos en extracto etéreo y cenizas en el modelo EMAn y los de almidón y extracto etéreo en el de EMAn/EB permitieron incrementar los coeficientes de determinación hasta 0,791 y 0,839, respectivamente. Un modelo incluyendo los efectos lineales y cuadráticos de la digestibilidad de la materia orgánica *in vitro* (dMOIV) dio lugar a una predicción similar de los valores de EMAn/EB ($R^2 = 0,833$). Sin embargo, la predicción de EMAn a partir de dMOIV fue peor ($R^2 = 0,62$), puesto que las variaciones entre partidas de la concentración en EB (desde 4.225 hasta 5.896 kcal kg^{-1} MS) estuvieron escasamente relacionadas con los valores de digestibilidad *in vitro*.

Palabras clave adicionales: concentración energética, eficacia energética, gallos adultos, modelos de predicción, NIRS.

Introduction

Direct determination of energy values of feeds in *in vivo* trials is expensive and time-consuming; it also requires animal facilities and relatively large amounts of experimental diets. Chemical analyses, *in vitro* methods and near infrared reflectance spectroscopy (NIRS) techniques have been used in several animal species to estimate energy content of feeds and diets. These methods are rapid and economical, which make them more adequate to take into account the variability of the raw materials used by the poultry feed industry. However, its capability to estimate feed energy contents must be validated with *in vivo* determined values.

At present, several regression equations are available in poultry for the estimation of apparent metabolisable energy (AME) values from chemical components in compound feeds (e.g. Carpenter and Glegg, 1956; Sibbald *et al.*, 1980; Fisher, 1982; Carré *et al.*, 1984; EEC, 1986). However, prediction equations for feed ingredients are scarcer and its validity is limited to the conditions where they were obtained (Dolz and De Blas, 1992; Francesch, 2001). This approach is also limited by the time required for the chemical analyses and their accuracy.

The use of multiple-enzymatic *in vitro* methods has been proven to be a good alternative to chemical analyses to simulate the digestive processes and to predict energy values with a greater precision in non ruminant species as pigs (Boisen and Fernández, 1997; Noblet and Jacquelin-Peyraud, 2007) and rabbits (Ramos *et al.*, 1992; Pascual *et al.*, 2000). A two-step *in vitro* method using pepsin, pancreatin, bile acids and enterokinase has been tested in poultry complete diets by Valdes and Leeson (1992c). Its repeatability was similar to *in vivo* trials but the residual standard deviation of the prediction was high for some of the diets studied.

Previous studies have also shown that NIRS technique allows estimating succesfully the major chemical constituents and the digestion efficiency in several animal species (Roberts *et al.*, 2004), including the energy values of complete poultry feeds (Valdes and Leeson (1992a). However, AME values of a limited number of single ingredients were not well predicted from NIRS, neither when using equations calculated for a limited number of ingredients or when using equations derived from complete diets (Pérez-Vendrell *et al.*, 1992; Valdes and Leeson 1992d, 1994; Garnsworthy *et al.*, 2000).

The aim of this research has been to establish a method of prediction of *in vivo* apparent metabolisable energy (AMEn) values in several poultry feed ingredients, using chemical analysis, *in vitro* digestion and NIRS techniques.

Material and methods

Ingredients

Sixty batches of six starchy grains: wheat (*Triticum aestivum* L., *Triticum turgidum* L.), barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), sorghum (*Sorghum vulgare* L.), rye (*Secale cereale* L.) and peas (*Pisum sativum* L.) and 34 batches of six cereal byproducts (corn gluten feed, rice bran, wheat bran and dry distillers grains and solubles (DDGS) from wheat, corn and sorghum), were sampled from the COREN SCL poultry feed manufacturing plant throughout a 3-yr period. The number of samples and the mean and range of chemical composition within each ingredient are shown in Table 1.

Apparent metabolisable energy determination

Energy values for feed ingredients were determined *in vivo* by using the difference method. Experimental diets were made by substituting with the ingredients studied a 40% of three basal diets formulated to avoid an excessive imbalance in dietary essential nutrients before and after substitution. The chemical and raw material composition of the basal diets is shown in Table 2.

Trials were conducted in 20 series, each series including the evaluation of four to five feed ingredients and the corresponding basal diet. Eight adult cockerels (Warren) were randomly assigned to each experimental

Abbreviations used: ADF (acid detergent fibre), ADL (acid-detergent lignin), AME (apparent metabolisable energy), AMEn (nitrogen-corrected AME), CF (crude fibre), CP (crude protein), DDGS (dry distillers grains and solubles), DM (dry matter), GE (gross energy), IVDMd (*in vitro* digestibility of dry matter), IVOMd (*in vitro* digestibility of organic matter), NDF (neutral detergent fibre), NIRS (near infrared reflectance spectroscopy), S (sugars), SEC (root mean square error), SECV (standard error of cross-validation), SEP (standard error of prediction).

