

## Using near infrared spectroscopy to predict metabolizable energy of corn for pigs

Silvia Leticia Ferreira<sup>1\*</sup>, Ricardo Souza Vasconcelos<sup>2</sup>, Robson Marcelo Rossi<sup>3</sup>, Vinicius Ricardo Cambito de Paula<sup>4</sup>, Marcelise Regina Fachinello<sup>1</sup>, Laura Marcela Díaz Huepa<sup>1</sup>, Paulo Cesar Pozza<sup>2</sup>

<sup>1</sup>State University of Maringá – Dept. of Animal Sciences, Av. Colombo, 5790 – 87020-900 – Maringá, PR – Brazil.

<sup>2</sup>State University of Maringá – Dept. of Animal Sciences.

<sup>3</sup>State University of Maringá – Dept. of Statistics.

<sup>4</sup>São Paulo State University/FMVZ – Dept. of Animal Production, R. Prof. Dr. Walter Mauricio Correa, s/n – 18618-681 – Botucatu, SP – Brazil.

\*Corresponding author <leticiaalif@gmail.com>

Edited by: Antonio Faciola

Received December 29, 2016

Accepted September 27, 2017

**ABSTRACT:** The chemical composition of corn is variable and the knowledge of its chemical and energetic composition is required for an accurate formulation of the diet. This study aimed to determine the chemical composition, that is, dry matter (DM), mineral matter (MM), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), crude protein (CP), gross energy (GE) and energetic values of different varieties (batches) of corn and validate mathematical models to predict the metabolizable energy values (ME) of corn for pigs using near infrared spectroscopy (NIRS). Corn samples were scanned in the spectrum range between 1,100 and 2,500 nm, the model parameters were estimated by the modified partial least squares (MPLS) method. Ten prediction equations were inserted into the NIRS and used to estimate the ME values. The first degree linear regression models of the estimated ME values in function of the observed ME values were adjusted. The existence of a linear ratio was evaluated by detecting the significance to posterior estimates of the straight line parameters. The values of digestible energy and ME ranged from 3,400 to 3,752 and 3,244 to 3,611 kcal kg<sup>-1</sup>, respectively. The prediction equations,  $ME_1 = 4334 - 8.1MM + 4.1EE - 3.7NDF$ ;  $ME_2 = 4,194 - 9.2MM + 1.0CP + 4.1EE - 3.5NDF$ ; and  $ME_3 = 16.13 - 9.5NDF + 16EE + (23CP \times NDF) - (138MM \times NDF)$  were the most adequate to predict the ME values of corn by using NIRS.

**Keywords:** chemical composition, prediction equations, validation, swine

### Introduction

Although corn (*Zea mays L.*) is considered food of well-defined chemical composition, with average values provided in food composition tables, factors such as soil fertility, genetic variety of cultivars, planting conditions, storage and processing can significantly alter the chemical composition of corn (Li et al., 2014). Determining nutrient composition of corn generally requires different procedures for each nutrient. In addition, energy value of feed ingredients is evaluated mainly through traditional digestion-metabolism experiments, which are labor intensive, time consuming and expensive, which may impair its use in the feed industry (Li et al., 2016).

Thus, other methods have attempted to determine the chemical composition and energetic value of corn, such as the near infrared spectroscopy (NIRS) technology, which is rapid, non-destructive and analyzes more than one component at the same time, avoiding the use of chemical reagents and producing no waste (Swart et al., 2012). Some studies have also indicated that NIRS is useful to predict the nutritive and energetic content of feed ingredients and diets for poultry (Valdes and Leeson, 1992, 1994), rabbits (Xiccato et al., 1999, 2003) and even swine (Aufrère et al., 1996; Van Barneveld et al., 1999). However, to the best of our knowledge there is just one report (Li et al., 2016) on the rapid prediction of DE and ME content in corn by NIRS in pigs.

NIRS, in conjunction with prediction equations to estimate the energy value of feed ingredients, is already used by some feed industries; however, the results need

validation, because they consider the prediction errors of estimates of chemical composition and ME, not only the prediction error of the adjusted models to estimate ME of feed ingredients. This study was conducted to determine the chemical composition and energetic values of different corn varieties and validate mathematical models to predict the ME values of corn for pigs by using NIRS.

### Materials and Methods

#### Samples preparation and laboratorial chemical analysis

Ninety-nine corn samples of different varieties and from different municipalities [Ângulo (23°12'52" S, 51°56'6" W, 300 m above sea level); Apucarana (23°31'30" S, 51°24'20" W, 988 m above sea level); Araçongas (23°25'8" S, 51°25'26" W, 816 m above sea level); Atalaia (23°10'05" S, 52°03'10" W, 630 m above sea level); Califórnia (23°39'00" S; 51°21'18" W, 800 m above sea level); Foz do Iguaçu (25°32'45" S, 54°35'07" W; 192 m above sea level); Maringá (23°25'31" S, 51°56'19" W, 555 m above sea level)] of Paraná State, Brazil, were ground through a 1 mm screen and stored in plastic pots, prior chemical analysis. The samples were analyzed using the methods of AOAC International (AOAC, 1990, 2002) for dry matter (DM; AOAC method 934.01), mineral matter (MM; AOAC method 942.05), neutral detergent fiber (NDF; AOAC method 2002:04, 2002), acid detergent fiber (ADF; AOAC method 973.18), ether extract (EE; AOAC method 920.39) and crude pro-

tein (CP; AOAC method 954.01). Eighty samples were chosen randomly as a calibration set and nine samples were used for external validation. The remaining ten samples were used in the metabolism assay. Corn, diets, and excreta were analyzed for gross energy (GE, kcal kg<sup>-1</sup>) using a bomb calorimeter.

### NIRS spectra collection

Spectral measurements were performed using a FOSS XDS rapid content with the spectrometer search grid in the full spectrum 400-2500 nm. Spectral data collection, processing and calibration were conducted with the software WinISI III. Raw (log (1/R)) data were corrected for the scatter effects using the standard normal variance (SNV) and detrend (DT) combined with a mathematical treatment (2, 4, 4, 1) to derive the NIRS spectrum. The first number indicates the order of derivative function, the second is the gap, the third represents the number of data points used in the first smoothing, and the fourth is the number of data points in the second smoothing, which is normally set at 1 for no second smoothing (Li et al., 2016).

