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The use of NIRS for prediction of intake, digestibility, diet composition and faecal concentration of n-alkanes in sheep fed different proportions of lucerne and rye grass (*Lolium rigidum*)

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SUMMARY – Sixteen sheep fed different proportions of lucerne and rye grass (100:0, 67:33, 33:67 and 0:100) were used to obtain regression equations for prediction, from the NIRS analysis of faeces, of dry matter intake (g/day or g/kg $LW^{0.75}$), digestibility coefficients of dry matter (DMD), organic matter (OMD) and neutral detergent fibre (NDFD), lucerne consumed (% of the diet, g DM/day or g DM/kg $LW^{0.75}$), and n-alkane concentrations in faeces. Sixteen pairs of faecal samples were collected in order to develop two calibration equations. Faeces were: (i) pooled (per animal) throughout a 7-day digestibility trial (S1); and (ii) taken daily from the rectum of the same animals at 9:00 hours (throughout the same 7 days of the digestibility trial) and pooled to obtain a unique sample per sheep (S2). All S2 equations had coefficients of determination in calibration (R^2) higher than 0.66, whereas R^2 in S1 equations was lower for the prediction of intake per kg metabolic weight (0.44), and OMD (0.46) and NDFD (0.56). The coefficients of determination in cross-validation (R^2cv) were much lower than those in calibration, with equations derived from S2 showing again better performances for intake prediction. Accuracy of equations for prediction of digestibility coefficients was reached for neither S1 nor S2 samples. Diet composition and faecal concentration of most n-alkanes was more accurately predicted in both cases (R^2cv>0.67).

Keywords: Near infrared reflectance spectroscopy, intake, digestibility, n-alkanes, sheep.

RESUME *– "Utilisation de la SPIR pour la prédiction de l'ingestion, de la digestibilité, de la composition du régime et de la concentration fécale des n-alcanes chez des ovins alimentés avec différentes proportions de luzerne et de ray grass (*Lolium rigidum*)". L'essai a été mené sur seize brebis alimentées avec différentes proportions de luzerne et de ray grass (100:0, 67:33, 33:67 et 0:100) pour obtenir des équations de régression pour la prévision des quantités ingérées de matière sèche (g/jour ou g/kg $PV^{0,75}$), la digestibilité de la matière sèche (DMS), la matière organique (DMO) et les parois cellulaires (DPC), la proportion de luzerne consommée (% du régime, g MS/jour ou g MS/kg $PV^{0,75}$) et la concentration fécale en n-alcanes à partir de l'analyse des fèces moyennant la SPIR. Seize paires d'échantillons ont été prélevées pour développer deux équations de calibration SPIR : (i) à partir du mélange des fèces de la collecte journalière (par animal durant toute la période de digestibilité) (S1) ; et (ii) à partir du mélange des prélèvements rectaux journaliers (par animal durant le même bilan de digestibilité) (S2). Toutes les équations de calibration S2 avaient des coefficients de détermination (R^2) supérieurs à 0,66, tandis que ceux de S1 étaient faibles pour la prévision des quantités ingérées exprimées par kg de poids métabolique (0,44), de la DMO (0,46) et de la DPC (0,56). Les coefficients de détermination de la validation croisée (R^2vc) ont été plus faibles que ceux de la calibration, tout en notant que les équations S2 sont plus précises pour la prévision des quantités ingérées. La précision des équations de prévision de la digestibilité a été faible dans S1 et S2, tandis que la composition du régime et la concentration fécale pour la plupart des n-alcanes ont été prévues avec une certaine précision dans les deux cas (R^2vc > 0,67).*

Mots-clés : *SPIR, quantités ingérées, digestibilité, n-alcanes, ovins.*

Introduction

Integration of grazing sheep in the crops' cycle of the arid and semi-arid areas of Spain is not only possible but also very interesting from the point of view of profitability and conservation of the environment (de Vega and Delgado, 2002). One of the proposed alternatives (Delgado, 2000) includes direct grazing of winter cereals, shrubs (*Atriplex halimus*), and associations of lucerne

(*Medicago sativa*) with rye grass (*Lolium rigidum*) along the year. The nutritive value of all these resources will depend, mainly, on their intake and digestibility properties, both variables being very difficult to estimate accurately in grazing animals (Mayes and Dove, 2000). The n-alkane methodology (Mayes *et al*., 1986) is currently one of the most used for this purpose, although the analysis of hydrocarbons is costly, time consuming, and requires the use of organic solvents which are potential contaminants for the environment.

