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Appraisal of ingestion and digestibility in growing rabbits using near infrared reflectance spectroscopy (NIRS) of feeds and faeces

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ABSTRACT

The aim of this work was to examine whether faecal profiling using NIRS could be profitable for promote the Best Available Techniques (BAT) in the rational feeding of rabbits. A set of 51 feed samples, taken from 12 experimental diets, and of 66 dried grouped faeces samples, belonging to four nutrition experiments, with 130 *ad libitum* registered feed intakes (CV=25%), were submitted to a UV-Vis-NIRS scan (350-2500 nm) in order to calibrate the chemical composition and nutritional parameters, the ingestion aptitude and digestibility. A chemometric system has made it possible to contemporary use the spectrum of the input diet concatenated together with the spectra of the related output pool of the dried faeces. The daily measured feed intake, in absolute or in relative terms as ingestion 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 faeces (0.81 and 0.80) and for the joint evaluation of the concatenated spectra (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) content in the feeds, and negatively correlated to the N-free extract, lignocellulose and all the digestibility coefficients, except crude fiber. Very significant improvements, on average equal to 0.20 R^2 points, were also provided to the digestibility coefficients when using the concatenated method; in decreasing order: neutral detergent fiber (R^2_{cv}=0.00, 0.18 and 0.50 for the feeds, faeces and concatenated, respectively), ADF (0.00, 0.45 and 0.62), ether extract (0.53, 0.52 and 0.86), crude protein (0.53; 0.53 and 0.75), and gross energy (0.61; 0.74 and 0.83). The results corroborate previous knowledge and show the possibility of using NIRS faecal profiling in rabbit nutrition, which together with the NIRS of the feeds, could contribute to nitrogen monitoring.

Key words: Rabbits, Digestibility, Intake, Faecal profiling, Concatenated NIR Spectra.

RIASSUNTO

VALUTAZIONE DELL'INGESTIONE E DELLA DIGERIBILITÁ DI MANGIMI IN CONIGLI IN ACCRESCIMENTO TRAMITE SPETTROSCOPIA NIR DEL MANGIME E DELLE FECI

Lo scopo del presente lavoro è rivolto all'uso della diagnostica faecale mirata alla promozione delle Migliori Tecniche Disponibili (MTD, direttiva 96/61/CE) nell'allevamento razionale del coniglio. Un gruppo di 51

mangimi derivati da 12 diete sperimentali e di 66 pool di feci provenienti da quattro esperimenti di ali-
mentazione, con 130 controlli inviduali di ingestione volontaria (CV=25%), furono sottoposti a scansione
UV-Vis-NIRS (350-2500 nm) al fine di correlare i parametri nutrizionali e di composizione chimica con
l'attitudine all'ingestione e la digeribilità. Un sistema chemometrico concatenato ha reso possibile il con-
temporaneo impiego degli spettri della dieta e dei relativi pool di feci. L'ingestione giornaliera misurata,
in assoluto come consumo volontario e in relativo come appetibilità per unità di peso metabolico, ha evi-
denziato una buona risoluzione per lo spettro degli alimenti (R^2_{CV}=0,80 e 0,75, rispettivamente), delle feci
(0,81 e 0,80) e degli spettri concatenati (0,87 e 0,81). Il consumo volontario era correlato positivamente
a componente minerale, ceneri acido insolubili, proteina grezza, energia grezza, fibra grezza e fibra resi-
stente al detergente acido (ADF) nei mangimi, e correlato negativamente a estrattivi inazotati, lignina e a
tutti i coefficienti di digeribilità, ad eccezione di quelli della fibra grezza. Elaborando spettri concatenati dei
mangimi (input) e delle relative feci (output), miglioramenti consistenti, mediamente pari a 0,20 punti di
R^2_{CV} assoluto, corrispondenti all'87% come relativo, sono stati conseguiti per i coefficienti di digeribilità di
fibra NDF (+41% per il metodo concatenato vs la media di mangimi e feci), ADF (+39%), estratto etereo
(+34%), proteina grezza (+22%), energia grezza (+15%), fibra grezza (+11%), sostanza secca (+7%),
sostanza organica (+6%) ed estrattivi inazotati (+3%). Questi risultati confermano quelli di precedenti
ricerche e dimostrano la possibilità d'impiego nello studio della nutrizione dei conigli della spettroscopia
NIRS, che, applicata alle feci unitamente alla lettura dei mangimi, potrebbe contribuire al monitoraggio
dell'escrezione azotata di questa specie.

