JOINT VIS-NIRS EVALUATION OF FEEDS AND DRIED FECES TO ESTIMATE INGESTION AND DIGESTIBILITY IN GROWING RABBITS

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ABSTRACT

A group of 51 feed samples taken from 12 experimental diets and of 66 dried grouped feces belonging to four nutrition experiments were submitted to a Uv-VIS-NIRS scan (350-2500 nm) in order to calibrate the chemical composition and nutritional parameters, that is namely the ingestion aptitude and digestibility. A new chemometric system has made it possible to contemporary use the spectrum of the diet together with the spectra of the relative pool of dried feces. The daily measured ingestion, in absolute as the intake and in relative as the palatability per unit of metabolic weight, obtained a good resolution for the spectra of the feeds (R^2_{cv}=0.80 and 0.75, respectively), for the feces (0.81 and 0.80) and for the joint evaluation (0.87 and 0.81). The intake was positively correlated to the mineral, insoluble ash, protein, gross energy, crude fiber and acid detergent fiber (ADF) in the feeds, and negatively correlated to N-free extract, lignocellulose and to all the digestibility coefficients except crude fiber. Very significant improvements, on the average of some 0.20 absolute R^2 points, corresponding to 87% as relative, were also provided to the digestibility coefficients by the joint method; in decreasing order: neutral detergent fiber (R^2_{cv}=0.00; 0.18 and 0.50, respectively, for the feeds, feces and joint: +41% vs. the average of feeds and feces), ADF (0.00; 0.45 and 0.62: +39%), ether extract (0.53; 0.52; and 0.86: +34%), crude protein (0.53; 0.53 and 0.75: +22%), gross energy (0.61; 0.74 and 0.83: +15%), crude fiber (0.61; 0.82 and 0.82: +11%), dry matter (0.60; 0.79 and 0.76: +7%), organic matter (0.65; 0.80 and 0.79: +6%) and the N-free extract (0.86; 0.82 and 0.87: +3%). The results corroborate previous knowledge and show the possibilities of using NIRS fecal profiling in rabbit nutrition, which joined together with NIRS of the feeds, could optimize nitrogen monitoring.

Key words: NIRS, Rabbits, Feed, Fecal profiling, Intake, Palatability, Digestibility, Chemometric.

INTRODUCTION

The past five decades have witnessed the emergence of rapid methods which have become useful tools in experimental biology and chemistry. Near infrared spectroscopy (NIRS) has been used for several years for the commercial testing of feeds; it is a rapid and convenient alternative to traditional chemical methods, which are time consuming, polluting and expensive. In EU research operations on rabbit nutrition (FAIR3-1651), the ERAFE group examined collective feed and feces databases from 164 experimental groups using NIRS (Xiccato *et al.*, 1999, 2003) and concluded that the method showed good reliability in the prediction of the chemical constituents, digestibility and energy values of rabbit compound feeds for a wide variation in chemical and ingredient composition. In the present work our approach was directed to fecal profiling using NIRS. This method was originally developed for free-range ruminants, where feeding is mainly based on natural pastures which can be highly variable in their nitrogen contents. The NIRS-NUTBAL program (Mc Dryden, 2003) rules on the surveillance of nutritional scarcity in the field. In the research field, NIRS has been extended to estimate feed intake in ruminants in an easier way than alkanes technique (Garnsworthy and Unal, 2004). In ruminants NIR fecal profiling capitalize on the linkage of the organic matter digestibility with the N contents of the forages (R^2=0.82; Garnsworthy and Unal, 2004).