### Calibration and validation process

The selection of the spectra / sample that best represented the data set and the separation of samples that showed large differences compared to the other calibration set of samples were analyzed by the principal component analysis (PCA). A standardized H static (GH = 3) and Neighborhood H (NH = 0) were adopted to detect outliers.

The regression model parameters were estimated by the modified partial least squares (MPLS) regression method. The calibration model was developed by regression of absorption spectra of standard samples (n = 80) and their respective values of DM, CP, EE, GE, ADF, NDF and MM, which were obtained by conventional laboratory analyses.

The calibration model was evaluated by cross-validation (made by NIRS) and through an external validation using the validation set (n = 9). The results of corn chemical composition, obtained from the conventional laboratory analyses and NIRS technology, were compared in a paired manner (observed and predicted values) using the Bayesian approach. The differences in the results of each pair of samples were written as  $DIF = Y_2 - Y_1$ , providing a sample of differences, normally distributed such as:

$$Dif_i \sim N(\mu, \tau)$$

with  $i = 1, 2, \dots, n$ , considering for  $\mu$  (average) and  $\tau$  (precision), noninformative prior distributions, such that  $\mu \sim N(0, 10^{-6})$  and  $\tau \sim \text{Gama}(10^{-3}, 10^{-3})$  with variance  $\sigma^2 = 1/\tau$ , according to the OpenBUGS parameterization computer program that simulates the chains of parameters through the Monte Carlo Markov Chain (MCMC) method.

To evaluate the existence (or absence) of the correlation between values obtained by NIRS and by the conventional laboratory analyses, we used a Bayesian model, assuming that the response  $Y$  has the characteristics of a bivariate normal distribution, that is:

$$Y \sim N_2 \left( \begin{bmatrix} \mu_{Y_1} \\ \mu_{Y_2} \end{bmatrix}, \begin{bmatrix} \sigma_{11}^2 & \sigma_{12} \\ \sigma_{21} & \sigma_{22}^2 \end{bmatrix} \right),$$

where:  $\mu_{Y_1}$  and  $\mu_{Y_2}$  correspond to  $Y_1$  and  $Y_2$  responses, respectively, and NIRS / predicted and Laboratory / observed for each composition parameter. Similarly,  $\sigma_{11}^2$  and  $\sigma_{22}^2$  represent their respective variances, and  $\sigma_{12} = \sigma_{21}$ , the covariance between  $Y_1$  and  $Y_2$ . For bivariate model parameters, a noninformative prior distribution was considered  $\mu(\cdot) \sim N(0, 10^{-6})$  and a precision matrix  $\Omega \sim W$  was deduced, where  $W$  is a Wishart distribution with matrix (co) variance given by  $\Sigma = \Omega^{-1}$ . The correlation  $\rho$  was given by  $\sigma_{12} / \sqrt{\sigma_{11} \sigma_{22}}$ , according OpenBUGS parameterization (Rossi et al., 2014).

In both models, posterior distributions were obtained through the Brugs package program R (R Development Core Team, 2014). Fifty thousand values were generated through the MCMC method, with a discharge of 1,000 initial values in jumps of size 1. The convergence of the chains was tested by the coda package R program according to the criterion of Heidelberger and Welch (1983).

The average posterior estimates were used for each composition parameter and a credibility interval was calculated for the average difference, where significance was observed in the parameters in which the value zero did not belong to the range of 95 % of credibility.

### Metabolism assay

The metabolism assay was carried out in Maringá, Paraná, Brazil (23°21' S, 52°04' W, altitude 564 m). All experimental procedures were previously submitted to the Ethics Committee on Animal use in trial (CEUA n° 8329240815). Forty-four crossbred barrows with average initial weights of  $25.05 \pm 2.01$  kg were distributed in a randomized block design with ten treatments plus the reference diet and four replicates per treatment. Corn cultivars replaced 25 % of the reference diet. The chemical composition of ten corn cultivars used in the metabolism assay was determined by the conventional laboratory analyses and the NIRS technology, using the previously developed calibration model.

The experimental period lasted 12 days, seven days for animal adaptation to metabolism cages and feed and five days of feces and urine collection, which were performed once a day, at 08h00. The beginning and the end of the collection period was determined using 2 % iron oxide (Fe<sub>2</sub>O<sub>3</sub>) as a fecal marker.

The reference diet was formulated based on corn, soybean meal, soybean oil, vitamins, minerals, amino acids and additives to meet, at minimum, the nutritional requirements proposed by the National Research

Council (2012). The ration provided daily was calculated based on metabolic weight ( $\text{kg}^{0.75}$ ) of each animal and on the average consumption recorded in the adaptation period. The diets were moistened with water, at approximately 30 % of the feed, and fed twice a day (07h30 and 15h00).

The digestible energy (DE), ME, digestibility coefficients of GE (DCGE), metabolizability coefficients of GE (MCGE), and ME: DE ratio were determined. The analysis of variance (ANOVA) was performed using PROC ANOVA using SAS (Statistical Analysis System, version 8.1), and the DCGE and MCGE were analyzed using the Student Newman Keuls test.

To estimate the ME values, ten equations were included in NIRS (Table 1). A database containing the observed metabolizable energy values (OME) and the estimated metabolizable energy values (EMElab and EMEnirs) were tabulated. The OME data were obtained in the metabolism assay, while the EMElab data were obtained from replacing the chemical composition of corn obtained by the conventional analyses (LAB) in the prediction equation. The EMEnirs data were obtained from reading the corn samples in NIRS, using the calibration model developed to predict the chemical composition of values associated with the prediction equations selected in the literature and inserted into NIRS.

The validation of prediction equations was initially assessed with the adjustment of linear regression models of the first degree of estimated metabolizable energy values (EMEnirs and EMElab) due to the observed metabolizable energy values (OME), following a Bayesian approach assuming that:

$$Y_i \sim N(\mu, \tau) \text{ such that } \mu = \beta_0 + \beta_1 X_i$$

where: Y is the EMEnirs and EMElab and X is OME. For parameters  $\beta_0$ ,  $\beta_1$  and  $\tau$  (precision), a noninformative prior distribution and independent were assumed, respectively,  $\beta_0 \sim N(0, 10^{-6})$ ,  $\beta_1 \sim N(0, 10^{-6})$  and  $\tau \sim \text{Gamma}(10^{-3}, 10^{-3})$  with variance  $\sigma^2 = 1/\tau$ , according to OpenBUGS parameterization.