Parole chiave: *Conigli, Digeribilità, Ingestione, Profilo fecale, Spettri NIR combinati.*

Introduction

The past five decades have witnessed the emergence of rapid methods which have become useful tools in the field of 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 programmes on rabbit nutrition (FAIR3-1651), the ERAFE group has examined collective feed and faeces databases from 164 experimental groups using NIRS (Xiccato *et al.*, 1999, 2003) and concluded that the method is reliable in the prediction of the chemical constituents, digestibility and energy values of rabbit compound feeds for a wide variation in chemical composition and ingredients. In the present work, our approach has been directed to faecal profiling using NIRS. This method was originally developed for free-range ruminants, where feeding is mainly based on natural pastures which can have highly variable chemical compositions. The NIRS-NUTBAL programme (Mc Dryden, 2003) rules on the surveillance of nutritional scarcity in the field. In the research field, NIRS has been extended to the estimation of feed intake in ruminants in an easier way than the alkanes technique (Garnsworthy and Unal, 2004). In ruminants, NIR faecal profiling capitalises on the connection of organic matter digestibility to the N contents of the forages (R^2=0.82; Garnsworthy and Unal, 2004). Decruyenaere *et al.* (2008) studied two large databases with 1034 forages and faeces in sheep and confirmed the relevance of faecal profiling. They also discovered the opportuneness of a joint elaboration of multi-NIRspectra, constituted by the input spectra of the feed concatenated with the output spectra of the individual or pooled faeces. In this way, the maximum R^2 was achieved by fitting the grass OM intake to OM digestibility. The aim of the present work has been to examine whether faecal profiling using NIRS could be profitable

to promote the Best Available Techniques (BAT) in the modern feeding of rabbits.

Material and methods

The database was obtained from four experiments related to studies on the inclusion of chia (*Salvia hispanica* L.) seeds (Meineri and Peiretti, 2007), false flax (*Camelina sativa* L.) seeds (Peiretti *et al.*, 2007), golden flax (*Linum usitatissimum*) seeds (Peiretti and Meineri, 2008b), and microalgae (*Spirulina platensis*) (Peiretti and Meineri, 2008a) in rabbit diets. The young crossbred rabbits, originating from New Zealand White females, were reared in individual cages. The collection of the faeces lasted for 4 days, after which the 130 rabbits weighed on average 2812±421 grams (CV=15%) and their individual daily intake, on a fed basis, was 160±40 grams d^{-1}(CV=25%). Daily samples of the single faeces of the rabbits were pooled by groups and then stored at -20 °C. After thawing, the samples were dried in a ventilated oven at 60 °C till constant weight and then ground to pass a 1mm sieve. Digestibility was calculated through the acid insoluble ash indirect method (Vogtmann *et al.*, 1975). After pooling of the faeces, the elaborated set consisted of 51 feed samples belonging to 12 experimental diets and 66 dried faeces pooled samples.

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. An A122100 ASD Model high-intensity reflectance probe was used as an external light source (a 2900 K colour temperature quartz halogen light) to illuminate the objects 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 jump cable that consisted of a bundle of forty-four 200-lm fibers. The spectra were collected with the LabSpec Pro software "sample spectrum count" option set at 20, meaning that 20 spectra were collected and averaged per sample.

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 Modified Partial Least Squares (MPLS) method of the WinISI II software (Infrasoft International, Port Matilda, PA, USA) which was chosen to perform the chemometric, 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 faeces. Then the two spectra of the feed and the corresponding faeces, each consisting of 2151 digits, were joined in a single concatenated spectrum (Figure 1) and mathematically pre-treated and calibrated in the same way as the 1441 mode. The coefficient of determination in cross validation mode (R^2_{cv}) was used for the assessment evaluation of the equations, while the Standard Errors of Validation were compared with the available reference papers for digestibility values.