The near infrared reflectance spectroscopy (NIRS) is being routinely used for prediction of chemical composition and energy value of feeds (Aufrère *et al*., 1996), intake and digestibility (Boval *et al*., 2003, 2004; Decruyenaere *et al*., 2003), and faecal concentration of n-alkanes in beef cattle (Garnsworthy and Unal, 2004) and deers (Ru *et al*., 2002), and seems to be a promising technique for estimation of intake and diet composition in sheep fed mixed barley grain:straw diets (Valiente *et al*., 2004). However, its application in grazing conditions, where sampling faeces more than once a day may represent a severe constraint, requires the description of a collection pattern that will produce spectra which may be used to obtain accurate prediction equations. The objectives of the present experiment were: (i) to check the possibility of using the NIRS methodology for estimation of intake, digestibility, botanical composition of the ingested diet and faecal concentration of n-alkanes in sheep fed different proportions of lucerne and rye grass; and (ii) to compare the performance of two faecal sampling patterns (daily pool or spot samples taken at 9:00 h) in terms of accuracy of the equations derived from the respective NIRS spectra.

Materials and methods

Sixteen adult Rasa Aragonesa ewes were randomly assigned to four treatments consisting in rations differing in proportions of lucerne (*Medicago sativa)* and rye grass (*Lolium rigidum*) (100:0, 67:33, 33:67 and 0:100) chopped to 5 cm. Following a 14-day adaptation period to the diets, during which voluntary intake was established and level restricted to 95% of *ad libitum* to avoid refusals, animals were introduced in metabolic cages where a 7-day digestibility trial was performed after a 3-day adaptation period to the crates. Diets were offered once daily (9:00 h), and samples of lucerne and rye grass taken and pooled for the whole balance. A subsample of the forages was dried at 60 ºC for 48 h to estimate their dry matter (DM) concentration, and another one ground through a 1 mm sieve and stored in plastic containers until analysis of organic matter (OM) and neutral detergent fibre (NDF). In the sporadic cases where animals left refusals, these were weighed and their DM concentration determined. A subsample was ground through the same sieve and treated the same way than forages. Daily faecal production was also registered and its DM concentration determined, and 5% of the weight frozen at -20 ºC, freeze-dried and pooled in a single sample per animal, which was ground through a 1 mm sieve and stored in plastic containers until analysis of OM, NDF and n-alkanes. The same samples were used for obtaining the NIRS spectra. Spot samples of faeces were also collected daily at 9:00 h, along the balance period, directly from rectum, frozen at -20 ºC, freeze-dried and pooled in a single sample per animal. The pooled spot samples were processed in the same way and analysed for the same constituents than faecal samples from daily production.

NIR data from 700 to 2500 nm were collected using a Foss NIRSystems 6500 monochromator equipped with a spinning module. Spectra and reference data were recorded with the Infrasoft International (ISI) software NIRS3 version 3.0 (NIRSystems Inc., Silver Spring, MD, USA). Modified partial least squares regression method (MPLS) was used for equations' development after a first derivative transformation of the spectral data and a scatter correction treatment (SNVD: standard normal variate and detrend) (Barnes *et al*., 1989). Calibration models were developed for dry matter intake (g/day or g/kg $LW^{0.75}$), digestibility coefficients of DM (DMD), OM (OMD) and NDF (NDFD), lucerne consumed (% of the diet, g DM/day or g DM/kg $LW^{0.75}$) and n-alkane concentrations in faeces, from faecal samples of daily production (S1) and from spot faecal samples (S2). 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 (SECV), the determination coefficient in calibration (R^2c) and the determination coefficient in cross-validation (R^2cv).

Results and discussion

Table 1 shows the performances of the different calibrations obtained from S1 samples, whereas the results from S2 samples are given in Table 2.

Table 1. Performances of NIR calibration equations obtained for dry matter intake (DMI; g/day or g/kg $LW^{0.75}$), dry matter (DMD), organic matter (OMD), and neutral detergent fibre (NDFD) digestibility, lucerne consumed (% of the diet, g dry matter (DM)/day and g DM/kg $LW^{0.75}$) and faecal concentrations of n-alkanes in sheep fed different proportions of lucerne (*Medicago sativa*) and rye grass (*Lolium rigidum*) hays (100:0, 67:33, 33:67 and 0:100). Equations obtained from faecal samples of daily production