Posterior distributions were obtained through the BRugs package from program R (R Development Core

Team, 2014), where one million values were generated in a MCMC process with a discharge of 100,000 initial values in jumps of size 1. The convergence of the chains was tested by the convergence diagnostics and output analysis (CODA) package R program, following the criterion of Heidelberger and Welch (1983).

The null hypothesis  $H_0: \beta_1 = 0$  was tested against the bilateral alternative hypothesis  $H_a: \beta_1 \neq 0$  for each parameter individually. The linear relationship between estimated and observed values was evaluated by detecting the significance of estimated a posterior parameter  $\beta_0$  and  $\beta_1$ , recorded where the null (zero) did not belong to the 95 % credible intervals for each parameter. The absence of significance for the angular coefficient ( $\beta_1$ ) of the model indicated no linear relationship between EME and OME.

The regression models adjusted for each equation were subjected to a comparison test intercept ( $\beta_0$ ) and the angular coefficient ( $\beta_1$ ) to verify whether the generated regression models were similar for the different methods of analysis. In this case, the null hypothesis  $H_0: \beta_{inirs} = \beta_{ilab}$  was tested against the two-sided alternative hypothesis  $H_a: \beta_{inirs} \neq \beta_{ilab}$ ,  $i = 0, 1$ , for  $\beta_0$  and  $\beta_1$  individually. In cases where the null (zero) did not belong to the 95 % credible interval for the difference between the respective coefficients of each model, the adjusted models were considered distinct.

## Results and Discussion

### Calibration and cross-validation

The calibration and cross-validation statistics are shown in Table 2. In the calibration set, the highest coefficients of determination were obtained for DM ( $R^2 = 0.99$ ), CP ( $R^2 = 0.94$ ), GE ( $R^2 = 0.86$ ) and EE ( $R^2 = 0.83$ ), with values for the standard error of cross-validation (SECV) of 0.39, 0.39, 19.62 and 0.24, for each parameter, respectively. The coefficient of determination indicates how much of the variation in the data is explained by the adjusted model; however,  $R^2$  should not be considered in isolation, as it is generally applicable to the dataset from which it was generated and is not a good parameter for independent data sets (Shenk et al., 2007).

**Table 1** – Prediction equations used to estimate ME of corn with NIRS.

Item <sup>1</sup>	Equation	(R <sup>2</sup> ) <sup>2</sup>
ME <sub>1</sub> (Noblet and Perez, 1993)	4,334 – 8.1MM + 4.1EE – 3.7NDF	0.91
ME <sub>2</sub> (Noblet and Perez, 1993)	4,194 – 9.2MM + 1.0CP + 4.1EE – 3.5NDF	0.92
ME <sub>3</sub> (Noblet and Perez, 1993)	1099 + 0.740GE – 5.5MM – 3.7NDF	0.91
ME <sub>4</sub> (Ferreira et al., 1997)	3221.47 + 60.91CP – 29.04MM	0.86
ME <sub>5</sub> (Li et al., 2014)	4,464.24 + 20.15EE – 17.84NDF – 233.72MM	0.44
ME <sub>6</sub> (Li et al., 2014)	4,289.74 + 20.02CP + 22.47EE – 18.40NDF – 245.20MM	0.49
ME <sub>7</sub> (Morgan et al., 1987)	16.13 – 9.5NDF + 16EE + (23CP × NDF) – (138MM × NDF)	0.39
ME <sub>8</sub> (Morgan et al., 1987)	17.50 – 15.3NDF + 16EE + 5.9CP – 34MM	0.40
ME <sub>9</sub> (Morgan et al., 1987)	18.47 – 21NDF + 16EE + (30CP × NDF) – 32MM	0.40
ME <sub>10</sub> (Morgan et al., 1987)	5.42 – 17.2NDF – 19.4MM + 0.709GE	0.43

<sup>1</sup>Suffix (ME<sub>x</sub>) corresponds to the number of equations; <sup>2</sup>Coefficient of determination.

**Table 2** – Results for the prediction equation in the set of calibration samples.

Variable	n	Average	SEC	R <sup>2</sup>	SECV	1-VR	RPD	SEP	Bias	GH
DM	73	90.30	0.18	0.99	0.39	0.98	6.93	0.33	0.055	0.986
MM	74	1.21	0.11	0.30	0.12	0.17	1.39	0.16	0.024	1.000
NDF	74	12.05	0.79	0.15	0.84	0.02	1.29	0.81	0.030	1.004
ADF	76	3.97	0.39	0.40	0.52	-0.05	1.04	0.43	0.034	1.003
EE	73	3.96	0.17	0.83	0.24	0.66	3.23	0.21	0.010	1.003
CP	74	9.02	0.16	0.94	0.39	0.64	1.12	0.21	0.017	0.988
GE	74	4,476	12.85	0.86	19.62	0.69	1.84	14.06	-0.763	0.977

DM = Dry matter; MM = Mineral matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; GE = Gross energy; SEC = Standard error of the calibration set; R<sup>2</sup> = Coefficient determination for the calibration set; SECV = Standard error of the cross-validation; 1-VR = Coefficient determination for the cross-validation; RPD = SD/SECV; SEP = Ratio of the standard deviation to the standard error for the cross-validation; Bias = Mean reference value minus the mean predicted value; GH = Global distance Mahalanobis.

All GH values were below the limit (GH < 3) indicated by Shenk et al. (2007), showing good accuracy of the adjusted models, because the higher the GH value, the lower the accuracy of the adjusted model. The parameters that showed the best results for the standard error of performance (SEP) were DM, EE, CP and MM; however, the coefficient of determination for the MM was low (R<sup>2</sup> = 0.30).

### External validation

The paired test showed differences between the methods of analysis used (NIRS and LAB) for DM, MM and ADF (Table 3). For CP, NDF and EE, although there was no difference ( $p > 0.05$ ) between the methods of analysis, the correlation between the methods was low, with values of 0.29, 0.14 and 0.27, respectively. Among all parameters, the DM showed the highest correlation ( $\rho$ ) between the LAB values and NIRS results, showing good precision of the fitted model for this parameter. The worst correlations were observed for MM, ADF and GE.