Results and discussion

In comparison to the EU database of Xiccato *et al.* (2003), the standard deviations of the feed compounds (Table 1) were higher for the fibrous components, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) and, consequently, for the gross energy and the organic matter content.

The correlation coefficients of the feed in-

Figure 1.   A concatenated UV-Vis-NIR spectrum of a feed (left) and its related fae-
ces (right).
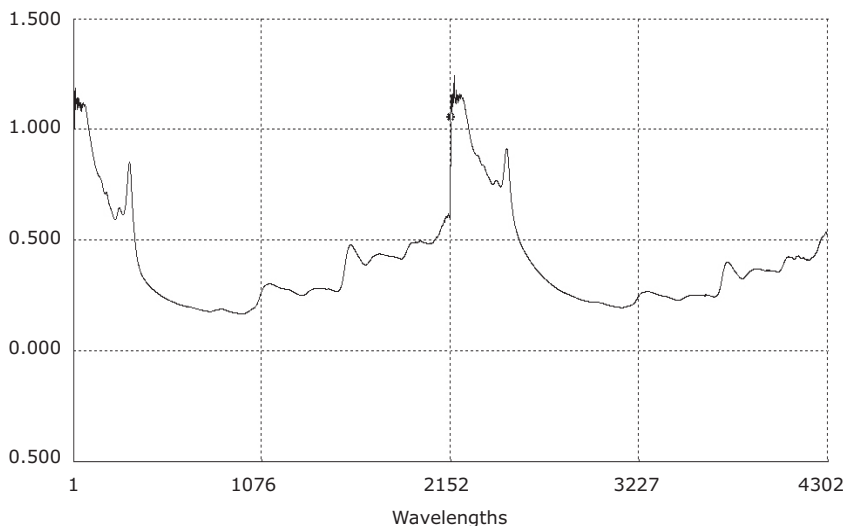
X: 350-2500 nm axis repeated on 4302 digits; Y=Log (1/R).

Figure 2.   Correlation coefficients of the daily feed intake with the chemical compo-
sition of the feeds and feces and with the digestibility coefficients.
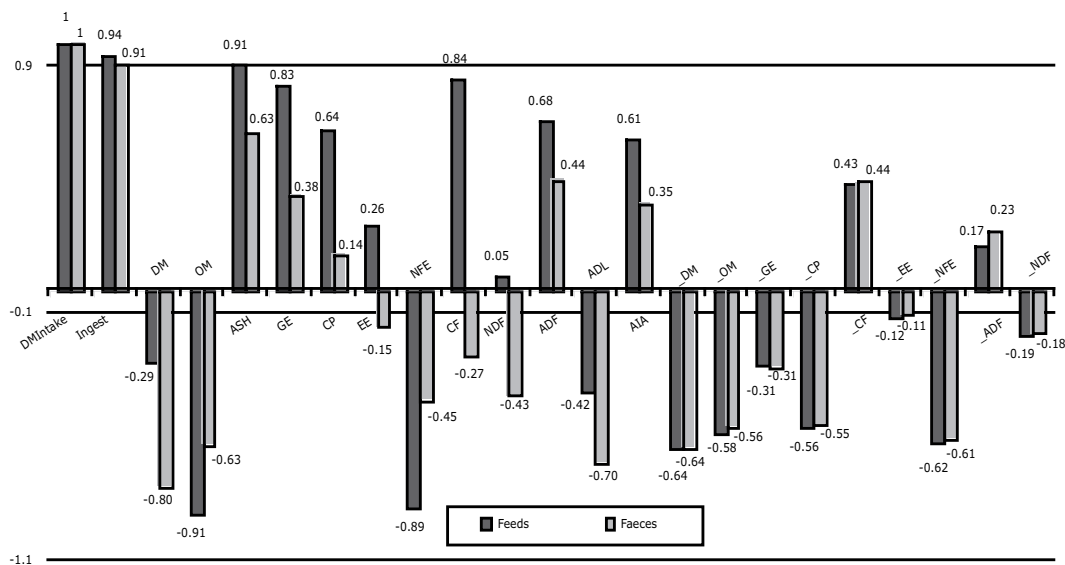
Note: abbreviation of variables are reported in table 1 and 2, first column.