Table 1. Chemical composition (%) of the feed ingredients studied

Ingredient	n[a]	Moisture	Ash	Crude protein	Ehter extract	Crude fibre	NDF[b]	ADF[c]	ADL[d]	Starch	Sugars	Gross energy (kcal g^{-1} DM)
Wheat grain	13											
Mean		13.0	1.4	11.5	1.5	2.5	13.1	3.3	1.0	58.8	2.5	4,408
SD[e]		0.9	0.2	1.0	0.3	0.4	1.1	0.4	0.1	1.5	0.2	29.1
Min		10.9	1.0	10.2	1.2	1.9	10.6	2.8	0.8	55.0	2.2	4,358
Max		14.4	1.6	13.6	2.2	3.2	14.7	4.0	1.1	61.1	2.8	4,455
Barley grain	12											
Mean		11.2	2.1	10.5	1.9	4.5	20.4	5.3	0.9	52.0	2.7	4,404
SD		1.1	0.2	1.0	0.2	0.3	1.6	0.3	0.2	1.6	0.3	65.0
Min		9.6	1.7	8.8	1.6	4.1	18.3	4.9	0.5	49.6	2.4	4,225
Max		13.2	2.4	12.2	2.2	4.8	23.1	6.0	1.2	55.1	3.4	4,457
Corn grain	12											
Mean		13.7	1.2	7.5	3.7	2.1	11.3	2.6	0.4	61.9	1.9	4,495
SD		1.2	0.1	0.5	0.3	0.2	0.8	0.3	0.1	1.4	0.2	38.9
Min		12.9	1.1	6.9	3.0	1.8	10.4	2.1	0.2	59.6	1.7	4,427
Max		16.7	1.4	8.9	4.1	2.5	12.9	3.1	0.6	63.9	2.2	4,551
Sorghum grain	11											
Mean		13	1.4	8.9	2.7	2.2	9.3	3.7	0.7	64.8	1.0	4,463
SD		0.2	0.2	1.0	0.2	0.3	1.1	0.5	0.1	1.7	0.3	37.0
Min		12.6	1.1	7.4	2.4	1.8	7.8	2.7	0.4	61.6	0.7	4,432
Max		13.2	1.6	11.1	2.9	2.7	11.3	4.3	0.8	67.6	1.7	4,568
Rye grain	9											
Mean		12.3	1.7	9.5	1.3	2.3	14.9	3.1	1.0	52.7	4.6	4,369
SD		0.4	0.2	0.7	0.2	0.4	2.0	0.5	0.2	1.1	0.2	15.3
Min		11.9	1.5	8.4	0.8	1.9	12.1	2.6	0.8	51.1	4.3	4,346
Max		13.1	2.0	10.7	1.5	3.1	17.5	4.0	1.5	54.4	4.9	4,398
Peas	4											
Mean		13.0	3.1	21.9	1.4	6.0	16.0	7.0	0.5	42.6	3.6	4,443
SD		0.6	0.4	3.8	0.1	0.6	1.5	0.1	0.1	4.0	0.4	24.8
Min		12.2	2.9	19.8	1.3	5.1	15.2	6.6	0.4	36.7	3.2	4,408
Max		13.5	3.7	27.6	1.5	6.3	18.2	7.6	0.6	44.9	4.0	4,467
Corn gluten feed	4											
Mean		11.6	6.1	18.9	3.8	6.7	36.0	9.0	1.3	25.1	2.2	4,528
SD		0.9	0.8	0.8	0.3	0.9	2.0	0.9	0.4	1.1	0.1	52.3
Min		10.6	5.2	18.2	3.4	5.6	33.3	8.1	0.8	23.8	2.0	4,486
Max		12.3	6.9	20.0	4.0	7.7	38.1	9.9	1.6	26.5	2.3	4,604
Rice bran	4											
Mean		8.7	6.4	15.1	22.4	9.9	26.4	11.8	4.4	18.5	5.1	5,707
SD		0.5	0.2	0.6	0.8	0.8	3.4	1.3	0.3	2.9	0.2	127
Min		8.2	6.2	14.3	21.6	8.8	23.1	9.8	4.2	14.7	4.8	5,624
Max		9.3	6.7	15.7	23.3	10.7	31.2	12.7	4.8	21.6	5.3	5,897
Wheat bran	10											
Mean		10.9	4.4	15.6	3.3	7.9	35.1	10.7	3.3	27.3	4.7	4,589
SD		0.9	0.4	1.2	0.5	0.6	3.6	1.8	0.8	4.1	0.4	39.2
Min		9.2	3.4	13.2	2.5	7.2	29.6	8.2	2.4	19.6	4.2	4,503
Max		12.1	5.0	17.0	4.0	8.9	40.3	14.2	4.7	32.5	5.7	4,651
Wheat DDGS	4											
Mean		7.9	4.5	33.6	4.3	6.6	27.6	8.8	4.4	7.4	4.6	4,970
SD		0.3	0.4	1.1	0.3	0.2	1.0	0.8	0.6	1.2	0.2	43.2
Min		7.5	4.1	32.6	4.0	6.4	26.2	7.9	3.4	5.8	4.4	4,906
Max		8.2	5.0	35.2	4.6	6.8	28.8	9.8	4.8	8.7	4.8	5,001

