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EUROPEAN HARMONISATION OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) ANALYSIS ON RABBIT FEEDS: FIRST STEPS[*]

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ABSTRACT

The harmonisation of experimental methodologies is the goal of several European projects allowing a more efficient comparison of scientific results among laboratories. Among these projects, the Concerted Action ERAFE (European harmonisation of rabbit feed evaluation; FAIR3-1651) involves six European laboratories working on rabbit nutrition. One of the ERAFE tasks is the harmonisation of methods to predict the nutritive value of feeds through Near Infrared Reflectance Spectroscopy (NIRS).

As a first step, the project aimed to compare NIRS accuracy in predicting chemical composition and nutritive value of 55 compound feeds for rabbits scanned in three laboratories (Belgium, France and Italy), following a common procedure of sample preparation. The pre-drying of samples was decided in order to homogenise moisture content, which is one of the most important factors of variation in NIRS analysis. The samples were scanned after drying in a ventilated oven at 60°C overnight. Scanning method and spectra analysis were not harmonised among laboratories. Calibration equations were calculated in all laboratories both on absorbance and transformed data by step-wise multiple linear regression (MLR) and Partial Least Square Regression (PLSR) in order to predict crude protein (%DM), crude fibre (%DM), gross energy (MJ/kg DM), dry matter digestibility (%), gross energy digestibility (%) and digestible energy (MJ/kg DM). Moreover, calibrations were recalculated in one laboratory by using a unique software.

The calibrations calculated in the three laboratories did not show large differences concerning the coefficient of determination or accuracy of prediction for all variables. The derivative treatment of spectra permitted to improve the accuracy of calibrations compared to absorbance data. No sensible advantage of MLR or PLSR was evidenced. When the different sets of spectra were submitted to calibration by using a unique software, no difference in comparison with the previous results was obtained. Principal component analysis separated spectral data according to the laboratory.

It can be concluded that despite the different hardware and software equipment used, the calibration results for all variables were substantially equivalent in all labs when NIRS analysis was performed with a harmonised sample preparation and following a similar mathematical and statistical procedure for data analysis.

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INTRODUCTION

The need of achieving comparable results induces the researchers towards a harmonisation of experimental methodologies in all fields. Since 1993, a group of six European laboratories called EGRAN (European Group on Rabbit Nutrition) is engaged in the harmonisation of analytical methodologies used for rabbit feed evaluation. Since 1997, this group participates in the EU Concerted Action named ERAFE (European harmonisation of rabbit feed evaluation; FAIR3-1651), whose main research objectives were recently presented by GIDENNE (1999). One of the ERAFE tasks is the harmonisation of methods to predict the nutritive value of feeds, *i.e.* chemical and *in vitro* analyses, and Near Infrared Reflectance Spectroscopy (NIRS).

Several factors may affect repeatability and reproducibility of NIRS analysis, from sample handling to instrumental equipment and the software used for data treatment. Among them, the effects of sample moisture concentration are well-known (SHENK ET AL., 1992). The overtones of O-H stretch and the combination bands of O-H stretch and bend of water have specific absorption bands which permit to determine its concentration in agricultural products. However, moisture strongly interacts with the other chemical constituents influencing their absorption (WILLIAMS, 1987) and affecting NIRS calibration and prediction performance.

The standardisation of sample preparation methods before NIRS analysis is controversial. Sample pre-drying is recommended to standardise moisture conditions and achieve more robust calibrations when analysing fresh forages (SHENK AND WESTERHAUS, 1994). However, compound feeds for ruminants (MURRAY AND HALL, 1983; DE BOEVER ET AL., 1995; AUFRÈRE ET AL., 1996) and monogastric species (CHEN ET AL., 1987; VALDES AND LEESON, 1992) were successfully analysed by NIRS without any pre-drying treatment.

In the ERAFE project, the first step of the harmonisation of NIRS analysis was to standardise the sample preparation through SPAN action (Sample Preparation for NIRS Analysis) and compare the predictive performance in three laboratories (Ghent, INRA and Padova).