The difference observed for MM can be related to the low specificity of the NIRS technology to predict the mineral content of food or ingredients. As NIRS relies on how infrared light is absorbed by organic compounds (Agelet et al., 2012), theoretically, there are no absorption bands for minerals in the near infrared region (Clark et al., 1987; Shenk et al., 2007). Minerals forming organic complexes or chelates may be detected, but there are no spectral matches for minerals in the ionic or salt form (Shenk et al., 2007).

Regarding the NDF and ADF, the difference between the methods of analysis and the low correlation between the LAB and NIRS results can be attributed to the difficulty of accurately determining the different fractions that comprise the fiber content of foods, which influences the reference values and consequently the predictive power of the fitted curve.

### Metabolism assay

The corn cultivars used in the metabolism assay showed DM values ranging from 86.57 % to 89.71 %

**Table 3** – Bayesian estimates for bromatological corn composition analyzed by reflectance spectroscopy in the near infrared (NIRS) and physico-chemical method (LAB).

Parameters	Method	Average <sup>1</sup>	Standard deviation	Median	ICr 95 % <sup>2</sup>	
					2.5 %	97.5 %
DM	NIRS	89.50 <sup>b</sup>	0.77	89.50	87.97	91.02
	LAB	90.16 <sup>a</sup>	0.91	90.16	88.36	91.97
	$\rho^3$	0.92	0.06	0.94	0.78	0.98
MM	NIRS	1.22 <sup>a</sup>	0.13	1.22	0.97	1.48
	LAB	1.13 <sup>b</sup>	0.14	1.13	0.86	1.41
	$\rho$	0.05	0.32	0.06	-0.57	0.65
NDF	NIRS	12.00 <sup>a</sup>	0.16	12.00	11.70	12.32
	LAB	12.21 <sup>a</sup>	0.35	12.21	11.52	12.91
	$\rho$	0.14	0.31	0.15	-0.50	0.70
ADF	NIRS	3.91 <sup>a</sup>	0.14	3.91	3.64	4.18
	LAB	3.61 <sup>b</sup>	0.17	3.61	3.28	3.95
	$\rho$	-0.07	0.32	-0.08	-0.65	0.56
EE	NIRS	3.82 <sup>a</sup>	0.15	3.82	3.53	4.12
	LAB	3.77 <sup>a</sup>	0.18	3.77	3.42	4.12
	$\rho$	0.27	0.30	0.30	-3.77	0.77
CP	NIRS	8.61 <sup>a</sup>	0.16	8.61	8.29	8.94
	LAB	8.97 <sup>a</sup>	0.27	8.98	8.44	9.53
	$\rho$	0.29	0.29	0.32	-0.36	0.78
GE	NIRS	4,464.13 <sup>a</sup>	5.76	4,464.13	4,452.65	4,475.63
	LAB	4,479.68 <sup>a</sup>	18.39	4,479.86	4,442.73	4,515.97
	$\rho$	-0.05	0.32	-0.06	-0.64	0.57

DM = Dry matter; MM = Mineral matter; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; EE = Ether extract; CP = Crude protein; GE = Gross energy. <sup>1</sup>Different letters in the columns indicate significant differences between the analysis methods through Bayesian comparisons; <sup>2</sup>Cr = Credibility interval ( $p \leq 0.05$ ); <sup>3</sup> $\rho$  = Correlation.

(Table 4). Mineral matter contents ranged from 0.90 % to 1.32 %, corroborating the findings of Pasquetti et al. (2015). The EE values varied from 3.32 % to 3.61 %, near the average (3.65 %) proposed by Rostagno et al. (2011) and also consistent with the value (3.48 %) displayed in the NRC (2012).

The CP contents found in this study resemble the average (7.88 %) proposed by Rostagno et al. (2011) and are similar to those found by Pasquetti et al. (2015). The ADF values were greater than 3.00 %, reaching 4.27 %. These figures contrast with those found by Kil et al. (2014), who assessed the digestibility of CP and amino acids in corn of different origins for pigs and obtained mostly values below 3.00 %.

The NDF had values between 9.66 % and 12.23 %, with the maximum value close to the value (11.93 %) submitted by Rostagno et al. (2011) and the lowest value similar to the default value (9.11 %) by the NRC (2012). This variation observed in the chemical and nutritional composition of corn may be related to soil fertility, the genetic variety of cultivars, planting conditions, anti-nutritional factors, storage and processing (Li et al., 2014).

The digestibility coefficients of GE (Table 5) ranged from 85.68 % to 95.66 %, with an average of 90.72 %, which is higher than the average of 88 % indicated by Rostagno et al. (2011) and the NRC (2012). Cultivar 7



**Table 4** – Chemical composition of corn of different cultivars expressed as feed basis.

Corn	DM <sup>1</sup>	MM	CP	%			GE
				EE	NDF	ADF	
1	86.57	1.32	9.27	3.49	10.31	3.71	3,838
2	88.12	1.14	8.22	3.60	9.66	3.30	3,946
3	88.33	1.13	7.75	3.40	10.89	3.12	3,933
4	88.64	1.04	7.38	3.32	10.73	3.38	3,915
5	89.61	1.20	7.49	3.56	11.74	3.56	3,969
6	88.35	1.17	7.83	3.53	12.15	3.16	3,924
7	88.56	1.11	7.68	3.41	10.76	3.18	3,922
8	87.79	1.09	7.80	3.60	12.23	3.62	3,909
9	88.57	0.94	7.33	3.55	10.83	4.27	3,911
10	89.71	0.90	7.93	3.61	11.37	3.11	3,939
Average	88.43	1.10	7.87	3.56	11.07	3.44	3,921
SD <sup>2</sup>	0.89	0.12	0.56	0.15	0.81	0.37	34.11
CV (%) <sup>3</sup>	1.00	11.79	7.97	4.81	6.91	10.90	0.55
Range	3.14	0.42	1.94	0.48	2.57	1.16	59.83

<sup>1</sup>DM = Dry matter; MM = Mineral matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; GE = Gross energy; <sup>2</sup>SD = Standard deviation; <sup>3</sup>CV% = Coefficient of variation.

