Evaluation of the repeatability of near infrared reflectance spectroscopy applied to faeces for predicting diet characteristics of grazing ruminants

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Introduction

Pasture management and economical ruminant productions are closely linked. Grazed grass can be an efficient feed for suckling or lactating cows as long as digestibility and intake are known in real time. The difficulty is precisely to measure these parameters continuously for grazing ruminants. The reference method usually mobilised for the determination of these *in vivo* parameters is the digestibility trial (Andueza et al., 2011) but the method is time consuming and difficult to implement over long period. To solve this problem, alternative methods linking *in vivo* measurements to diverse analytical procedures have been developed. For instance, the 'rumen fluid pepsin' method of Tilley and Terry (1963), enzymatic mixtures simulating rumen activities, such as the cellulase-method (De Boever et al., 1988; Aufrère et al., 2007) are used with success for estimating *in vivo* digestibility of a large range of forages. In parallel, the development of near infrared spectroscopy (NIRS) databases for predicting the chemical composition and the digestibility of forages is a real progress. Intake appears more difficult to estimate, probably due to the selective behaviour of herbivore under grazing. Currently, there is no method for determining the individual diet selection and intake of grazing herbivores with a sufficient precision (Walker et al., 2010). Nevertheless, during these 15 last years, several studies have highlighted the potential of NIRS applied to faeces (F.NIRS) to predict *in vivo* organic matter digestibility and intake of grazed grass (Stuth et al., 2003; Decruyenaere et al., 2009; Dixon and Coates, 2009). FNIRS calibrations for predicting *in vivo* organic matter digestibility and intake are derivative calibrations because the sample analysed for reference values (diet samples) differs from the samples submitted to NIRS analyses (faeces). So, the accuracy of a NIRS model depends largely on the repeatability of the reference method and on the reliability of diet-faecal pairs. The aim of this study is to estimate the repeatability of NIRS applied to faeces to predict *in vivo* organic matter digestibility (OMD) and organic matter voluntary intake (OMVI) of grazing ruminants.

Materials and methods

Repeatability of prediction

Ten faecal samples were selected in a database generated during grazing season 2006. The objective was to select samples as heterogeneous as possible. There were faeces of sheep (Swifter ewes, samples 1 and 2) and heifers (Belgian Blue White, samples 3 to 10) grazing alone or according mixed grazing schemes. During grazing season (mid April to November), fresh faeces were sampled on the pasture two times a week. Each faecal sample was oven dried (65°C for 36 h), roughly ground in a hammer mill (1 mm screen) before NIRS scanning (NIRSystems monochromator 5000, FOSS Electric, Hillerød, Denmark) following two samples presentation. The first sample presentation consisted to fill ten small ring cups with the homogenized sample (cup presentation 1). The second sample presentation consisted to fill one small ring cup and to transfer the powder sample successively 10 times to other cups (cup presentation 2). The absorbance data were recorded as log 1/R from 1100 to 2498 nm by 2 nm steps.

Thus, 200 spectra were generated (10 samples x 2 cup presentations x 10 repetitions). For each faecal spectrum, *in vivo* organic matter digestibility (OMD) and organic matter voluntary intake (OMVI, g/kg metabolic weight) were predicted according to F.NIRS databases previously developed (Decruyenaere et al., 2009). The predictions were obtained using the GLOBAL or LOCAL MPLS model. The calibration and database statistics were listed in Table 1.

Table 1. Statistics of the database and global MPLS NIRS model used for predicting OMD and OMVI (Decruyenaere et al., 2009)

Parameter	N	mean	SD	SEC	R^2	SECV
OMD	951	0.710	0.0698	0.0200	0.92	0.207
OMVI	936	51.27	10.46	4.28	0.83	4.53

N : number of diet faecal pairs; SD : standard deviation of reference database; OMD : *in vivo* organic matter digestibility obtained by digestibility trial on sheep; OMVI : organic matter voluntary intake (g/kg metabolic weight) obtained by weighing diet proposed to sheep

The repeatability of predicted values was estimated through analysis of variance (ANOVA-full crossed model) performed after outlier detection (Cochran and Grubbs tests, ISO 5725-2, 1994). The variance components were sample (n=10), sample presentation (n=2) and NIRS model (n=2). The repeatability standard error (s_r) was the residual error of the ANOVA (Genot et al. 2011):

$$s_r = \sqrt{MS_{error}}$$

where MS_{error} = residual mean square error of the ANOVA; s_r = standard error of repeatability.