MATERIALS AND METHODS

The set of samples

Fifty-five compound feeds used in formulation for weaning, growing and reproducing rabbits were analysed; some were commercial, others experimental, already used in other common EGRAN activities (XICCATO ET AL., 1999). Thirty-six feeds were from Belgium (Ghent) and 19 from Italy (Padova). The *in vivo* digestibility trials and the chemical analysis of the compound feeds were performed by the laboratory of origin. About 15 grams of sample (1 mm ground) were stored in plastic cups with labels assigned by the owner laboratory.

Sample preparation for NIRS analysis (SPAN)

Because of the different origin and storage conditions before the interlaboratory study, SPAN procedure was concerted in order to homogenise moisture condition.

SPAN consisted in drying the sample before scanning into a plate (approx. 13.0×9.5×3.5 cm) in a ventilated oven during the night. A moderate temperature (60°C) was chosen to avoid possible damage to chemical constituents (SHENK ET AL., 1992). After drying, the samples were cooled in a desiccator until they reached room temperature and then analysed by NIRS.

NIRS equipment and software

Within three months, the samples were scanned at Padova, INRA and Ghent, following SPAN procedure. Scanning method and spectra collection were not harmonised among laboratories. In all labs, NIRS analysis was performed using a monochromator spectrometer in the range from 1100 to 2500 nm with a 2 nm step. Ghent and Padova used an InfraAlyzer 500 (Bran+Luebbe, Germany), while INRA was equipped with a NIRS-System 6500 (Foss). Ghent collected spectra and analysed data by IDAS (version 1.41, Bran+Luebbe), Padova by "Sesame" (version 2.10, Bran+Luebbe) and INRA by ISI NIRS3 (version 3.0). Padova scanned the samples once. Ghent collected two spectra per sample by scanning it twice, after refilling the cup while INRA collected three series of spectra along three days. The average spectra were used for calibrations.

Mathematical and statistical treatment of NIR spectra

In all laboratories, spectral data were transformed in absorbance and then in (1,4,4) and (2,4,4) derivatives. INRA also used the Standard Normal Variate (SNV) algorithm. Calibration equations were calculated by step-wise multiple linear regression (MLR) and Partial Least Square Regression (PLSR) on the whole set of samples, without excluding any possible outlier. When using PLSR, full cross validation was applied. The maximum number of wavelengths or independent factors used as variables in the prediction equations was 6 (*i.e.* 10% of the number of samples used in the calibration) in order to avoid overfitting (SHENK AND WESTERHAUS, 1994). Calibration equations were calculated for crude protein (CP, % DM), crude fibre (CF, %DM), gross energy (GE, MJ/kg DM), dry matter digestibility (DMd, %), gross energy digestibility (GEd, %) and digestible energy (DE = GE x GEd, MJ/kg DM). Moreover, the three series of spectra collected at Ghent, INRA and Padova were reanalysed in Padova by using a unique software (Sesame) and method (MLR on 2,4,4 data). Calibration performance was compared in terms of multiple coefficient of determination (R^2), standard error of calibration (SEC) and/of cross validation (SECV). The three sets of spectra were then gathered and submitted to Principal Component Analysis (PCA) by using Unscrambler 7.01 (Camo, Norway).

RESULTS AND DISCUSSION

Prediction of chemical constituents and nutritive characteristics

The chemical composition and the nutritive value of the feeds used are shown in Table 1. Their characteristics covered the range of the compound feeds usually fed to different categories of rabbits.

Table 1. Chemical composition and nutritive value of rabbit compound feeds

Variable		Min	Max	Average	SD
Crude fiber	% DM	12.0	22.5	15.9	2.7
Crude protein	% DM	13.1	25.1	18.0	2.5
Gross energy	MJ/kg DM	17.78	21.32	18.59	0.64
DM digestibility	%	48.9	71.0	61.4	4.9
GE digestibility	%	47.4	70.6	60.9	5.0
Digestible energy	MJ/kg DM	8.85	14.57	11.36	1.08

The statistical parameters of the prediction regressions for chemical composition and nutritive characteristics are listed in Table 2; only the best calibration results obtained by each laboratory are reported. The mathematical treatment adopted and the statistical method used are also specified.