**Table 5** – Energetic values and coefficients of metabolizable energy of different corns for pigs, as feed basis.

Corn	GE <sup>1</sup>	DCGE <sup>2</sup>	DE <sup>3</sup>	CMGE <sup>4</sup>	ME <sup>5</sup>	ME:DE <sup>6</sup>
	kcal kg <sup>-1</sup>	%	kcal kg <sup>-1</sup>	%	kcal kg <sup>-1</sup>	
1	3,838	90.33 <sup>abc</sup>	3,467	84.51 <sup>b</sup>	3,244	93
2	3,946	91.18 <sup>abc</sup>	3,598	88.61 <sup>ab</sup>	3,497	97
3	3,933	90.49 <sup>abc</sup>	3,559	86.83 <sup>ab</sup>	3,415	96
4	3,915	90.76 <sup>abc</sup>	3,553	88.75 <sup>ab</sup>	3,474	97
5	3,969	85.68 <sup>c</sup>	3,400	83.00 <sup>b</sup>	3,294	96
6	3,924	92.07 <sup>abc</sup>	3,613	88.40 <sup>ab</sup>	3,469	96
7	3,922	95.66 <sup>a</sup>	3,752	92.07 <sup>a</sup>	3,611	96
8	3,909	92.97 <sup>ab</sup>	3,634	90.02 <sup>ab</sup>	3,518	96
9	3,911	90.13 <sup>abc</sup>	3,525	87.98 <sup>ab</sup>	3,441	97
10	3,939	87.97 <sup>bc</sup>	3,465	85.74 <sup>ab</sup>	3,377	97
CV% <sup>7</sup>	-	3.06	-	3.41	-	-

<sup>abc</sup>Means followed by different letters in the column differ by Student Newman Keuls Test ( $p \leq 0.05$ ); <sup>1</sup>Gross energy; <sup>2</sup>Digestibility coefficients of gross energy; <sup>3</sup>Digestible energy; <sup>4</sup>Coefficients of metabolizability of gross energy; <sup>5</sup>Metabolizable energy; <sup>6</sup>Relation ME:DE; <sup>7</sup>Coefficient of variation.

showed higher DCGE ( $p \leq 0.05$ ) compared to Cultivars 10 and 5 that had higher NDF contents compared to other cultivars (except Corns 6 and 8), which may have contributed to the lower DCGE and GE (Le Goff and Noblet, 2001; Noblet and van Milgen, 2004).

The average ME value was 3,434 kcal kg<sup>-1</sup>, ranging from 3,244 to 3,611 kcal kg<sup>-1</sup>, similar to the average value of 3,430 kcal kg<sup>-1</sup> found by Li et al. (2014), who evaluated different cultivars of corn for growing pigs. ME variation is a reflection of the different chemical compositions of the evaluated values found for corn, since the ME and chemical composition of the feed are directly correlated and can be predicted directly from nutrient composition (NRC, 2012).

Although the DCGE and MCGE are higher than the values reported in the literature, the ratio ME:DE remained close to the calculated values (0.96) using the data presented by Rostagno et al. (2011) and reflected the value (0.98) suggested by the NRC (2012). Even with the higher CP content, Cultivar 1 had the lowest ME:DE ratio (0.94) because, according to Noblet and Perez (1993), the ratio ME:DE is linearly (inversely) related to the CP content of the diet, which was observed in this study.

Among all evaluated prediction equations, six had significance ( $p \leq 0.05$ ) for both coefficients ( $\beta_0 \neq 0$  and  $\beta_1 \neq 0$ ) and the set straight (ME<sub>1</sub>, ME<sub>2</sub>, ME<sub>3</sub>, ME<sub>5</sub>, ME<sub>7</sub> and ME<sub>10</sub>). Thus, the observed metabolizable energy values explained the estimated values for these equations (Table 6).

The equations ME<sub>1</sub>, ME<sub>2</sub> and ME<sub>7</sub> (Table 1) were effective in predicting ME values only when associated with NIRS technology. The equations ME<sub>3</sub>, ME<sub>5</sub> and ME<sub>10</sub> were effective in predicting ME values only when associated with corn chemical composition obtained by the conventional methods (LAB). The effectiveness of different equations for each method of analysis can be justified by the fact that most equations tested had MM as regressive variable, as explained earlier. In addition, MM has a negative effect on ME, since it acts as a diluent of GE, reducing the organic matter content in foods (Morgan et al., 1987; Pasquetti et al., 2015).

The NDF is another regressive variable that may have contributed to the variation in performances of the same equation due to the difficulty to determine accurately the different fractions that comprise the food fiber. This influences the reference values and, consequently, the predictive power of the adjusted model. In addition, the fibrous fraction is considered an important predictor of the energy value of food.

Fiber has a negative effect due to its lower digestibility and its ability to cause changes in the apparent digestibility of the other nutrients (Noblet et al., 1993). As the NDF content increases, there is a reduction in the digestibility of CP and EE (Le Goff and Noblet, 2001; Noblet et al., 1993; Noblet and van Milgen, 2004), which contribute to the energy values of food.

Thus, the significance observed for the equations (ME<sub>1</sub>, ME<sub>2</sub> and ME<sub>7</sub>), when associated with the NIRS method, is explained by the other regressive variables of the model (Table 6). Variables, such as GE and EE, when together with NDF, form a reasonable basis for a predictive equation (Morgan et al., 1987). Similarly, EE is considered a good predictor of corn ME due to its high energy value (Li et al., 2014) and has a positive effect on ME (Morgan et al., 1987). The equations ME<sub>4</sub>, ME<sub>6</sub>, ME<sub>8</sub> and ME<sub>9</sub> showed no significance for the angular coefficient ( $\beta_1$ ), demonstrating the absence of a linear relationship between EME and OME for the evaluated methods (NIRS or LAB).

The use of prediction equations associated or not to NIRS requires caution, since predicted ME values using NIRS (EMEnirs) are often similar to laboratory val-

**Table 6** – Posterior Bayesian estimates for regression equations of the estimated values of metabolizable energy (EME) as a function of the observed values of metabolizable energy (OME), for the corn analyzed by near-infrared spectroscopy (NIRS) and physico-chemical methods (LAB).