take with the chemical parameters on the DM basis of the feeds and faeces are shown in Figure 2 together with the digestibility coefficients. Strong positive relationships appeared for the ash, acid insoluble ash, crude protein, gross energy, crude fiber and ADF in the feeds, while the intake was depressed by high contents of N-free extract and ADL. Unlike to ruminants, where NDF plays a central role on the intake, the NDF does not apparently regulate ingestion (r=0.05) in rabbits. On the other hand, the ADF in the feeds appeared more active in increasing the intake of rabbits (0.68) and this correlation was also reflected by the ADF of the faeces (0.44). The chemical composition of the faeces also appeared to be linked to the intake, 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 of the crude fiber in the feeds (0.43) and in faeces (0.44). The higher intakes were related to lower levels of the digestible energy contents in the experimental diets. The measured *ad-libitum* feeding parameters, in absolute terms as intake, and in relative terms as ingestion per unit of metabolic weight, obtained a good resolution from the spectra of the feeds (R^2cv=0.80 and 0.75, respectively), from the faeces (0.81 and 0.80) and from the joint evaluation of the concatenated spectra (0.87 and 0.81) (Tables 1 and 2).

Garnsworthy and Unal (2004) concluded that using NIRS to predict alkane concentrations in bovine faeces did not lead to accurate estimates of DMI, but a direct prediction of DMI by NIRS instead gave estimates with a similar accuracy to that derived using the traditional alkane technique.

Very significant improvements, on average by some 0.20 absolute R^2cv points, corresponding to 87% as relative, were also

provided for the digestibility coefficients estimated by the concatenated spectra. The following appeared to be advantaged: NDF (+41% for concatenated *vs* the average of feeds and faeces), ADF (+39%), ether extract (+34%), crude protein (+22%), gross energy (+15%), crude fiber (+11%), dry matter (+7%), organic matter (+6%) and the N-free extract (+3%). These results, in spite of our higher standard deviations, agree well with the findings reported in EU experiments conducted by Xiccato *et al.* (1999; 2003). The Standard Error of Validation are compared in Tables 1 and 2, which show some improvement in the NIRS estimation of apparent digestibility coefficients for crude protein (SEV 1.3% *vs* 2.6%) and gross energy (1.5 *vs* 1.9 MJ kg^{-1} DM). Caution must be taken in extrapolating the NIRS estimates of the intake and ingestion parameters because, in spite of the 130 individual values of the intakes, with a 25% coefficient of variation, there were only 12 spectra in the experimental rations in the database, a rather limited number. This ingestion aspect, which is fundamental in ruminant nutrition, has been poorly investigated by rabbit scientists especially from the point of view of rapid NIRS utilization, considering the fact that no feeds tables exist that report information about the ingestion of simple feeds or forages.

Conclusions

The results confirm the ability of the new chemometric method which uses the concatenated spectra of feeds and faeces in the analyses of experimental trials. They also corroborate results by previous researches and show the possibility of using NIRS faecal profiling in rabbit nutrition, which joined together with NIRS of the feeds, could optimize nitrogen monitoring. In the BAT challenge, further samples of dried and ground

Table 1. Calibration and cross-validation of feed composition and apparent digestibility to the UV-Vis-NIR Spectra of the feed or the concatenated feed-and-faeces spectra.