The calibrations calculated in the three laboratories for all variables did not show large differences in terms of coefficient of determination or accuracy of prediction. Similar results were found by Bartl et al. (1996), working on the harmonisation of Fourier transform infrared spectroscopy analysis.

On average, R^2 for CF was quite satisfactory when considering that the analysis on feeds had been performed in two laboratories and the reproducibility of the analytical method for CF determination among the EGRAN laboratories is not high (Xiccato et al., 1996).

The R^2 for CP was good in all laboratories (>0.90) with SEC lower than 0.8% on DM. The NIRS ability to predict CP in compound feeds for rabbits had been already showed by Xiccato et al. (1999), as well as in compound feed for ruminants (De Boever et al., 1995).

The high NIRS accuracy in predicting GE concentration (R^2: 0.96-0.97 and SEC: 0.12-0.15 MJ/kg DM) may be explained in terms of the high correlation of this variable with the C-H and O-H bonds of the main chemical constituents easily detected by NIRS (Osborne et al., 1993). When predicting DMd and GEd, a lower accuracy was obtained because these characteristics are linked also to the animal's response to feeding, as already found by Aufrère et al. (1996) in compound feeds for swine and ruminants. The same result was observed for NIRS prediction of DE concentration.

Table 2. Statistical parameters of the best calibration equations calculated by each lab

Variable	Mathematical statistical treatment	R^2	SEC	SECV[1]
Crude fibre				
Ghent	(1,4,4)-PLSR	0.929	0.68	1.36
INRA	(2,4,4) SNV-PLSR	0.884	0.88	0.94
Padova	(2,4,4)-MLR	0.899	0.89	
Crude protein				
Ghent	(1,4,4)-PLSR	0.903	0.77	1.04
INRA	(2,4,4) SNV-PLSR	0.911	0.74	0.79
Padova	(2,4,4)-MLR	0.924	0.74	
Gross energy				
Ghent	(2,4,4)-MLR	0.968	0.12	
INRA	(2,4,4) SNV-PLSR	0.956	0.13	0.14
Padova	(2,4,4)-MLR	0.951	0.15	
DM digestibility				
Ghent	(2,4,4)-MLR	0.830	2.14	
INRA	(2,4,4)-PLSR	0.824	2.04	2.12
Padova	(2,4,4)-MLR	0.886	1.77	
GE digestibility				
Ghent	(1,4,4)-MLR	0.856	1.98	
INRA	(2,4,4)-PLSR	0.836	1.97	2.08
Padova	(2,4,4)-MLR	0.843	2.08	
DE				
Ghent	(1,4,4)-MLR	0.901	0.36	
INRA	(2,4,4)-PLSR	0.869	0.39	0.41
Padova	(2,4,4)-MLR	0.906	0.36	

[1] Standard error of cross validation

Looking at the most accurate calibrations calculated by each laboratory (Table 2), the derivative treatment of spectral data always permitted better prediction performance compared to calibrations calculated on absorbance spectra. In fact, the derivative treatment allows to correct the signal for the noise due to the particle size (FEARN, 1999), by adjusting the baseline shift, and to resolve overlapping bands (WILLIAMS, 1987). The second derivative seems to work better than the first derivative transformation for almost all variables and in all laboratories.

Comparing the statistical algorithms, no sensible advantage of MLR or PLSR was evidenced, even though PLSR is usually preferred to overcome inter-correlations among variables (SHENK ET AL., 1992).

The results of the calibrations performed in Padova on the three series of spectra collected by Ghent, INRA and Padova are listed in table 3. On average, the statistical parameters calculated by using the same software and statistical method showed minor differences in comparison with the domestic methods.

PCA showed a clear separation of the same outliers from the three sets of data along the first principal component (PC), explaining 38% of spectra variability (Figure 1). The separation of the three sets of spectra was observed along the second, the third and the fourth PCs, which accounted for 21%, 19% and 11% of data variability, respectively (Figure 2).

Table 3. Calibration results on the three series of spectra obtained in Ghent, INRA and Padova and analysed by using the same software (Sesame) and statistical method (MLR on 2,4,4 data).