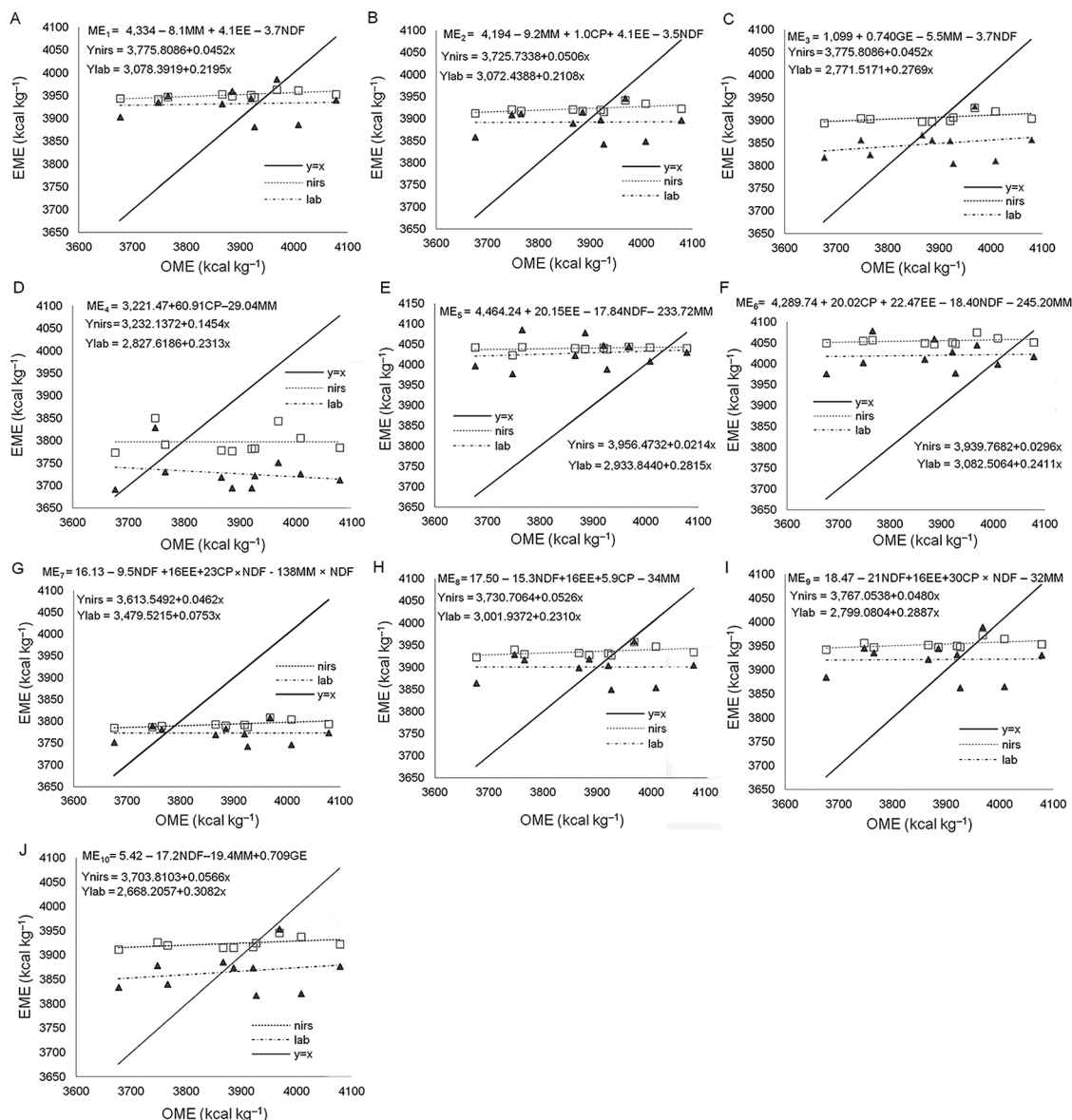
Equations	Methods	Regression models <sup>1</sup>	Parameters	Average	SD <sup>2</sup>	Median	ICr 95 % <sup>3</sup>	
							2.5 %	97.5 %
ME <sub>1</sub>	Nirs*	$\hat{Y} = 3,775.81 + 0.0452x^a$	$\beta_0$	3,775.81	64.40	3,777.52	3,641.10	3,898.17
			$\beta_1$	0.0452	0.0165	0.0447	0.0137	0.0798
	Lab	$\hat{Y} = 3,078.40 + 0.2195x^a$	$\beta_0$	3,078.40	663.08	3,196.16	1,429.33	4,039.80
			$\beta_1$	0.2195	0.1706	0.1892	-0.0278	0.6437
ME <sub>2</sub>	Nirs*	$\hat{Y} = 3,725.73 + 0.0506x^a$	$\beta_0$	3,725.73	102.36	3,730.42	3,506.20	3,914.23
			$\beta_1$	0.0506	0.0263	0.0494	0.0021	0.1071
	Lab	$\hat{Y} = 3,072.44 + 0.2108x^a$	$\beta_0$	3,072.44	666.01	3,190.57	1,416.93	4,038.74
			$\beta_1$	0.21086	0.1714	0.1804	-0.0377	0.6368
ME <sub>3</sub>	Nirs	$\hat{Y} = 3,679.42 + 0.0582x^a$	$\beta_0$	3,679.42	128.33	3,686.88	3,400.24	3,910.73
			$\beta_1$	0.0582	0.0330	0.0563	-0.0012	0.1301
	Lab*	$\hat{Y} = 2,771.52 + 0.2769x^b$	$\beta_0$	2,771.52	646.69	2,869.84	1,203.24	3,757.40
			$\beta_1$	0.2769	0.1664	0.2516	0.0232	0.6804
ME <sub>4</sub>	Nirs	$\hat{Y} = 3,232.14 + 0.1454x^a$	$\beta_0$	3,232.14	542.66	3,325.72	1,880.12	4,017.98
			$\beta_1$	0.1454	0.1396	0.1214	-0.0568	0.4931
	Lab	$\hat{Y} = 2,827.62 + 0.2313x^a$	$\beta_0$	2,827.62	806.77	2,955.60	895.6702	4,041.39
			$\beta_1$	0.2313	0.2076	0.1984	-0.0810	0.7286
ME <sub>5</sub>	Nirs	$\hat{Y} = 3,956.47 + 0.0214x^a$	$\beta_0$	3,956.47	75.98	3,959.12	3,795.83	4,098.99
			$\beta_1$	0.0214	0.0195	0.0207	-0.0152	0.0627
	Lab*	$\hat{Y} = 2,933.84 + 0.2815x^b$	$\beta_0$	2,933.84	730.05	3,057.47	1,143.46	4,012.65
			$\beta_1$	0.2815	0.1878	0.2498	0.0039	0.7421
ME <sub>6</sub>	Nirs	$\hat{Y} = 3,939.77 + 0.0296x^a$	$\beta_0$	3,939.77	111.01	3,945.71	3,699.55	4,141.41
			$\beta_1$	0.0296	0.0285	0.0280	-0.0222	0.0914
	Lab	$\hat{Y} = 3,082.51 + 0.2411x^b$	$\beta_0$	3,082.51	707.68	3,211.17	1,322.00	4,102.99
			$\beta_1$	0.2411	0.1820	0.2080	-0.0215	0.6939
ME <sub>7</sub>	Nirs*	$\hat{Y} = 3,613.54925 + 0.0462x^a$	$\beta_0$	3,613.55	79.89	3,616.18	3,444.99	3,763.81
			$\beta_1$	0.0462	0.0205	0.0455	0.0075	0.0895
	Lab	$\hat{Y} = 3,479.52154 + 0.0753x^a$	$\beta_0$	3,479.52	352.91	3,529.37	2,632.36	4,018.89
			$\beta_1$	0.0753	0.0908	0.0625	-0.0634	0.2932
ME <sub>8</sub>	Nirs	$\hat{Y} = 3,730.70640 + 0.0526x^a$	$\beta_0$	3,730.71	128.38	3,738.29	3,451.14	3,961.73
			$\beta_1$	0.0526	0.0330	0.0507	-0.0067	0.1246
	Lab	$\hat{Y} = 3,001.93720 + 0.2310x^b$	$\beta_0$	3,001.94	710.94	3,126.26	1,245.56	4,041.53
			$\beta_1$	0.2310	0.1829	0.1991	-0.0364	0.6830
ME <sub>9</sub>	Nirs	$\hat{Y} = 3,767.05383 + 0.0480x^a$	$\beta_0$	3,767.05	104.06	3,771.97	3,543.48	3,958.20
			$\beta_1$	0.0480	0.0267	0.0467	-0.0011	0.1055
	Lab	$\hat{Y} = 2,799.08043 + 0.2887x^a$	$\beta_0$	2,799.08	778.53	2,920.99	929.99	3,975.91
			$\beta_1$	0.2887	0.2003	0.2573	-0.0141	0.7697
ME <sub>10</sub>	Nirs	$\hat{Y} = 3,703.81032 + 0.0566x^a$	$\beta_0$	3,703.81	130.40	3,711.58	3,419.69	3,938.26
			$\beta_1$	0.0566	0.0335	0.0546	-0.0036	0.1297
	Lab*	$\hat{Y} = 2,668.20576 + 0.3082x^b$	$\beta_0$	2,668.21	700.00	2,770.60	989.63	3,748.60
			$\beta_1$	0.3082	0.1801	0.2818	0.0301	0.7400

