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Faecal NIRS to assess the chemical composition and the nutritive value of dairy sheep diets

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Abstract. This study explored the value of faecal near infrared reflectance spectroscopy (NIRS) in predicting the chemical composition and the nutritive value of pelleted diets offered to dairy ewes. Reference data and faecal spectra were measured from three experiments in which dry, mid- and late-lactating Sarda dairy ewes were individually fed 8 different complete pelleted diets. The pelleted diets used featured different energy contents (1.2-1.9 Mcal/kg DM of NE_L), NDF (23.9-51.9% DM) and fairly similar CP levels (17.5-19.8% DM). In each experiment, intake, chemical composition and *in vivo* digestibility of diets were measured. Individual faecal samples (n = 120) were collected, dried, milled and scanned between 1100-2500 nm in 2 nm increments using a Foss NIR-Systems 5000 monochromator spectrometer. Calibration equations were developed for predicting intake, dietary chemical composition on DM basis (CP, N fractions, NDF, ADF, ADL, NSC, WSC), *in vivo* digestibility coefficients (OMD, CPD, NDFD), and energy concentration (Mcal of NE_L), by using the modified partial least squares procedure (MPLS). Values for R^2 and the standard error of cross validation (SEC-V) were used as estimates of calibration equation prediction accuracy. Overall, the parameters of dietary chemical composition showed high R^2 (>0.90) and low SECV, with best values for CP (R^2 = 0.95, SECV = 0.24). Regarding the digestibility coefficients, OMD and NFCD presented the best values of R^2 and SECV (0.90, 3.44; and 0.91, 2.51, respectively). Dietary energy concentration showed an R^2 of 0.88 and SECV 0.09 Mcal/kg DM. This study confirmed the accuracy of the predictions based on faecal NIR spectra with reference to dietary chemical composition and, to a lesser extent, to its nutritive value.

Keywords. Faecal near infrared reflectance spectroscopy – Diet chemical composition – Dairy sheep.

Utilisation du NIRS pour la prévision de la composition et de la valeur nutritive de la ration chez les brebis laitières

Résumé. *L'essai a été mené pour évaluer la capacité du NIRS fécal pour la prévision de la composition chimique et de la valeur nutritive des rations en pellets offerts à des brebis laitières. Les données de référence et les spectres fécaux ont été mesurés dans trois essais avec des brebis sardes respectivement taries, au milieu et à la fin de la lactation, alimentées individuellement avec 8 différentes diètes complètes. Les rations étaient caractérisées par leur différente teneur énergétique (1,2-1,9 Mcal/kg MS de NE_L) et MAT assez similaires (17,5-19,8% MS). L'ingestion, la composition chimique et la digestibilité* in vivo *des rations ont été mesurées dans chaque essai. Des échantillons de fèces individuels (n = 120) ont été séchés, broyés et scannés avec le Foss NIR-Systems 5000 monochromateur spectromètre entre 1100-2500 nm avec 2 nm d'incréments. Des équations de calibration ont été développées pour prédire l'ingestion, la composition chimique en % de la MS (MAT, fractions azotées, NDF, ADF, ADL, NSC, WSC) la digestibilité* in vivo *(DMO, DMAT, DNFC) et la concentration énergétique (Mcal de NE_L) avec la "modified partial least squares procedure" (MPLS). Pour évaluer la précision de la calibration le coefficient de détermination R^2 et l'erreur standard de la validation croisée (SEC-V) ont été utilisés. En général les valeurs plus hautes en R^2 et plus faibles en SECV ont été enregistrées pour MAT (R^2 = 0,95, SECV = 0,24). Concernant les coefficients de digestibilité les meilleures valeurs de R^2 et SECV ont été trouvées pour DMO et DNFC (0,90 et 3,44 ; 0,91 et 2,51, respectivement). La concentration énergétique a montré un R^2 de 0,88 et un SECV de 0,09 Mcal/kg MS. Cette étude confirme l'exactitude de la prévision avec les spectres des fèces par NIR pour la composition chimique de la diète et même à un moindre degré pour la valeur nutritionnelle.*

Mots-clés. *Spectroscopie dans le proche infrarouge fécal – Composition chimique de la ration – Brebis laitières.*

I – Introduction

Near infrared reflectance spectroscopy (NIRS) is a long established, and now mature technology to predict the chemical composition and the digestibility of forages and other foods, as reviewed by different authors (Givens and Deaville, 1999; Landau *et al*., 2006), and it is now well accepted as alternative to laboratory chemical procedures. One of the relatively new applications of near infrared reflectance spectroscopy is devoted to predict diet quality on the basis of faecal spectra. Different researches were carried out in this field with cattle (Lyons and Stuth, 1992; Boval *et al*., 2004), sheep (Li *et al*., 2007) and goats (Leite and Stuth, 1995; Landau *et al*., 2004; Walker *et al*., 2007). The NIRS methodology presents many advantages over standard methods used for dietary evaluation, and, in particular, low cost, chemical-free, rapid and non-destructive analyses. But this method requires accurate reference values for developing faecal NIRS calibrations to predict functional properties, like digestibility and intake. For this reason, *in vivo* data sets coming from confined feeding experiments are the most valuable. The nutritional variables that have been estimated with the high accuracy using faecal NIRS method were the dietary CP content and the *in vitro* OM digestibility (Landau *et al*., 2006). Other very important nutrients for ruminants like N fractions, NFC, WSC, starch and fat content have been less studied, even though these nutrients can provide valuable information about the energy and N level of the diet.