Results and discussion

FNIRS predictions of OMD and OMVI are presented in Table 2. At the sample level, OMD predictions varied between 0.690 to 0.792 and the OMVI prediction varied between 57.88 to 74.90 g/kg metabolic weight. As expected and linked to the evolution of grass quality and availability during the grazing season, samples were significantly different between them for the both predicted parameters. Per sample, the OMD coefficient of variation (CV=SD/mean) was good and lower than 5 % reflecting the good repeatability of the prediction. OMVI coefficient of variation was higher than 5 % and for some samples higher than 20 %. According to Andueza et al. (2011), the variability of the intake measurement, in standardised conditions, was higher and usually close to 10 percent of the mean.

Samples appeared as the higher variance component for OMD prediction. NIRS model (GLOBAL or LOCAL) and cup presentation had less importance on the OMD prediction variability. NIRS model (GLOBAL or LOCAL) was the higher variance component for OMVI prediction, samples and cup presentation had lower impact. Indeed, the difference between GLOBAL and LOCAL OMVI predictions was high (12.60 g/kg metabolic weight on average). The nature of our F.NIRS database, including several intake levels (from maintenance to *ad libitum*), used under LOCAL or GLOBAL procedure could explain this difference. In the GLOBAL MPLS, maintenance level was automatically included in the calibration. The LOCAL MPLS procedure consisted to select a sample subset which was similar to the unknown sample and to develop a specific calibration for predicting the sample. It was so possible that maintenance level, automatically included in GLOBAL MPLS procedure allowed to predict lower intake level. Tran et al. (2010) have compared GLOBAL and LOCAL procedure applied to F.NIRS and conclude that LOCAL procedure was more precise that GLOBAL procedure.

Table 2. F.NIRS prediction reported to sample, NIR model and cup presentation.

Sample	N	OMD Mean	SD	SD/mean	OMVI Mean	SD	SD/mean
s1	40	0.781	0.008	0.98	66.89	7.09	10.60
s2	40	0.712	0.030	4.23	74.90	3.87	5.17
s3	40	0.690	0.021	3.02	69.26	6.14	8.86
s4	40	0.758	0.010	1.31	70.63	8.46	11.98
s5	40	0.744	0.014	1.87	67.38	9.94	14.75
s6	40	0.694	0.020	2.94	70.55	4.87	6.90
s7	40	0.792	0.005	0.59	57.88	9.55	16.49
s8	40	0.783	0.012	1.55	58.95	12.50	21.20
s9	40	0.719	0.026	3.62	64.80	3.76	5.81
s10	40	0.752	0.017	2.28	59.78	14.50	24.26
NIR model							
Global	200	0.749	0.031	4.08	59.80	9.75	16.31
Local	200	0.736	0.046	6.28	72.40	5.84	8.06
Sample presentation							
sp1	200	0.750	0.035	4.65	66.41	9.80	14.75
sp2	200	0.736	0.043	5.85	65.80	10.62	16.14
ANOVA		MS-OMD			MS-OMVI		
Sample		0.0558			1290.6		
NIRS model		0.0184			15876.4		
Sample presentation		0.0198			36.7		

OMD : *in vivo* organic matter digestibility; OMVI : organic matter voluntary intake (g/kg metabolic weight); sp1 : ten small ring cups were filled with the homogenized sample; sp2 : one small ring cup was filled and the matter was transferred into another cup, 10 times; MS : mean square error of the ANOVA

The variation mainly linked to samples suggested that OMD F.NIRS calibration leads to robust predictions. At the reverse, the variation linked to the model suggested that OMVI F.NIRS calibration was not sufficiently representative of the field variations. These results highlighted also the difficulty of building an efficient F.NIRS database for predicting intake. As described by Williams (2001, cited by Walker, 2010), five or six growing seasons were needed to represent grain variability adequately. It was probably the same for F.NIRS. So, increasing the heterogeneity of the F.NIRS databases would probably improve the precision of intake prediction. This hypothesis will be tested in future developments.