\*Indicates significance for the intercept  $\beta_0$  and for the angular coefficient  $\beta_1$  of the adjusted line; <sup>1</sup>Different letters in the columns indicate significant differences between the models adjusted by means of the coefficient comparison test; <sup>2</sup>SD = Standard deviation; <sup>3</sup>ICr = Credibility interval ( $p \leq 0.05$ ).

ues (EMElab), but both predictions may differ from the real value of ME, determined in the metabolism assay (OME) (Figures 1A, B, and G).

Based on the comparison test of parameters of the adjusted regression models for the different methods of analysis, equations ME<sub>1</sub>, ME<sub>2</sub>, ME<sub>4</sub>, ME<sub>7</sub> and ME<sub>9</sub> were considered to be the same (Figures 1A, B, D, G and I). However, ME<sub>4</sub> and ME<sub>9</sub> equations were not significant for the angular coefficient of the straight line (Figures

1D and I) adjusted between the values estimated and observed in the metabolism test for the evaluated methods (NIRS and LAB) and cannot be used to predict the ME values of corn. The adjusted regression models for equations ME<sub>3</sub>, ME<sub>5</sub>, ME<sub>6</sub>, ME<sub>8</sub> and ME<sub>10</sub> were considered distinct by the comparison test of parameters (Table 6 and Figures 1C, E, F, H and J). Equations ME<sub>1</sub>, ME<sub>2</sub> and ME<sub>7</sub> comprise up to four variables of chemical corn composition, which are all well predicted us-



**Figure 1** – Regression models adjusted for the estimated metabolizable energy (EME) values by equations ME1 (A), ME2 (B), ME3 (C), ME4 (D), ME5 (E), ME6 (F), ME7 (G), ME8 (H), ME9 (I), ME10 (J) as a function of the observed metabolizable energy (OME) values for corn analyzed by near infrared spectroscopy (NIRS) and physical chemical methods (LAB).

ing NIRS (Figures 1A, B and G). The use of these NIR-predicted parameters allows for easy and economical application (Pozza et al., 2008). Developing prediction equations with few variables is important because it facilitates the maintenance of robustness and predictive power of the NIRS calibration / prediction curve. Moreover, it allows for the periodical expansion of the reference database.

### Conclusion

Near infrared spectroscopy was effective in determining the NDF, EE, CP and GE contents compared

to the conventional method of laboratory analysis, indicating that NIRS could be used as an analysis method to determine corn chemical composition. The calibration set with a larger number of samples and the optimization of spectral pre-treatment could improve the NIRS calibration and prediction performance for MM. The prediction equations ME<sub>1</sub> = 4,334 - 8.1MM + 4.1EE - 3.7NDF; ME<sub>2</sub> = 4,194 - 9.2MM + 1.0CP + 4.1EE - 3.5NDF and ME<sub>7</sub> = 16.13 - 9.5NDF + 16EE + (23CP × NDF) - (138MM × NDF) were the most adequate to predict the ME values of corn for pigs using NIRS compared to the laboratory tests; however, neither method was accurate.

## Acknowledgements

We thank the State University of Maringá, for supporting this work, the Coordination for the Improvement of Higher Level Personnel (CAPES), for the scholarship grant, and the Complex of Research Support Centers (COMCAP) and Research and Projects Financing (FINEP) for enabling the analysis of the adiabatic calorimeter.