	Observed		Calibration		Cross-validation	
	N	Mean±σ	SEC	R^2c	SECV	R^2cv
DM						
g/day	15	1058±326.0	151.00	0.78	193.00	0.65
g/kg $LW^{0.75}$	15	48.2±13.00	9.71	0.44	11.56	0.20
DMD (%)	15	59.4±2.56	0.64	0.94	2.19	0.30
OMD (%)	16	61.0±2.97	2.19	0.46	3.00	0.06
NDFD (%)	16	55.8±4.86	3.22	0.56	4.39	0.24
Lucerne consumed						
% of the diet	16	50.0±38.56	1.40	0.99	5.52	0.98
g DM /day	15	590±518.0	121.00	0.95	192.00	0.86
g DM /kg $LW^{0.75}$	14	27.4±21.83	7.22	0.89	8.24	0.85
Faecal concentration of n-alkanes (mg/kg DM)						
C_{21}	16	1.9±1.52	0.39	0.93	1.09	0.51
C_{23}	15	3.4±2.14	0.28	0.98	0.44	0.96
C_{25}	14	31.1±17.01	1.58	0.99	3.62	0.95
C_{26}	16	3.6±0.76	0.40	0.73	0.53	0.50
C_{27}	15	95.1±31.60	1.62	0.99	5.45	0.97
C_{28}	16	14.7±3.07	0.14	0.99	0.79	0.94
C_{29}	16	172.0±18.09	4.32	0.94	7.59	0.85
C_{30}	16	25.8±5.12	0.42	0.99	1.12	0.95
C_{31}	16	702.3±197.59	13.84	0.99	46.61	0.95
C_{32}	15	199.6±65.40	34.30	0.73	65.43	0.05
C_{33}	16	76.4±6.40	1.16	0.97	2.80	0.83
C_{35}	15	3.1±0.95	0.19	0.96	0.44	0.78
C_{36}	15	180.9±70.44	33.68	0.77	61.32	0.27

N: number of samples; SEC: standard error of calibration; R^2c: coefficient of determination in calibration; SECV: standard error of cross-validation; R^2cv: coefficient of determination in cross-validation.

All S2 equations had coefficients of determination in calibration higher than 0.66, whereas R^2 in S1 equations was lower than this figure for the prediction of intake per kg $LW^{0.75}$ (0.44), and organic matter (0.46) and neutral detergent fibre (0.56) digestibility. The coefficients of determination in cross-validation were much lower than those in calibration, with equations derived from S2 showing again better performances for intake prediction. Accuracy of equations for prediction of digestibility coefficients was reached neither for S1 or S2 samples, featuring R^2cv values lower than 0.31 (S1-DMD) and 0.49 (S2-NDFD), respectively, and indicating that the low number of samples may not be enough to obtain robust equations. Diet composition (% of lucerne in the diet or g lucerne DM consumed either per day or per kg $LW^{0.75}$) and faecal concentration of most n-alkanes (except C_{21}, C_{26}, C_{32} and C_{36} for S1-equations, and C_{29}, C_{32}, C_{33} and C_{36} for S2-equations) was more accurately predicted in both cases ($R^2cv>0.67$), especially for lucerne intake ($R^2cv>0.82$). The fact that faecal concentration of external n-alkanes (C_{32} and C_{36} in our case) could not be predicted from NIRS analysis was also pointed out by Ru *et al*. (2002) who argued that the lack of accuracy might be due to the absence of structural interactions between dosed n-alkanes and the rest of chemical entities.

Table 2. Performances of NIR calibration equations obtained for dry matter intake (DMI; g/day or g/kg $LW^{0.75}$), dry matter (DMD), organic matter (OMD), and neutral detergent fibre (NDFD) digestibility, lucerne consumed (% of the diet, g dry matter (DM)/day and g DM/kg $LW^{0.75}$) and faecal concentrations of n-alkanes in sheep fed different proportions of lucerne (*Medicago sativa*) and rye grass (*Lolium rigidum*) hays (100:0, 67:33, 33:67 and 0:100). Equations obtained from spot faecal samples

	Observed		Calibration		Cross-validation	
	N	Mean±σ	SEC	R^2c	SECV	R^2cv
DMI						
g/day	15	1058±326.0	115.00	0.88	173.00	0.72
g/kg $LW^{0.75}$	15	46.5±14.34	1.37	0.99	4.52	0.90
DMD (%)	16	59.5±2.51	1.35	0.71	2.22	0.26
OMD (%)	16	61.0±2.97	1.43	0.77	2.39	0.41
NDFD (%)	16	55.8±4.86	1.86	0.85	3.63	0.48
Lucerne consumed						
% of the diet	16	50.0±38.56	2.23	0.99	4.92	0.98
g DM /day	13	572±533.0	43.00	0.99	74.00	0.98
g DM /kg $LW^{0.75}$	16	27.2±22.36	7.90	0.88	9.43	0.82
Faecal concentration of n-alkanes (mg/kg DM)						
C_{21}	16	1.7±1.30	0.10	0.99	0.75	0.67
C_{23}	15	3.6±2.16	0.47	0.95	0.72	0.89
C_{25}	14	32.5±18.14	0.77	0.99	4.00	0.95
C_{26}	15	3.5±1.30	0.03	0.99	0.14	0.99
C_{27}	15	100.4±34.79	5.73	0.97	9.98	0.92
C_{28}	15	15.2±2.49	0.41	0.97	0.74	0.92
C_{29}	13	174.7±10.84	3.54	0.89	6.83	0.64
C_{30}	15	26.5±4.70	0.76	0.97	0.94	0.96
C_{31}	13	694.6±184.91	5.91	0.99	17.17	0.99
C_{32}	16	200.4±72.36	41.17	0.68	60.04	0.35
C_{33}	16	77.2±5.74	2.74	0.77	5.43	0.15
C_{35}	15	3.3±1.26	0.05	0.99	0.20	0.97
C_{36}	15	179.7±74.38	40.55	0.70	57.43	0.43