Table 1 (cont.). Chemical composition (%) of the feed ingredients studied

Ingredient	n[a]	Moisture	Ash	Crude protein	Ehter extract	Crude fibre	NDF[b]	ADF[c]	ADL[d]	Starch	Sugars	Gross energy (kcal g^{-1} DM)
Corn DDGS	6											
Mean		9.7	5.3	27.1	7.6	6.9	33.6	10.3	2.9	11.9	2.2	5,039
SD		1.8	0.4	1.1	1.1	0.4	2.6	1.3	1.0	3.1	0.3	72.0
Min		7.2	4.9	25.6	6.4	6.3	29.9	8.5	1.4	8.7	1.6	4,962
Max		11.4	6.0	28.2	8.9	7.2	36.1	11.8	4.2	15.4	2.5	5,115
Sorghum DDGS	6											
Mean		8.3	5.3	30.1	9.1	7.2	25.8	9.6	3.3	8.3	2.4	5,352
SD		0.4	0.7	2.1	0.9	1.0	1.4	0.9	0.4	2.3	0.3	143
Min		7.5	4.7	27.5	7.9	6.0	23.9	7.9	3.0	5.6	2.0	5,154
Max		8.7	6.2	32.3	10.2	8.2	27.7	10.3	4.0	11.7	2.8	5,508

[a] n: number of samples. [b] NDF: neutral detergent fibre. [c] ADF: acid detergent fibre. [d] ADL: acid-detergent lignin. [e] SD: standard deviation.

diet. Animals were housed in individual metabolic cages (0.3 × 0.5 × 0.4 m high) with wire floors, and kept in an environmentally controlled room. Feed ingredients were ground (in a hammer mill, 6 mm of grill size), mixed with basal diets and given in mash form to birds.

Determination of AME of the experimental diets was made following the European reference method (Bourdillon *et al.*, 1990). The droppings were dried in a forced-draught oven at 80°C to constant weight. After drying, the excreta samples were ground in a coffee mill and then stored in a sealed container at 4°C prior to chemical analysis.

The AME values were calculated using the following formula with appropriate corrections made for differences in DM content:

$$AME\left(\frac{kcal}{kg}\ of\ diet\right) =$$

$$= \frac{(Feed\ intake \times GE_{diet}) - (Excreta\ output \times GE_{excreta})}{Feed\ intake}$$

Nitrogen-corrected AME (AMEn) was calculated by correction to zero nitrogen retention by simple multiplication with 8.22 kcal g^{-1} of nitrogen retained in the body as described by Hill and Anderson (1958).

Chemical analyses

The proximate composition of feed ingredients, experimental diets and bird excreta were analyzed in duplicated samples using the procedures of AOAC (2000) for dry matter (DM) (930.15), ash (923.03), total sugars (974.06), ether extract (920.39) and crude fibre (978.10). Concentration of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid-detergent lignin (ADL) was determined according to the sequential method of Van Soest *et al.* (1991) using heat stable amylase (A3306, Sigma) and sodium sulfite, and expressed without residual ash. Starch content was measured following the alpha-amyloglucosidase method (996.11; AOAC, 2000). Nitrogen was measured by combustion (method 968.06; AOAC, 2000) using a VarioMax ELEMENTAR analyzer (Hanau, Germany). Gross energy was determined in an adiabatic bomb calorimeter (Parr Instruments, USA) standardized with BIPEA reference samples.

In vitro technique

Determination of *in vitro* digestibility of dry matter (IVDMd) and organic matter (IVOMd) of feed ingredient samples was based on the multi-enzymatic method proposed for pigs by Boisen and Fernández (1997). In each of the series, a reference sample (corn grain) was included. These samples were used as a blanck to correct IVDMd and IVOMd values for differences among the successive series. In the first digestion step, series of up to 30 duplicated samples were incubated with pepsin at pH 2 and 39°C during 2 h. In the second digestion step, samples were incubated with pancreatin (a mixture of protease, amylase and lipase) at pH 6.8 and 39°C during 7 h.