Composition			#	Mean	SD	CV%	Feeds			Joint			SEV Xic2	SEV Xic1
							RSQ	SECV	R²cv	RSQ	SECV	R²cv		
DMIntake	DM Intake	kg d⁻¹	45	0.1612	0.0303	19%	0.99	0.01	0.80	0.99	0.0091	0.87	-	-
Ingest	Ingestion, DMIntake	LW kg⁻·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	-	-	-	0.48	-
OM	Organic Matter	%DM	48	92.45	1.30	1%	0.97	0.56	0.81	-	-	-	0.86	-
ASH	Ash	"	48	7.55	1.30	17%	0.97	0.56	0.81	-	-	-	-	-
GE	Gross Energy	MJ kg⁻¹ DM	49	18.01	1.56	9%	0.99	0.64	0.83	-	-	-	0.25	0.26
CP	Crude Protein	%DM	47	17.35	1.44	8%	0.99	0.53	0.86	-	-	-	0.56	0.77
EE	Ether Extract	"	47	6.31	1.48	23%	0.95	0.68	0.78	-	-	-	0.42	-
NFE	N-Free Extract	"	47	54.25	4.81	9%	0.98	2.08	0.81	-	-	-	-	-
CF	Crude Fiber	"	49	14.81	2.13	14%	0.97	1.07	0.74	-	-	-	1.60	-
NDF	Neutral Detergent Fiber	"	48	24.65	5.56	23%	0.96	1.87	0.89	-	-	-	3.20	-
ADF	Acid Detergent Fiber	"	47	19.89	5.70	29%	0.95	2.69	0.78	-	-	-	1.40	-
ADL	Lignocellulose	"	48	3.97	0.96	24%	0.89	0.66	0.53	-	-	-	1.10	-
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	1.90	1.75
_OM	Organic Matter		46	68.19	4.19	6%	0.69	2.50	0.65	0.94	1.68	0.79	-	-
_GE	Gross Energy		48	66.65	3.67	6%	0.66	2.31	0.61	0.94	1.49	0.83	1.90	1.99
_CP	Crude Protein		46	68.99	2.90	4%	0.82	2.02	0.53	0.92	1.30	0.75	2.60	-
_CF	Crude Fiber		46	27.98	7.38	26%	0.91	4.61	0.61	0.97	3.06	0.82	-	-
_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	-	-
_NDF	Neutral Detergent Fiber		44	28.58	3.80	13%	0.14	3.89	0.00	0.77	2.85	0.50	-	-

#Number of valid spectra after removing of outliers; 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 concatenated feed-and-faeces spectra.

Composition			#	Mean	SD	CV%	RSQ	SECV	R²cv	RSQ	SECV	R²cv	SEV
						Faeces				Joint			Xic2
DMIntake	DM Intake	kg d⁻¹	58	0.1669	0.0248	15%	0.94	0.01	0.81	0.99	0.0091	0.87	1.90
Ingest	Ingestion, DMIntake	LW kg⁻·⁷⁵	58	0.0733	0.0072	10%	0.97	0.00	0.80	0.95	0.0030	0.81	-
DM	Dry Matter	%DM	60	42.11	10.69	25%	0.97	3.67	0.88	-	-	-	-
OM	Organic Matter	%DM	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	MJ kg⁻¹ DM	59	17.90	0.47	3%	0.85	0.22	0.77	-	-	-	-
PG	Crude Protein	%DM	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	1.90
_OM	Organic Matter		59	68.34	3.49	5%	0.94	1.55	0.80	0.94	1.68	0.79	-
_GE	Gross Energy		59	67.60	3.05	5%	0.83	1.58	0.74	0.94	1.49	0.83	1.90
_CP	Crude Protein		64	68.66	2.81	4%	0.70	1.92	0.53	0.92	1.30	0.75	2.60
_CF	Crude Fiber		56	30.19	7.03	23%	0.96	2.99	0.82	0.97	3.06	0.82	-
_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	-
_NDF	Neutral Detergent Fiber		58	27.90	3.45	12%	0.52	3.14	0.18	0.77	2.85	0.50	-

Xic2: Xiccato et al., 2003.

rabbit faeces examined via NIRS, could substantially improve the estimation of the apparent digestibility of nitrogen in feeds obtained only through the NIRS of feeds, with mutual advantages for the feed industry and herdsmen.

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