Table 3. Repeatability of predicted OMD and OMVI

	SECV	Mean	s_r	s_r %
OMD	0.0207	0.742	0.0099	1.33
OMVI	4.53	66.10	1.80	2.72

OMD : *in vivo* organic matter digestibility; OMVI : organic matter voluntary intake (g/kg metabolic weight); s_r : repeatability standard error; s_r% : s_r/mean x 100; SECV : standard error of cross-validation of FNIRS calibration.

For both OMD and OMVI, repeatability standard errors (s_r %) were lower than 5 % of the mean (Table 3). OMD repeatability (s_r %) was better than OMVI one. The repeatability standard error (s_r %) obtained with F.NIRS appeared similar to those reported by Genot et al. (2011) on soil samples. Compared to the accuracy of the calibrations used in our study, all repeatability standard error (s_r) of the FNIRS prediction were lower than the SECV of calibrations for both parameters tested. It means that repeatability of OMD and OMVI prediction was very good. To compare, the repeatability value (s_r) of the reference method reached 0.015 unit of digestibility for OMD and 6.00 g/kg metabolic weight for DMI (Andueza et al., 2011, 2007). These values, higher than those obtained by F.NIRS, suggested that F.NIRS could be as repeatable as reference measures.

Conclusion

In regard to the difficulty to obtain *in vivo* diet characteristics with the reference method (digestibility trial), NIRS analysis of faeces could be used to predict diet characteristics with a sufficient accuracy. OMD predictions appeared sufficiently accurate and repeatable for a routine use while OMVI predictions are repeatable but not accurate enough for such a use. OMVI spectral databases must be improved to cover field variability and to be used for ruminant diet management.

Reference

Andueza D., Picard F., Pradel Ph., Egal D., Hassoun P., Pecatte J.R., Beaumont R. (2011). Reproductibility and repeatability of forage in vivo digestibility and voluntary intake of permanent grassland forages in sheep. Livestock Sci., 140, 42-48.

Aufrère J., Baumont R., Delaby L., Peccate J.R., Andrieu J., Andrieu J.P., Dulphy J.P. (2007). Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. INRA Prod. Anim., 20(2), 129-136.

De Boever J.L., Cottyn B.G., Andries J.I., Buysse F.X., Vanacker J.M. (1988). The use of pepsin cellulase technique to predict digestibility metabolizable and net energy of forages. Anim. Feed. Sci. Technol., 19, 247-260.

Decruyenaere V., Lecomte Ph., Demarquilly C., Aufrere J., Dardenne P., Stilmant D., Buldgen A. (2009). Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Anim Feed Sci. Technol., 148, 138-156.

Dixon R., Coates D. (2009). Near infrared spectroscopy of faeces to evaluate the nutrition and physiology of herbivores. J. Near Infrared Spectrosc. 17, 1-31.

Genot V., Colinet G., Bock L., Vanvyve D., Reusen Y., Dardenne P. (2011). Near infrared reflectance spectroscopy for estimating soil characteristics valuable in the diagnosis of soil fertility. J. Near Infrared Spectrosc. 19, 117-138.

ISO 5725-1 (1994). Accuracy (trueness and precision) of measurement methods and results -- Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method General principles and definitions. International organisation for standardization, Geneva, Switzerland, 17p.

Stuth J., Jama A., Tolleson D. (2003). Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crop Research, 84, 45-56.

Tilley J.H.A, Terry R.A. (1963). A two stage technique for ‘in vitro’ digestion of forage crops. Grass Forage Sci., 18, 104-111.

Tran, H., Salgado, P., Tillard, E., Dardenne, P., Nguyen, X.T., Lecomte, P. (2010). “Global” and “local” predictions of dairy diet nutritional quality using near infrared reflectance spectroscopy. J. Dairy Sci. 93, 4961-4975.

Walker, J.W., Campbell, E.J., Kott, R.W., Landau, S.Y., Lupton, C.J., Scott, C.B., Surber, L., Taylor Jr, C.A., Whitworth, W.R. (2010). Fecal NIRS for predicting botanical composition of herbivore diets. In Walker J and Tolleson D. Eds. Shining light on manure improves livestock and land management, Texas Agrilife Research and Society for Range Management, Technical bulletin, 53-65.