A preliminary *in vitro* trial was done to set the digestion duration at the second step (by comparing eight incubation times increasing from 4 to 19 h with wheat and

Table 2. Ingredient and chemical composition of the basal diets (% as fed basis)

	Group of ingredients		
	Cereal grains	Peas	Cereal byproducts
Ingredients			
Corn grain	34.4	86.0	65.6
Soybean meal 44% crude protein (CP)	22.0	1.0	5.4
Sunflower 33% CP	14.3	—	6.0
Lard	2.9	0.95	1.0
Wheat bran	13.9	—	10.0
Calcium carbonate	5.6	5.8	6.1
Calcium bicarbonate	0.1	0.1	—
Dicalcium phosphate	3.9	4.3	4.0
Sodium chloride	0.9	0.9	1.0
Formic acid	0.9	0.3	0.3
Alimet	0.1	—	—
Lysine, 50%	0.2	—	—
Threonine	0.02	—	—
Vitamin-mineral premix[a]	0.6	0.3	0.6
Chemical analysis			
Crude protein	19.2	6.7	10.9
Lysine[b]	1.0	0.2	0.4
Threonine[b]	0.74	0.24	0.40
Methionine[b]	0.44	0.14	0.21
Crude fibre	6.2	2.3	4.03
Starch	25.3	53.8	43.4
Ether extract	5.0	3.8	3.6
Calcium[b]	3.0	3.1	3.2
Available phosphorus[b]	0.8	0.8	0.8

[a] Provides the following nutrients (per kg of diet): vitamin A (trans-retinyl acetate): 12,000 (IU); vitamin D_3 (cholecalciferol): 3,000 (IU); vitamin E (all-rac-tocopherol-acetate): 18 (IU); vitamin K_3 (bisulphate menadione complex): 2 mg; pantothenic acid (D-Ca pantothenate): 10 mg; nicotinic acid: 40 mg; vitamin B_{12} (cobalamin): 15 µg; D-biotin: 80 µg; folic acid: 0.5 mg; Se (Na_2SeO_3): 0.25 mg; I (KI): 1.9 mg; Cu ($CuSO_4 \cdot 5H_2O$): 12 mg; Fe ($FeSO_4 \cdot 7H_2O$): 60 mg; Mn ($MnSO_4 \cdot H_2O$): 100 mg; Zn (ZnO): 80 mg. [b] According to FEDNA (2003).

corn grain samples). Another preliminary test was done to determine in the same samples the value of doing a third digestion step using microbial carbohydrases (Viscozyme 120 L, 120 FBG g^{-1}) at pH = 4.8 and 39°C, as described by Boisen and Fernández (1997). The *in vitro* digestibilities of dry matter and organic matter were calculated from the difference between concentrations in the sample and the indigested residue, after corrections for values obtained with reference samples.

NIRS analysis

NIRS analysis was performed on ground (0.7 mm) samples of the ingredients studied using a near-infrared reflectance spectrophotometer (model 6500; FOSS-NIR System, Silver Spring, MD) equipped with spinning sample cup module. Samples were scanned between 400 and 2,498 nm and spectra were recorded with the ISI NIRS 3 software version 3.11 (Infrasoft International, Port Matilda, PA). Measurements were performed in duplicate with repacking of the cup, and spectra for the subsamples were averaged to provide one spectra per sample.

Statistics

Prediction equations of AMEn *in vivo* values from chemical and *in vitro* analysis were developed by stepwise regression analysis, using PROC REG of SAS (1990). The stepwise procedure introduced variables in the model only if they contributed to a significant improvement ($P < 0.05$) in the estimation of the dependent variable.

The NIRS calibrations were developed using the all-sample set of full-scan mean spectra (n = 94). The population boundaries for calibration were set with a maximum standardized H (distance between a sample and the centroid of the group) value of 3.0 (Shenk and Westerhaus, 1991), and no samples were marked as outliers. The NIRS models were set up with a modified partial least squares regression, after scattering correction with the standard normal variate transformation combined with detrending. Additional mathematical pretreatments were performed by second derivative treatment. Cross-validation was used to select the optimal number of partial least squares factors and to avoid overfitting (Shenk and Westerhaus, 1995). No outlier elimination pass was accepted. Calibration equations were evaluated in terms of coefficient of determination (R^2c) and root mean square error (SEC). Validation errors were combined in a standard error of cross-validation (SECV). Prediction error was measured by dividing the calibration samples into subsets (n = 4) with one subset reserved for validation and the remaining for calibration. Cross-validation was repeated until all subsets were used for validation once. Shenk and Westerhaus (1996) reported that the SECV is the best single estimate of the prediction capability of NIRS equations, and that this statistic is similar to the average

standard error of prediction (SEP) from 10 randomly-chosen prediction sets. The repeatability standard deviation and coefficient of variation of laboratory procedures and NIRS spectra was also determined from ten subsamples of two batches, one of corn grain and another one of peas.