N: number of samples; SEC: standard error of calibration; R^2c: coefficient of determination in calibration; SECV: standard error of cross-validation; R^2cv: coefficient of determination in cross-validation.

Conclusions

Intake, DMD, OMD, NDFD and lucerne consumed (as percentage of the diet or in terms of g DM consumed either per day or per kg $LW^{0.75}$) could be successfully predicted in sheep fed mixed lucerne:rye grass (*Lolium rigidum*) diets by NIRS. Prediction of faecal concentration of internal n-alkanes may also be accurately performed despite their low contents. However, this seems not to be the case for dosed (external) hydrocarbons, suggesting that more research is needed to clarify this aspect.

The results obtained with spot faecal samples open the possibility of a broad use of the method in grazing conditions where sampling faeces more than once daily might represent a severe constraint. Nevertheless, a larger number of data is needed in order to improve the practical usefulness of NIRS models for prediction of many of the parameters studied in the present experiment.

The good R^2cv values obtained for diet composition prediction (lucerne consumed) are in agreement with those found by Valiente *et al*. (2004) for prediction of straw consumed by sheep fed

mixed barley grain:straw diets, and seem to guarantee the success of further experiments aiming at validating the usefulness of the NIRS technique for diet composition studies, possibly also when including multiple ingredients.

References

Aufrère, J., Graviou, D., Demarquilly, C., Pérez, J.M. and 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.

Barnes, R.J., Dhanoa, M.S. and Lister, S.J. (1989). Standard normal variate transformation and detrending of near infrared diffuse reflectance spectra. *Appl. Spectrosc*., 43: 772-777.

Boval, M., Lecomte, P. and Archimède, H. (2003). Faecal near infrared reflectance spectroscopy (NIRS) to assess chemical composition, digestibility and intake of tropical fresh grasses by Creole cattle. *Tropical and Subtropical Agroecosystems*, 3: 453-456.

Boval, M., Coates, D.B., Lecomte, P., Decruyenaere, V. and Archimède, H. (2004). Faecal near infrared reflectance spectroscopy (NIRS) to assess chemical composition, in vivo digestibility and intake of tropical grass by Creole cattle. *Anim. Feed Sci. Technol*., 114: 19-29.

de Vega, A. and Delgado, I. (2002). Recursos pastables y utilización del territorio en zonas áridas y semiáridas. *Ovis*, 81: 45-60.

Decruyenaere, V., Peters, M., Stilmant, D., Lecomte, P. and Dardenne, P. (2003). Near infrared reflectance spectroscopy applied to faeces to predict dry matter intake of sheep under grazing, comparison with n-alkanes and direct biomass measurement methods. *Tropical and Subtropical Agroecosystems*, 3: 471-476.

Delgado, I. (2000). *Base forrajera para el establecimiento de ganaderías de ovino en el secano cerealista*. Institución "Fernando el Católico", Zaragoza, Spain.

Garnsworthy, P.C. and Unal, Y. (2004). Estimation of dry-matter intake and digestibility in group-fed dairy cows using near infrared reflectance spectroscopy. *Anim. Sci*., 79: 327-334.

Mayes, R.W. and Dove, H. (2000). Measurement of dietary intake in free-ranging mammalian herbivores. *Nutr. Res. Rev.*, 13: 107-138.

Mayes, R.W., Lamb, C.S. and Colgrove, P.M. (1986). The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. *J. Agric. Sci.*, 107: 161-170.

Ru, Y.J., Kruk, J.A., Fischer, M., Choct, M. and Glatz, P.C. (2002). Predicting N-alkane concentration in pastures and deer faeces for dietary composition and digestibility measurement using near infrared spectroscopy. *Asian-Aust. J. Anim. Sci*., 15: 1611-1616.

Valiente, O.L., Andueza, D., de Vega, A., Olmos, G. and Muñoz, F. (2004). The use of NIRS for prediction of intake, digestibility and diet composition in sheep fed mixed grain:roughage diets. *J. Anim. Feed Sci*., 13 Suppl. 1: 227-230.