MATERIALS AND METHODS

Animals and laboratory analyses

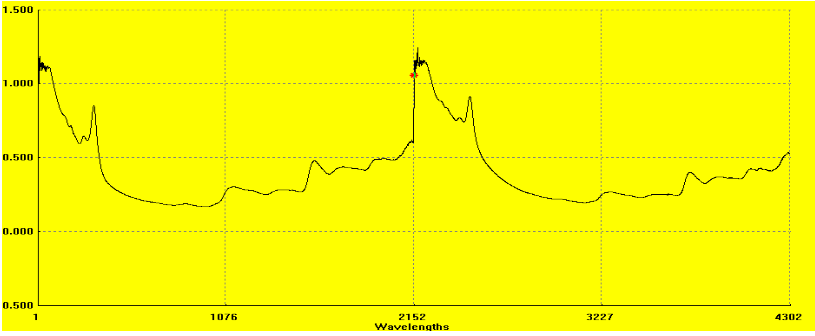
A group of 51 feed samples from 12 experimental diets and of 66 dried grouped feces belonged to 4 nutrition experiments (Meineri and Peiretti, 2007; Peiretti and Meineri, 2008a, b; Peiretti *et al.*, 2007) were examined. The young crossbred rabbits, came from New Zealand white females, were reared in individual cages. The collection of the feces lasted 4 days, when the 130 rabbits weighed on average 2812±421 g and their daily intake as fed basis was 160±40 g/d. Daily samples of the rabbit feces were pooled by groups and then stored at –20°C; after thawing the samples were dried in a ventilated oven at 60°C till to constant weight, then ground to pass a 1mm sieve. Digestibility was calculated through the indirect method of insoluble acid ash (Vogtmann *et al.*, 1975). The spectroscopy scan was conducted using a Model LSP 350-2500P LabSpec Pro portable spectrophotometer (ASD, Analytical Spectral Devices Inc., Boulder, CO) which was equipped to collect spectra from 350 to 2500 nm. The probe was an A122100 ASD Model high-intensity reflectance probe that served as an external light source (2900 K color temperature quartz halogen light) to illuminate the object of interest. This probe can be used to collect reflectance spectra on an area as large as 25 mm in diameter. The reflected light was collected through a 04-14766 ASD Model 1-m long fiber optic jumper cable that consisted of a bundle of forty-four 200-lm fibers. A spectrum was collected for each specimen, on a fixed glass-Petri dish surface. The spectra were collected with the LabSpec Pro software ''sample spectrum count'' option set to 20, that is, 20 spectra were collected and averaged per sample.

Chemometric analysis

All the spectra were mathematically pre-treated as Standard Normal Variates with Detrend (SNVD), derived and then smoothed using the 1441 mode. Multivariate statistical evaluation was performed through the Partial least squares (PLS) method of the WinISI II software (Infrasoft International, Port Matilda, PA, USA) which was chosen to perform the chemometrics, using a cross-validation system to assess the optimal number of latent variables, and allowing one passage for the elimination of any outliers (t>2; H>10).

The spectra were processed separately according to their origin, feed or feces, then the two spectra of the feed and the corresponding feces, each consisting of 2151 digits, were jointed in a unique spectrum (Figure 1) and mathematically pre-treated and calibrated at the 1441 mode. The coefficient of determination in cross validation mode (R^2_{val}), which is equivalent to 1-Variance Ratio (1-VR), was used for the evaluation of the provisional method.

Figure 1: A sample joint spectrum of a feed (left) and its related feces (right), x 350-2500 nm axis repeated on 4302 digits



RESULTS AND DISCUSSION

The database obtained from the four experiments was related to studies about the inclusion of chia seeds (*Salvia hispanica* L.), false flax seeds (*Camelina sativa* L.), golden flax seeds, and of microalgae (*Spirulina platensis*) in rabbit diets. In reference to the whole comprehensive report by Xiccato *et al.* (2003), the global variability was steadily increased in the fibrous components, that is, NDF, ADF and ADL and, consequently, in the gross energy and in the organic matter. Instead, our experiences were more homogenous for the ether extract (SD=4 vs. 12 g/kg DM). The correlation coefficients of the daily intake with the DM composition of the feeds and feces are shown in Figure 2 together with the digestibility coefficients.