Results and discussion

Average and standard deviation of AMEn values in the feedstuffs studied

Values of AMEn of the ingredients studied determined by difference are shown in Table 3. Mean values varied from 2,464 (wheat bran) to 3,595 kcal kg^{-1} DM (corn grain). Standard deviation of AMEn was 490 kcal kg^{-1} DM for all the samples studied and 154 kcal kg^{-1} DM for average variation within ingredients. The degree of metabolizity of gross energy (GE), expressed as the proportion AMEn/GE (%) for each ingredient was also calculated, and the average values are shown in Table 3. Absolute AMEn concentrations determined in the current study for high starch cereal grains (corn, sorghum, wheat) were slightly below (by about 4%) than the average values assigned to these ingredients by several international Feed Tables (NRC, 1994; INRA, 2002; FEDNA, 2003; CVB, 2004). This difference might be explained by a higher proportional weight of endogenous energy losses in the birds used in the current study (fed near maintenance level), with respect to productive animals. In the same way, AMEn values of corn grain and corn DDGS were 3.75% higher in layer hens than in cockerels (Losada *et al.*, unpublished data). However, *in vivo* AMEn values measured for low starch grains (rye and barley) were close, whereas those obtained for cereal byproducts were clearly higher (between 8 and 29%) than those assigned as average in the Feed Tables. This result indicates a relative underestimation of this group of feed ingredients when using mean table values, which was directly proportional to its NDF content ($r = 0.71$; $P < 0.01$). The highest deviations were observed for DDGS, which might also reflect recent improvements of the method of production of these byproducts still not considered in Feed Tables. In the same way, recent work in poultry (Batal and Dale, 2006; Fastinger *et al.*, 2006) has reported AMEn values for corn DDGS even higher than those obtained in the current study.

Prediction of AMEn/GE and AMEn from chemical composition

A stepwise regression analysis was made to predict AMEn/GE and AMEn of all the ingredients studied from the determined chemical composition traits. The regression equations obtained are presented in Table 4. The NDF concentration was the first independent variable included in both models, explaining 73.8 and 61.6% of the variation of AMEn/GE and AMEn, respectively. This relationship indicates the strong negative effect

Table 3. *In vivo* apparent metabolisable energy (AMEn, kcal kg^{-1} DM), degree of metabolizity of gross energy (AMEn/GE[a], %) and *in vitro* dry matter (IVDMd, %) and organic matter (IVOMd, %) digestibilities of the ingredients studied

Ingredient	AMEn		AMEn/GE		IVDMd		IVOMd	
	Mean	SD[b]	Mean	SD	Mean	SD	Mean	SD
Wheat grain	3,380	150	76.7	3.4	89.8	0.8	89.7	0.8
Barley grain	3,127	217	71.0	5.2	82.2	1.0	82.5	1.1
Corn grain	3,595	148	80.0	3.3	90.0	1.5	90.0	1.5
Sorghum grain	3,530	149	79.2	3.5	91.0	1.9	91.1	1.8
Rye grain	3,118	258	71.3	5.9	87.0	0.8	86.1	1.8
Peas	2,668	44	60.0	0.7	83.1	0.5	83.3	0.4
Corn gluten feed	2,383	71	52.6	0.9	71.0	1.3	72.0	1.3
Rice bran	3,420	76	59.9	1.7	69.1	3.1	70.0	3.0
Wheat bran	2,464	264	53.7	5.6	64.6	3.3	64.7	3.2
Wheat DDGS[c]	2,761	164	55.6	3.7	75.0	0.8	75.4	0.8
Corn DDGS	2,806	108	55.7	2.1	73.8	1.1	74.1	1.1
Sorghum DDGS	2,922	202	54.6	3.3	74.7	1.3	75.5	0.4

[a] GE: Gross energy. [b] SD: Standard deviation. [c] DDGS: dry distillers grains and solubles.