Variable	Ghent spectra		INRA spectra		Padova spectra	
	R^2_c	SEC	R^2_c	SEC	R^2_c	SEC
Crude fibre	0.897	0.89	0.908	0.83	0.899	0.89
Crude protein	0.899	0.84	0.897	0.85	0.924	0.74
Gross energy	0.968	0.12	0.958	0.14	0.951	0.15
DM digestibility	0.859	1.95	0.826	2.16	0.886	1.77
GE digestibility	0.867	1.89	0.843	2.06	0.843	2.08
Digestible energy	0.891	0.38	0.893	0.38	0.906	0.36

Figure 1. Scores plot 1st *vs* 2nd PCs: Ghent (G), INRA (I) and Padova (P) spectra (2,4,4)

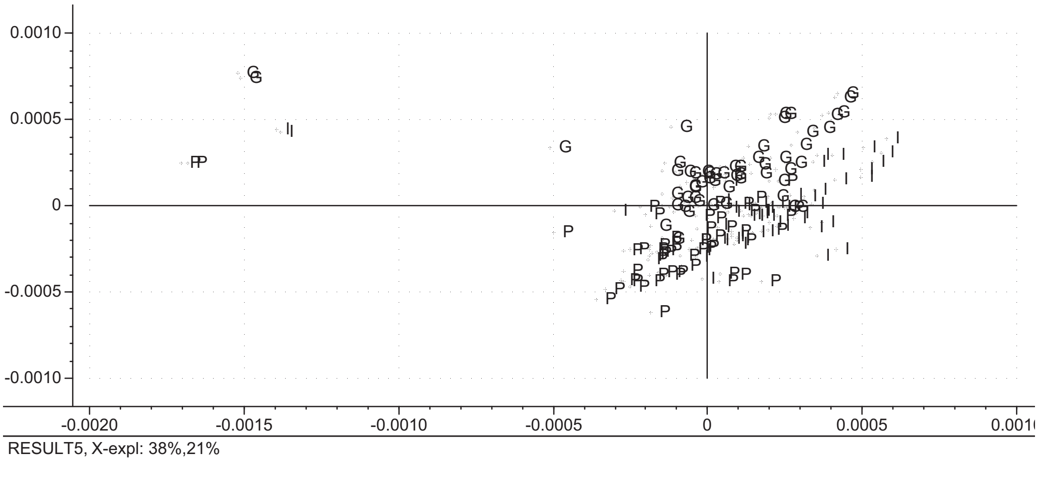
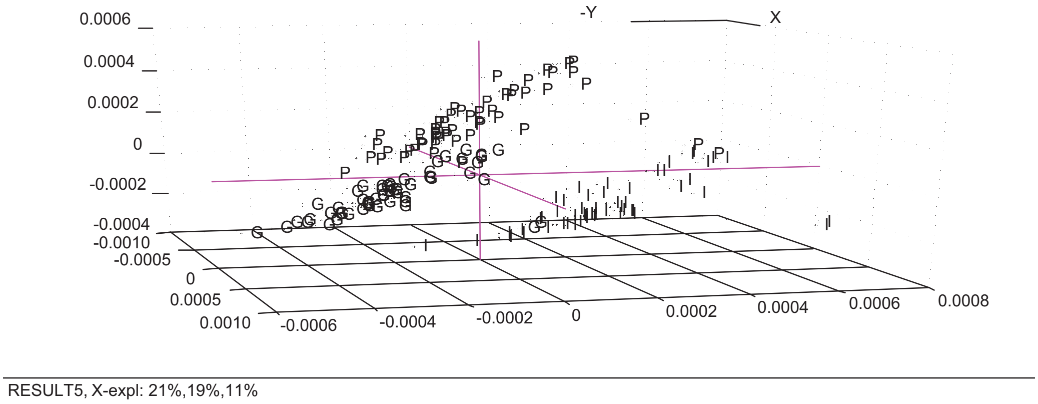


**Figure 2. Scores plot 2nd *vs* 3rd *vs* 4th PCs:
Ghent (G), INRA (I) and Padova (P) spectra (2,4,4)**



CONCLUSIONS

Despite the differences of hardware and software equipment, and the differences in spectral information evidenced by PCA, the use of a common method for sample preparation and a similar statistical approach permitted calibration results to be substantially equivalent in all laboratories. The use of a unique software to analyse spectral data coming from different instrumentation did not reduce the variability in prediction accuracy.