For this reason, this study explored the accuracy of faecal near infrared reflectance spectroscopy (NIRS) in predicting the chemical composition of pelleted diets for which detailed analyses of carbohydrate and N fractions were available and their nutritive value when fed at different levels of intake.

II – Materials and methods

Three experiments were carried out from December to July, using Sarda dairy sheep. In all experiments 40 sheep were used, divided in 8 homogeneous groups. In the first experiment dry sheep (LW = 46.8 ± 2.9 kg, mean ± SD) received the 8 diets twice a day, for a supply of 600 g/d as fed. In the second and third experiments mid (DIM = 112 ± 7 d; milk yield = 1657 ± 153 g/d, LW = 43.6 ± 3.9 kg) and late lactation sheep (DIM = 200 ± 10 d; milk yield = 1213 ± 355 g/d; LW = 46.9 ± 4.2 kg) were fed the pelleted diets *ad libitum*. The composition of the 8 complete pelleted diets is reported in Table 1.

Table 1. Ingredients of the experimental diets (% DM)

Ingredients	Diet†							
	CM	**WM**	**CF**	**BM**	**CC**	**BP**	**AA**	**SH**
Barley meal				47.0				
Corn flaked			51.6					
Corn meal	67.5	9.8	8.0	1.7	5.8	1.9		5.5
Alfalfa hay	26.7	28.0	27.0	28.0	26.6	26.0	89.4	26.2
Beet pulp						40.9		
Corn cobs					32.5			
Corn germ			6.4	12.9	20.0	20.0		
Soybean hulls								62.3
Wheat middlings		56.7			10.0	6.1	5.0	
Minerals and vit.	5.8	5.5	7.0	7.0	5.1	5.1	5.6	6.0

†Acronyms indicate the main ingredient of each diet: CM = corn meal; WM = wheat middlings; CF = corn flakes; BM = barley meal; CC = corn cobs; BP = beet pulps; AA = alfalfa hay; SH = soybean hulls.

Dehydrated alfalfa hay as common basis was used and was added to other ingredients in order to obtain different fiber (NDF, ADF and ADL) and energy contents, with fairly similar CP level. Before the beginning of each experiment the animals were fed at pasture with a mixture of the experimental pelleted diets as supplement. The ewes were then confined in pen and adapted to consume only the mixture of the pelleted diets (preliminary period, 7 days in total). After this period, the ewes were put in individual metabolic cages and submitted to the experimental feeding treatments. They stayed in cages for 23 days in total, 14 days were for adaptation (adaptation period) and 9 days for experimental measurements (experimental period). Since only 20 metabolic cages were available, the experiments were divided in two sequential trials, testing 4 diets at each time. The same diets were used throughout the three experiments. In all experiments, during the first 6 days of each experimental period, individual intake and total faeces and urine excreted were measured daily. The chemical composition of diets was measured and the energy content calculated according to NRC (1994) based on the digestibility coefficients measured in this experiment. The faeces of each ewe were mixed up each day and an aliquot was sampled and immediately stored at -20°C. Daily individual samples of faeces were pooled to have individual weekly samples for chemical analysis. *In vivo* digestibility was measured by collecting separately faeces and urine. Individual faecal samples (n = 120) were collected, dried, milled and scanned between 1100-2500 nm in 2 nm increments using a Foss NIR-Systems 5000 monochromator spectrometer. One sample was discarded because the ewe showed signs of acidosis. Before calibration reflected energy, data were transformed using scatter correction treatment (SNVD: standard normal variate and detrend) and different mathematical treatments using WINISI software (ISI, 1999). Modified partial least square regression (MPLS) was used to develop calibration equations with faecal spectra samples as independent variables and dietary chemical composition on DM basis, CP, N fractions (Licitra *et al*., 1996), NDF, ADF and ADL (van Soest, 1994), NFC calculated as [100 - (NDF - NDIP) - CP - EE - Ash], EE, WSC, starch, OM intake (OMI), CP intake (CPI, g/day or g/kg $LW^{0.75}$) digestibility coefficients of OM (OMD), CP (CPD), NFC (NFCD), energy concentration (Mcal/kg DM of NE_L; NRC, 1994) and CP/NE_L ratio (g/Mcal/kg DM), as dependent reference data. To assess the prediction performance of NIR equations, the main statistics used were the standard error of calibration (SEC), the standard error of cross-validation (SEC-V), the determination coefficient in calibration (R^2) and in cross-validation (R^2_{cv}).