Table 4. Stepwise regression analysis for dependent variables AMEn/GE (%) and AMEn (kcal kg^{-1} DM) using chemical composition traits (% DM) as predictors (n = 94)

Dependent variable	Step	Regression equation	R^2	RSD
AMEn/GE	1	AMEn /GE = 88.6 (±1.43)[a] – 0.920 (±0.057) NDF[b]	0.736	5.76
AMEn/GE	2	AMEn/GE = 64.0 (±3.69) – 0.407 (±0.086) NDF + 0.265 (±0.038) starch	0.829	4.66
AMEn/GE	3	AMEn/GE = 58.3 (±4.32) – 0.341 (±0.089) NDF + 0.322 (±0.044) starch + + 0.291 (±0.123) EE	0.839	4.55
AMEn	1	AMEn = 3,840 (±66.0) – 32.1 (±2.64) NDF	0.616	265
AMEn	2	AMEn = 3,810 (±58.1) – 36.5 (±2.45) NDF + 28.2 (±5.25) EE	0.718	232
AMEn	3	AMEn = 3,697 (±52.9) – 11.7 (±4.63) NDF + 57.1 (±6.58) EE – 177 (±29.7) Ash	0.791	198

[a] Values in parentheses are standard errors. [b] NDF: neutral detergent fibre.

of dietary fibre on energy utilization in poultry, an effect that was consistent throughout the whole interval of NDF studied (see Fig. 1). Type of fibre, expressed either as proportion of ADL on NDF or ADF, or by the concentration of hemicelluloses and cellulose (calculated, respectively, from the differences NDF-ADF and ADF-ADL), had no significant influence beyond that of dietary level of fibre. This result could reflect the inability of birds to digest any of the constituents of the insoluble fibre from these ingredients. In the model of prediction of AMEn/GE, the stepwise procedure included in two further steps significant corrections ($P < 0.05$) to take into account the relatively high digestion efficiency of starch and ether extract fractions. In the case of the AMEn model, ether extract and ash content were included in steps 2 and 3, as they were able to explain part of the variation of GE concentration among batches (from 4,225 to 5,896 kcal kg^{-1} DM). The inclusion of these additional independent variables allowed to decrease the RSD of the models from 5.76 to 4.55% (AMEn/GE) and from 265 to 198 kcal AMEn kg^{-1} DM (see Table 4).

Another stepwise regression equation was calculated to predict AMEn including as independent variables the ingredient concentrations (% DM) of ether extract (EE), ash, total sugars (S) and that of crude fibre (CF) instead of NDF. This equation had a RSD similar to that in the third step in Table 4 and is useful for feed manufacturers using Weende instead of Van Soest fibre analysis method:

AMEn (kcal kg^{-1} DM) =
= 3,775 (±48.7) – 47.7 (±18.7) CF+ 65.9 (±4.93) EE –
– 170 (±25.1) ash – 50.3 (±15.2) S;
RSD = 177; n = 96; R^2 = 0.833.

Prediction from *in vitro* digestibilities

In the first preliminary *in vitro* trial, organic matter digestibility (IVOMd) increased with time at the second incubation step from 4 to 7 h, especially in the case of corn grain, and reached a plateau after that (see Table 5). Accordingly, the relative IVOMd values of wheat and

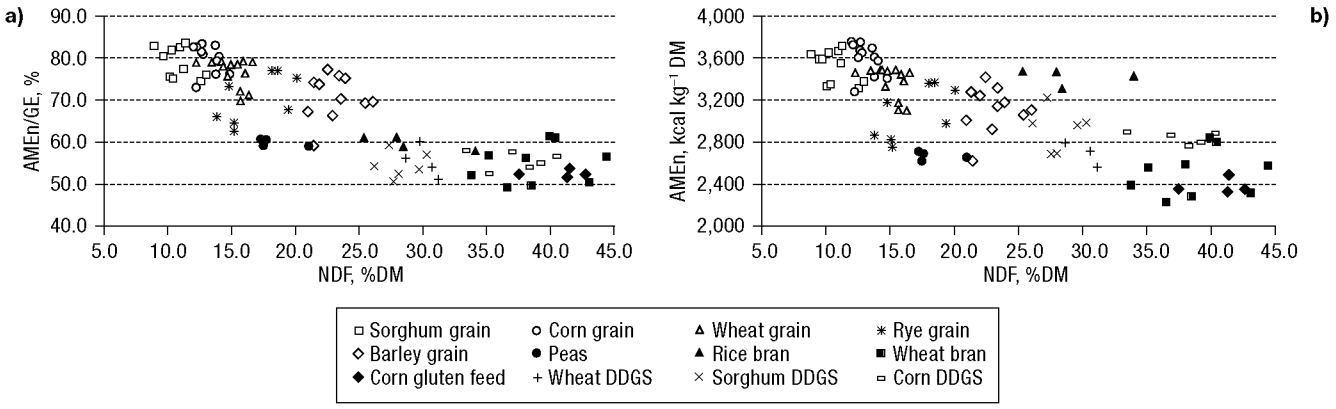

Figure 1. Relationship between: a) AMEn/GE and NDF content, and b) AMEn and NDF content of the samples studied.