The effectiveness of sample pre-drying on NIRS analysis of low moisture samples, like compound feeds for rabbits, was not investigated and needs further studies. However, the harmonisation of NIRS analysis among laboratories working on rabbit feed evaluation remains a fundamental step towards the possibility of transferring calibration equations.

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REFERENCES

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. *Anim. Feed Sci. Technol.,* **62:** 77-90.

BARTL, F., DELGADILLO, I., DAVIES, A.N., HUVENNE, J.P., MEURENS, M., VOLKA, K., WILSON, R.H., 1996. An interlaboratory comparison of sample presentation methods for the analysis of aqueous solution using fourier transform infrared spectroscopy. *J. Anal. Chem.,* **354**: 1-5.

CHEN, S.L.Y., HSU, A., LEE, M.L., 1987. Application of near infrared reflectance spectroscopy to compositional analysis of commercial pig feed mixes. *J. Assoc. Off. Anal. Chem.,* **70:** 420-423.

De Boever, J.L., Cottyn, B.G., Vanacker, J.M., Boucqué, Ch.V., 1995. The use of NIRS to predict the chemical composition and the energy value of compound feeds for cattle. *Anim. Feed Sci. Technol.,* **51:** 243-253.

Fearn T., 1999. A look at some standard pre-treatments for spectra. *NIR News,* **10 (3):** 10-11.

Gidenne, T., 1999. EGRAN: an European group for rabbit nutrition, presentation and activity. *World Rabbit Science*, **7:** 101-106.

Murray, I., Hall, P.A., 1983. Animal feed evaluation by use of near infrared reflectance (NIR) spectrocomputer. *Anal. Proc.,* **20:** 75-79.

Osborne, B.G., Fearn, T., Hindle, P.H., 1993. Pratical NIR Spectroscopy with Application in Food and Beverage Analysis. *Longman Scientific &Technical, Singapore*, p. 227.

Shenk, J.S., Westerhaus, M.O., 1994. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In: Fahey, G.C., Collins, M., Mertens, D.R., Moser, L.E. (Eds.), Forage Quality, Evaluation and Utilization. *Crop Science Society of America, Lincoln, Nebraska*, pp. 406-449.

Shenk, J.S., Workman, J.J., Westerhaus, M.O., 1992. Application of NIR spectroscopy to agricultural products. In: Burns, D.A., Ciurczak, E.W. (Eds.), Handbook of Near-Infrared Analysis. *Marcel Dekker, Inc., New York,* pp. 383-431.

Valdes, E.V., Leeson, S., 1992. Near infrared reflectance analysis as a method to measure metabolizable energy in complete poultry feeds. *Poultry Sci.* **71:** 1179-1187.

Williams, P.C., 1987. Variables affecting near-infrared reflectance spectroscopy analysis. In: Williams P.C., Norris K.H. (Eds.), Near Infrared Technology in the Agriculture and Food Industries. *Am. Assoc. Cereal Chem., Inc., St. Paul, MN, USA*, pp. 143-167.

Xiccato, G., Carazzolo, A., Cervera, C., Falcao e Cunha, L., Gidenne, T., Maertens, L., Perez, J.M., Villamide, M.J., 1996. European ring-test on the chemical analysis of feed and faeces: influence on the calculation of nutrient digestibility in rabbits. *Proc. 6th World Rabbit Congress*, 9-12 Juillet, Toulouse, France, Vol. **1**, 293-297.

Xiccato, G., Trocino, A., Carazzolo, A., Meurens, M., Maertens, L., Carabaño, R., 1999. Nutritive evaluation and ingredient prediction of compound feeds for rabbits by Near Infrared Reflectance Spectroscopy. *Anim. Feed Sci. Technol.,* **77:** 201-212.