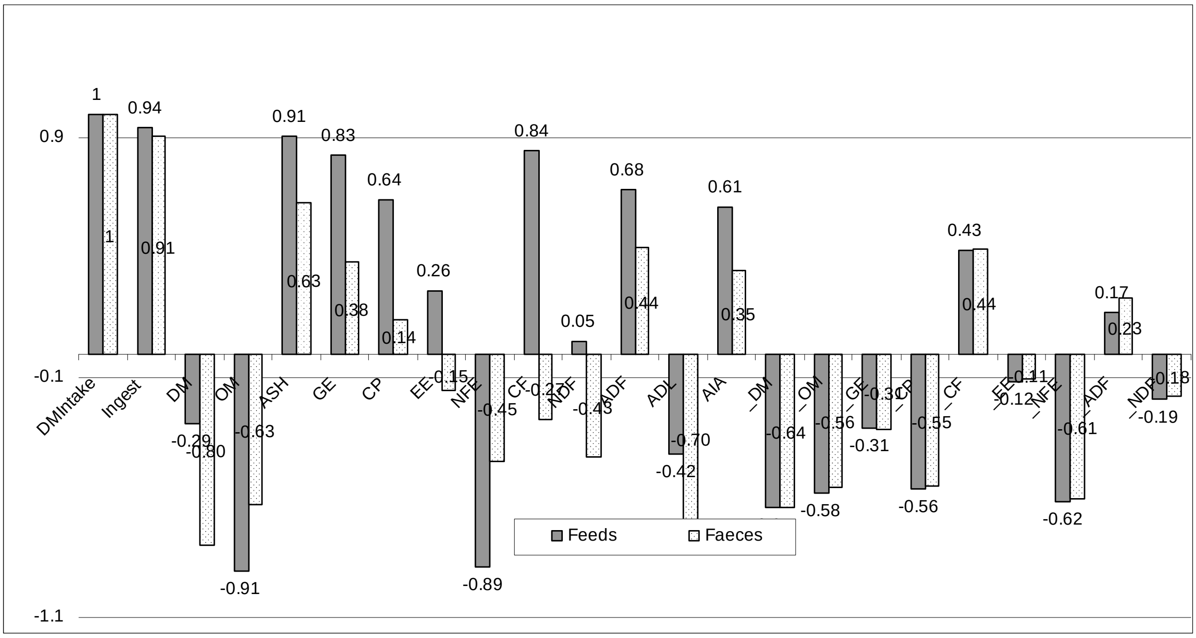


Figure 2: Correlation coefficients of the daily feed intake with the composition of the feeds and feces and with the digestibility coefficients

Some strong positive links emerged for the mineral, insoluble ash, crude protein, gross energy, crude fiber and ADF in the feeds, while appetite was depressed by high contents of N-free extract and of lignocellulose; obviously, contrary to ruminants, where NDF has a central role in the intake, the NDF in rabbit did not regulate the ingestion (r=0.05). Instead, the ADF in the feeds appeared more active in increasing the appetite of rabbits (r=0.68) and this relation was also translated in the ADF of the feces (0.44). The chemical composition of the feces in fact also appeared to be linked to appetite, but only weakly. On the other hand, a corresponding lowering in the digestibility coefficients should be expected for an increased level of intake and this was true for all the components of the feed with the exception for the crude fiber in feeds (0.43) and in feces (0.44). The differences in palatability were classically linked to digestible energy contra-variation. The measured feed ingestion parameters in absolute as intake and in relative as palatability per unit of metabolic weight, in fact obtained a good resolution from the spectra of the feeds (R^2cv= 0.80 and 0.75, respectively), from the feces (0.81 and 0.80) and from the joint evaluation (0.87 and 0.81) (Tables 1 and 2).

In cattle, Garnsworthy and Unal (2004) concluded that using NIRS to predict alkane concentrations of bovine feces does not give accurate estimates of DMI, but a direct prediction of DMI by NIRS gives estimates with similar accuracy to estimates derived from the traditional alkane technique.