Table 5. Effect of the incubation time with pancreatin on the 2-steps *in vitro* digestibility of the organic matter (%) of wheat and corn samples

Incubation time (h)	Wheat grain	Corn grain	Relative value wheat/corn
4	83.2	79.4	1.05
5	84.4	81.6	1.03
6	85.1	85.8	0.991
7	85.2	88.1	0.967
10	85.4	89.2	0.957
13	86.3	89.7	0.962
16	86.2	89.6	0.962
19	86.7	90.5	0.958

corn grain samples decreased from 1.05 at 4 h to 0.967 at 7 h, with little variation at higher incubation times (see Table 5). Furthermore, relative values between wheat and corn after 7 h of incubation were close to the relative *in vivo* AMEn value obtained for the same samples (0.95). In the second preliminary trial, the addition of fibrolytic enzymes in a third incubation step led to IVOMd values much higher (92.2 and 90.9% for the wheat and corn samples, respectively) than those obtained with the two-steps technique and than those determined for the proportion AMEn/GE in the same samples. Moreover, the relative value wheat/corn obtained (1.01), led to a worse prediction of *in vivo* AMEn relative value than that determined with the two-steps technique. According to the results obtained in these preliminary trials, the duration of the digestion in the second *in vitro* step was set at 7 h, and third step was not done in the further *in vitro* trials of this experiment. The results of IVDMd and IVOMd obtained with this procedure for each of the ingredients studied are presented in Table 3.

A regression analysis showed a significant ($P < 0.001$) linear effect of IVDMd and IVOMd on *in vivo* AMEn/GE and AMEn values (see Table 6 and Fig. 2). A significant ($P < 0.01$) quadratic effect was also observed, as the differences between *in vivo* AMEn/GE and IVd values were smaller in cereal byproducts than in starchy grains. In the case of AMEn, prediction was significantly improved ($P < 0.001$) when GE or chemical constituents related to GE concentration, as ether extract, ash or crude protein, were also included in the model (up to RSD values of 211 or 171 kcal kg^{-1} DM, respectively). Absolute values of IVDMd and IVOMd were similar for each of the ingredients studied (see Table 3), although the RSD of the regression equations to predict *in vivo* energy values were slightly improved when including IVOMd instead of IVDMd as independent

Table 6. Equations for prediction of AMEn/GE (%) and AMEn (kcal kg^{-1} DM) from *in vitro* digestibilities of dry matter (IVDMd, %) and organic matter (IVOMd, %) (n = 94)

Regression equation	R^2	RSD
AMEn/GE = 162 (±41) – 3.59 (±1.05) iVDMd + 0.030 (±0.0066) $IVDMd^2$	0.813	4.87
AMEn/GE = 181 (±39) – 4.12 (±1.00) IVOMd + 0.033 (±0.0063) $IVOMd^2$	0.833	4.61
AMEn = 4,687 (±2,316) – 79.1 (±59.5) IVDMd + 0.72 (±0.38) $IVDMd^2$	0.591	275
AMEn = 5,339 (±2,240) – 97.6 (±57.5) IVOMd + 0.85 (±0.36) $IVOMd^2$	0.621	265