## References

- Association of Official Analytical Chemists - International [AOAC]. 1990. Official Methods of Analysis. 15ed. AOAC, Gaithersburg, MD, USA.
- Association of Official Analytical Chemists - International [AOAC]. 2002. Official Method 2002:04. AOAC, Rockville, MD, USA.
- Agelet, L.E.; Ellis, D.D.; Duvick, S.; Goggi, A.S.; Hurburgh, C.R.; Gardner, C.A. 2012. Feasibility of near infrared spectroscopy for analyzing corn kernel damage and viability of soybean and corn kernels. *Journal of Cereal Science* 55: 160-165.
- Aufrère, J.; Graviou, D.; Demarquilly, C.; Perez, J.M.; Andrieu, J. 1996. Near infrared reflectance spectroscopy to predict energy value of compound feeds for swine and ruminants. *Animal Feed Science and Technology* 62: 77-90.
- Clark, D.H.; Mayland, H.F.; Lamb, R.C. 1987. Mineral analysis of forages with near infrared reflectance spectroscopy. *Agronomy Journal* 79: 485-490.
- Ferreira, E.R.A.; Fialho, E.T.; Teixeira, A.S. 1997. Evaluation of the chemical composition and determination of energetics values and prediction equation of some feedstuffs for swine. *Revista Brasileira de Zootecnia* 26: 514-523 (in Portuguese, with abstract in English).
- Heidelberger, P.; Welch, P.D. 1983. Simulation run length control in the presence of an initial transient. *Operations Research* 31: 1109-1144.
- Kil, D.Y.; Park, C.S.; Son, A.R.; Ji, S.Y.; Kim, B.G. 2014. Digestibility of crude protein and amino acids in corn grains from different origins for pigs. *Animal Feed Science and Technology* 196: 68-75.
- Le Goff, G.; Noblet, J. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. *Journal of Animal Science* 79: 2418-2427.
- Li, Q.; Zang, J.; Liu, D.; Piao, X.; Lai, C.; Li, D. 2014. Predicting corn digestible and metabolizable energy content from its chemical composition in growing pigs. *Journal of Animal Science and Biotechnology* 5: 11.
- Morgan, C.A.; Whittemore, C.T.; Phillips, P.; Crooks, P. 1987. The prediction of the energy value of compounded pig foods from chemical analysis. *Animal Feed Science and Technology* 17: 81-107.
- National Research Council [NRC]. 2012. Nutrient Requirements of Swine. 11ed. The National Academies Press, Washington, DC, USA.
- Noblet, J.; Fortune, H.; Dupire, C.; Dubois, S. 1993. Digestible, metabolizable and net energy values of 13 feedstuffs for growing pigs: effect of energy system. *Animal Feed Science and Technology* 42: 131-149.
- Noblet, J.; Perez, J.M. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. *Journal of Animal Science* 71: 3389-3398.
- Noblet, J.; van Milgen, J. 2004. Energy value of pig feeds: effect of pig body weight and energy evaluation system. *Journal of Animal Science* 82: 229-238.
- Pasquetti, T.J.; Pozza, P.C.; Furlan, A.C.; Marcato, S.M.; Castilha, L.D.; Diaz-Huepa, L.M.; Fachinello, M.R.; Tanamati, W. 2015. Adjustment of equations to predict the metabolizable energy of corn for meat type quails. *Semina: Ciências Agrárias* 36: 2861-2870.
- Pozza, P.C.; Gomes, P.C.; Donzele, J.L.; Rostagno, H.S.; Pozza, M.S.S.; Rodrigueiro, R.J.B.; Nunes, R.V. 2008. Determination and prediction of the digestible and metabolizable energy values of poultry by-products for swine. *Revista Brasileira de Saúde e Produção Animal* 9: 734-742 (in Portuguese, with abstract in English).
- Rossi, R.M.; Martins, E.N.; Lopes, P.S.; Silva, F.F. 2014. Univariate and bivariate Bayesian analysis for feed conversion of the Piau swine breed. *Pesquisa Agropecuária Brasileira* 49: 754-761 (in Portuguese, with abstract in English).
- Rostagno, H.S.; Albino, L.F.T.; Donzele, J.L.; Gomes, P.C.; Oliveira, R.F.; Lopes, D.C.; Ferreira, A.S.; Barreto, S.L.T.; Euclides, F.R. 2011. Brazilian Tables for Poultry and Pigs: Food Composition and Nutritional Requirements = Tabelas Brasileiras para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais. 3ed. UFV, Viçosa, MG, Brazil (in Portuguese).
- Swart, E.; Brand, T.S.; Engelbrecht, J. 2012. The use of near infrared spectroscopy (NIRS) to predict the chemical composition of feed samples used in ostrich total mixed rations. *South African Journal of Animal Science* 42: 550-554.
- Shenk, J.S.; Westerhaus, M.O.; Workman, J.J. 2007. Application of NIR spectroscopy to agricultural products. p. 347-386. In: Burns, D.A.; Ciurczak, E.W., eds. *Handbook of near-infrared analysis*. CRC Press, Boca Raton, FL, USA (Practical Spectroscopy, 35).
- Valdes, E.V.; Leeson, S. 1992. The use of near-infrared reflectance spectroscopy to measure metabolizable energy in poultry feed ingredients. *Poultry Science* 71: 1559-1563.
- Valdes, E.V.; Leeson, S. 1994. Measurement of metabolizable energy, gross energy, and moisture in feed grade fats by nearinfrared reflectance spectroscopy. *Poultry Science* 73: 163-171.
- Van Barneveld, R.J.; Nuttall, J.D.; Flinn, P.C.; Osborne, B.G. 1999. Near infrared reflectance measurement of the digestible energy content of cereals for growing pigs. *Journal of Near Infrared Spectroscopy* 7: 1-7.
- Xiccato, G.; Trocino, A.; Carazzolo, A.; Meurens, M.; Maertens, L.; Carabano, R. 1999. Nutritive evaluation and ingredient prediction of compound feeds for rabbits by near-infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology* 77: 201-212.
- Xiccato, G.; Trocino, A.; De Boever, J.L.; Maertens, L.; Carabano, R.; Pascual, J.J.; Perez, J.M.; Gidenne, T.; Cunha, F.E. 2003. Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology* 104: 153-168.