Very significant improvements, on average by some 0.20 absolute R^2 points, corresponding to 87% as relative, were also provided for the digestibility coefficients estimated by the joint method; in decreasing order: NDF (R^2_{cv}= 0.00; 0.18 and 0.50, respectively, for feeds, feces and joint: +41% *vs.* the average of feeds and feces), ADF (0.00; 0.45 and 0.62: + 39%), ether extract (0.53; 0.52; and 0.86: +34%), crude protein (0.53; 0.53 and 0.75: +22%), gross energy (0.61; 0.74 and 0.83: +15%), crude fiber (0.61; 0.82 and 0.82: +11%), dry matter (0.60; 0.79 and 0.76: +7%), organic matter (0.65; 0.80 and 0.79: +6%) and the N-free extract (0.86; 0.82 and 0.87: +3%). These results (Tables 1 and 2) agree with the wider EU results by Xiccato *et al.* (1999; 2003) and show some improvement in the NIRS estimation of organic matter (0.79 *vs.* 0.25), crude protein (0.75 *vs.* 0.44) and crude fiber (0.82 *vs.* 0.60) digestibility, which capitalize on the NIRS information from fecal profiling.

Table 1: Calibration and cross-validation of feed composition and apparent digestibility to the UV-Vis-NIR Spectra of the feed or the joint feed-and-feces spectra

		#	Mean	SD	CV%	RSQ	SECV	1-VR	RSQ	SECV	1-VR	1-VR	1-VR
	Composition (%)							Feeds			Joint	*Xic2*	*Xic1*
DMIntake	DM Intake (g/d)	45	0.1612	0.0303	19%	0.99	0.01	0.80	0.99	0.0091	0.87	-	-
Palatab	Palatability (g kg$^{-0.75}$)	49	0.0722	0.0086	12%	0.98	0.00	0.75	0.95	0.0030	0.81	-	-
DM	Dry Matter	49	91.11	0.85	1%	0.67	0.56	0.59	-	-	-	*0.70*	-
OM	Organic Matter	48	92.45	1.30	1%	0.97	0.56	0.81	-	-	-	*0.25*	-
ASH	Ash	48	7.55	1.30	17%	0.97	0.56	0.81	-	-	-	-	-
GE	Gross Energy	49	18.01	1.56	9%	0.99	0.64	0.83	-	-	-	*0.57*	*0.92*
CP	Crude Protein	47	17.35	1.44	8%	0.99	0.53	0.86	-	-	-	*0.86*	*0.87*
EE	Ether Extract	47	6.31	1.48	23%	0.95	0.68	0.78	-	-	-	*0.93*	-
NFE	N-Free Extract	47	54.25	4.81	9%	0.98	2.08	0.81	-	-	-	*0.90*	-
CF	Crude Fiber	49	14.81	2.13	14%	0.97	1.07	0.74	-	-	-	*0.60*	-
NDF	Neutral Detergent Fiber	48	24.65	5.56	23%	0.96	1.87	0.89	-	-	-	*0.50*	-
ADF	Acid Detergent Fiber	47	19.89	5.70	29%	0.95	2.69	0.78	-	-	-	*0.82*	-
ADL	Lignocellulose	48	3.97	0.96	24%	0.89	0.66	0.53	-	-	-	*0.59*	-
AIA	Acid Insoluble Ash	45	1.04	0.23	23%	0.97	0.09	0.86	-	-	-	-	-
	Apparent Digestibility (%)												
_DM	Dry Matter	46	67.09	4.09	6%	0.64	2.62	0.60	0.92	1.74	0.76	*0.79*	*0.81*
_OM	Organic Matter	46	68.19	4.19	6%	0.69	2.50	0.65	0.94	1.68	0.79	*0.25*	-
_GE	Gross Energy	48	66.65	3.67	6%	0.66	2.31	0.61	0.94	1.49	0.83	*0.81*	*0.82*
_CP	Crude Protein	46	68.99	2.90	4%	0.82	2.02	0.53	0.92	1.30	0.75	*0.44*	-
_CF	Crude Fiber	46	27.98	7.38	26%	0.91	4.61	0.61	0.97	3.06	0.82	*0.60*	-
_EE	Ether Extract	49	88.48	3.22	4%	0.90	2.20	0.53	0.98	1.22	0.86	-	-
_NFE	N-Free Extract	43	76.81	3.67	5%	0.96	1.43	0.86	0.95	1.25	0.87	-	-
_ADF	Acid Detergent Fiber	46	25.29	4.19	17%	0.22	4.24	0.00	0.90	3.49	0.62	*0.59*	-
_NDF	Neutral Detergent Fiber	44	28.58	3.80	13%	0.14	3.89	0.00	0.77	2.85	0.50	*0.50*	-