III – Results and discussion

The best predictions of dietary composition were obtained with the transformation values equal order of derivative function, gap (nm), segment length of first and second smoothing (nm) of 2,6,6,2 for chemical composition, digestibility and energy contents and 2,4,4,1 for intakes (ISI, 1999). Nutrient composition, calibration and cross-validation statistics of nutrients are presented in Table 2.

Overall the predictions of dietary chemical composition showed high R^2 and low SEC-V, with best values for OM (R^2 = 0.95, SEC-V = 0.31) and CP (R^2 = 0.95, SEC-V = 0.24). The SEC and SEC-V values found for CP were lower than those found in other faecal NIRS studies (Leite and Stuth, 1995; Boval *et al*., 2004; Landau *et al*., 2004). Fiber fractions as well as NFC, WSC and starch presented very high R^2 (≥0.95) but with great SEC-V values as compared to lab accuracy. Fat composition (EE) showed a relatively low R^2 in calibration and cross validation. The N fractions showed also good statistics, in particular NPN (non protein N), BSP (buffer soluble protein) NDIN (neutral detergent insoluble protein) and ADIN (acid detergent insoluble protein), whereas TP (true protein) and IP (insoluble protein) had less accurate values (Table 2).

Intake of nutrients was predicted with lower accuracy (Table 3) than dietary composition, as already observed in other studies (Coates, 2000; Landau *et al*., 2004). Expression of intake on a $LW^{0.75}$ basis did not improve calibrations, compared with absolute daily values (Table 3). Regarding digestibility coefficients, OMD and NFCD presented the best values of R^2 and SEC-V (0.9, 3.44 and 0.91, 2.51 respectively). Dietary energy concentration showed an R^2 of 0.88 and SEC-V 0.09. The CP/NE_L ratio had good statistics (R^2 = 0.90, SEC-V = 6.95). This is particularly interesting because this ratio was well correlated with milk urea in stall-fed (Giovanetti, 2007) and grazing dairy sheep (Molle *et al*., 2009).

Table 2. Prediction of chemical dietary composition (% DM) for the whole dataset (n = 119) using second derivatized spectra for calibration

Constituent	n	Mean	SD	Calibration		Cross-validation	
				SEC	R^2	SEC-V	R^2_{cv}
OM	116	91.28	1.11	0.24	0.95	0.31	0.92
CP	116	18.43	0.84	0.19	0.95	0.24	0.92
TP	111	14.73	0.52	0.18	0.88	0.24	0.80
NPN	117	3.67	0.60	0.15	0.94	0.18	0.91
BSP	109	1.52	0.41	0.10	0.94	0.15	0.87
NDIN	110	4.66	0.92	0.21	0.95	0.31	0.89
ADIN	116	1.63	0.27	0.08	0.92	0.10	0.86
IP	115	13.30	0.71	0.29	0.84	0.34	0.77
EE	114	3.01	0.62	0.24	0.85	0.34	0.74
NDF	115	38.58	9.91	1.92	0.96	2.70	0.93
ADF	115	20.99	7.72	1.43	0.97	1.95	0.94
ADL	114	3.26	1.14	0.18	0.97	0.26	0.95
NFC	118	36.08	10.66	2.09	0.96	2.89	0.93
WSC	113	3.84	0.45	0.10	0.95	0.13	0.91
Starch	115	22.90	11.21	2.32	0.96	3.14	0.92

Table 3. Prediction of digestibility (%) and intake (g, and g/kg $LW^{0.75}$) for the whole dataset (n = 119) using second derivatized spectra for calibration

Constituent	n	Mean	SD	Calibration		Validation	
				SEC	R^2	SEC-V	R^2_{cv}
OMI (g)	115	1262.19	671.03	177.47	0.93	290.79	0.81
OMI (g/kg $LW^{0.75}$)	117	72.23	37.43	11.78	0.90	15.57	0.83
CPI (g)	115	255.66	138.74	36.52	0.93	55.86	0.84
CPI (g/kg $LW^{0.75}$)	116	14.62	7.76	2.05	0.93	3.11	0.84
OMD (%)	114	72.33	8.03	2.55	0.90	3.44	0.82
CPD (%)	113	77.03	5.19	2.43	0.78	2.93	0.72
NFCD (%)	116	88.48	6.28	1.84	0.91	2.51	0.84
NE_L (Mcal/kg DM)	117	1.68	0.21	0.07	0.88	0.09	0.79
CP/NE_L (g/Mcal/kg DM)	113	109.83	16.43	5.24	0.90	6.95	0.84

IV – Conclusions

This study confirms the accuracy of the predictions based on faecal NIR spectra with reference to dietary chemical composition and, to a lesser extent, its nutritive value. The results obtained demonstrate also that *in vivo* feeding trials offer the best data for developing robust faecal NIRS calibrations. More studies are needed to evaluate this approach on grazing system, which is the most common for sheep in Mediterranean regions.

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