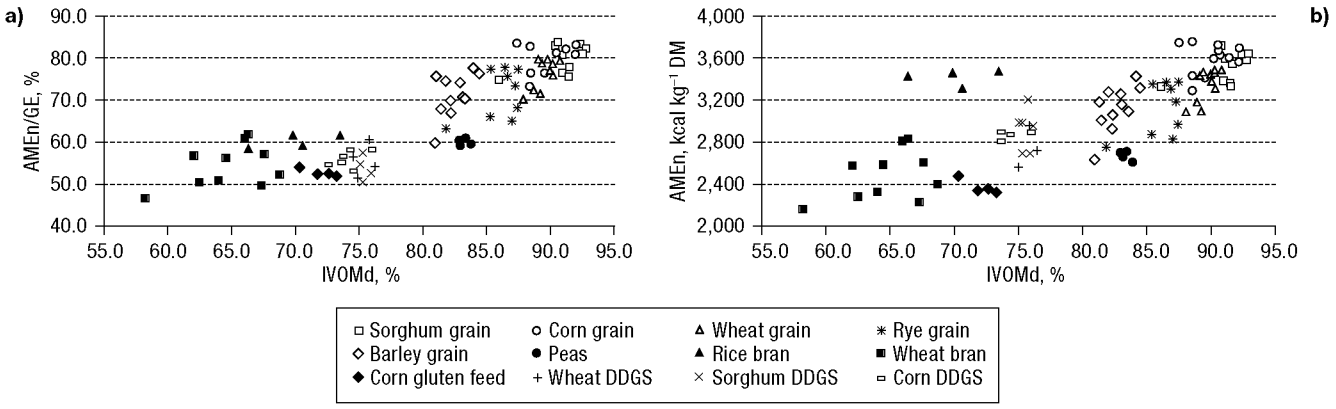

Figure 2. Relationship between: a) AMEn/GE and IVOMd, and b) AMEn and IVOMd of the samples studied.

variable in the model (see Table 6). The comparison between regression equations based on chemical constituents (Table 4) or the combination of *in vitro* digestibilities and chemical constituents (see above), shows that both models led to a similar accuracy of prediction of AMEn/GE and AMEn values. Otherwise, the repeatability of IVDMd and IVOMd ($CV_R = 1.05\%$) was similar to that reported in complete diets for pigs (0.9%, Noblet and Jaguelin-Peyraud, 2007) and rabbits (1.09%, Carabaño *et al.*, 2008). This value was better than that obtained for NDF analyses (3.5%).

Prediction from NIRS analysis

Calibration and cross validation statistics of prediction of nutrient composition, *in vitro* digestibility and energy value of the ingredients tested from NIRS analysis is shown in Table 7. The repeatability of the NIRS method was estimated from the variability of the energy values predicted in homogeneous analytical conditions. The coefficient of variation obtained for AMEn/GE and AMEn were, respectively, 0.393 and 0.497% (corn grain) and 0.504 and 0.479% (peas subsamples). The coefficients of determination and values of SECV obtained confirm the utility of NIRS to predict chemical composition (Pérez-Marín *et al.*, 2004) and *in vitro* digestibility in poultry diets (Valdes and Leeson, 1992b). These relationships among NIRS and laboratory analyses could explain its accuracy to predict the energy value of feed ingredients. The ratio of SD to SECV values in Table 7 for AMEn and AMEn/GE was 2.72 and 2.70, which makes the prediction «good», according to Williams and Sobering (1996). This ratio should be ideally at least of three, unless variance of the reference data is low, as it is the case in the current study. Differences in the variance of data also explain the higher coefficients of determination observed in the current study with respect to those determined in a shorter range of ingredients variation, as samples of barley ($R^2 = 0.61$, Pérez-Vendrell *et al.*, 1992) or wheat ($R^2 = 0.45$, Garnsworthy *et al.*, 2000). On the other hand, prediction results in the current study were poorer than those obtained by Valdes and Leeson (1992a) for complete poultry diets, which might be explained by a higher error of the determination of AMEn by the difference with respect to the direct method.

Conclusions

The results of the current study indicate that AMEn/GE values for poultry of starchy grains and cereal byproducts can be predicted with a good precision using different regression models. The accuracy of the equations was slightly higher for NIRS than for regression models including *in vitro* digestibility or a combination of chemical constituents of the ingredients studied. All the techniques were less accurate for predicting AMEn than AMEn/GE. This was especially the case of *in vitro* digestibility, where additional inclusion of several chemical constituents was required to reach a comparable accuracy level than that obtained with the other methods.

Table 7. Coefficients of determination and root mean square errors of calibration (R^2c, SEC) and cross validation (R^2cv, SECV) to predict the chemical composition (%) and the energy value of the ingredients studied (n = 94)

	R^2c	SEC	R^2cv	SECV	SD/SECV
Dry matter	0.958	0.41	0.932	0.53	3.75
Ash	0.982	0.23	0.971	0.30	5.97
Crude protein	0.998	0.42	0.997	0.56	13.6
Ether extract	0.961	0.93	0.950	0.96	4.55
Crude fibre	0.980	0.36	0.952	0.56	4.45
NDF	0.969	1.81	0.958	2.22	4.21
ADF	0.937	0.79	0.910	1.02	3.21
ADL	0.916	0.38	0.878	0.48	2.81
Starch	0.999	1.35	0.998	1.56	13.7
Total sugars	0.971	0.24	0.939	0.34	3.79
Gross energy, kcal kg^{-1} DM	0.984	49.1	0.970	61.7	5.83
IVOMd, %	0.970	1.49	0.958	1.87	4.83
AMEn/GE, %	0.882	3.79	0.861	4.14	2.70
AMEn, kcal k^{-1} DM	0.863	160	0.823	180	2.72

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