Xic1: Xiccato *et al.*, 1999; Xic2: Xiccato *et al.*, 2003

Table 2: Calibration and cross-validation of feed composition and apparent digestibility to the UV-Vis-NIR Spectra of the feces or the joint feed-and-feces spectra

		#	Mean	SD	CV%	RSQ	SECV	1-VR	RSQ	SECV	1-VR	1-VR
	Composition (%)							Feces			Joint	Xic2
DMIntake	DM Intake (g/d)	58	0.1669	0.0248	15%	0.94	0.01	0.81	0.99	0.0091	0.87	-
Ingest	Ingest (g kg$^{-0.75}$)	58	0.0733	0.0072	10%	0.97	0.00	0.80	0.95	0.0030	0.81	-
DM	Dry Matter	60	42.11	10.69	25%	0.97	3.67	0.88	-	-	-	-
OM	Organic Matter	64	88.81	2.28	3%	0.99	0.66	0.92	-	-	-	-
ASH	Ash	64	11.19	2.28	20%	0.99	0.66	0.92	-	-	-	-
GE	Gross Energy	59	17.90	0.47	3%	0.85	0.22	0.77	-	-	-	-
PG	Crude Protein	59	16.29	1.60	10%	0.96	0.51	0.90	-	-	-	-
EE	Ether Extract	59	1.99	0.36	18%	0.95	0.23	0.58	-	-	-	-
NFE	N-Free Extract	56	38.42	2.38	6%	0.96	1.05	0.80	-	-	-	-
CF	Crude Fiber	57	31.85	1.73	5%	0.97	0.72	0.83	-	-	-	-
NDF	Neutral Detergent Fiber	55	50.02	10.82	22%	1.00	1.89	0.97	-	-	-	-
ADF	Acid Detergent Fiber	58	47.77	10.15	21%	0.99	1.97	0.96	-	-	-	-
ADL	Lignocellulose	58	10.37	1.82	18%	0.93	0.98	0.71	-	-	-	-
AIA	Acid Insoluble Ash	57	3.09	0.46	15%	0.95	0.19	0.83	-	-	-	-
	Apparent Digestibility (%)											
_DM	Dry Matter	59	67.08	3.38	5%	0.93	1.57	0.79	0.92	1.74	0.76	*0.79*
_OM	Organic Matter	59	68.34	3.49	5%	0.94	1.55	0.80	0.94	1.68	0.79	*0.25*
_GE	Gross Energy	59	67.60	3.05	5%	0.83	1.58	0.74	0.94	1.49	0.83	*0.81*
_CP	Crude Protein	64	68.66	2.81	4%	0.70	1.92	0.53	0.92	1.30	0.75	*0.44*
_CF	Crude Fiber	56	30.19	7.03	23%	0.96	2.99	0.82	0.97	3.06	0.82	*0.60*
_EE	Ether Extract	59	89.11	3.16	4%	0.73	2.20	0.52	0.98	1.22	0.86	-
_NFE	N-Free Extract	58	76.15	3.51	5%	0.95	1.50	0.82	0.95	1.25	0.87	-
_ADF	Acid Detergent Fiber	56	26.17	3.75	14%	0.78	2.78	0.45	0.90	3.49	0.62	*0.59*
_NDF	Neutral Detergent Fiber	58	27.90	3.45	12%	0.52	3.14	0.18	0.77	2.85	0.50	*0.50*

Xic2: Xiccato *et al.*, 2003

CONCLUSIONS

The results corroborate previous knowledge and show the possibility of using NIRS fecal profiling in rabbit nutrition, which joined together with NIRS of the feeds, could optimize nitrogen monitoring.

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