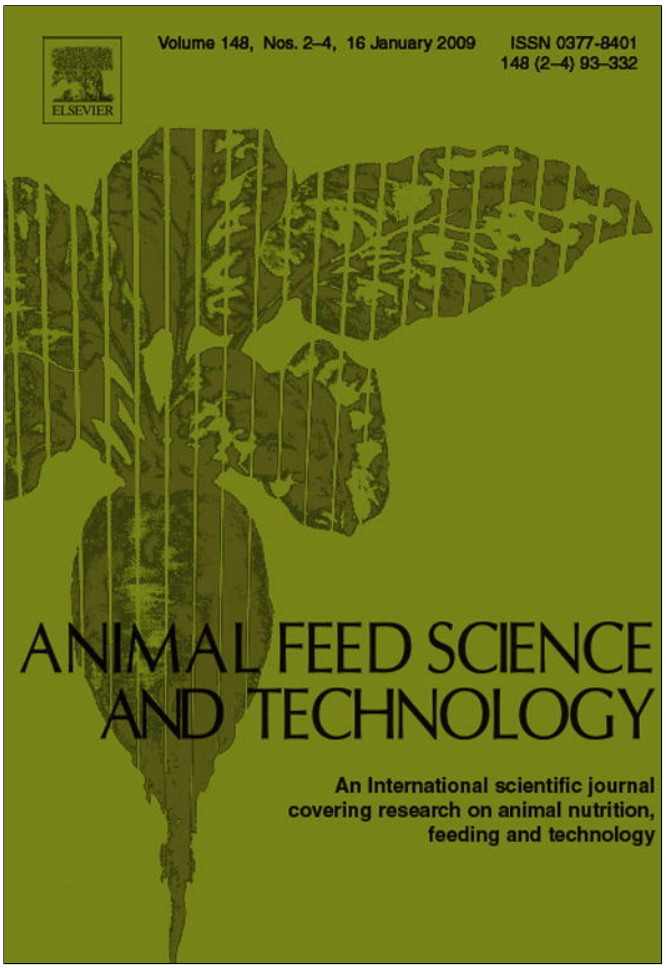

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Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration

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Abstract

The objective of this study was to evaluate the potential of near infrared reflectance spectroscopy (NIRS), applied to forage and/or faeces, to estimate the *in vivo* organic matter digestibility (OMD) and the organic matter voluntary intake (OMVI, g/kg metabolic weight [$BW^{0.75}$]) for a wide range of

Abbreviations: BW, body weight; CEL, cellulose; CP, crude protein; DM, dry matter; H, standardized distance; ADL, acid detergent lignin; NIRS, near infrared reflectance spectroscopy; OM, organic matter; OMDcel, *in vitro* organic matter digestibility coefficient; OMD, *in vivo* organic matter digestibility coefficient; OMVI, organic matter voluntary intake; PCA, principal components analysis; R, reflexion; R^2, coefficient of determination; RPD, standard error of reference database/standard error of cross validation; S.D., standard error of reference database; S.E., standard error of regression; SEC, standard error of calibration; SECV, standard error of cross validation.

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temperate forages. Two different databases, in terms of forage species and development stages were studied. The first one included two grass species and two forage mixtures for which OMD and OMVI were continuously measured during the grass-growing seasons (spring and summer). The second one contained a large set of grass and legume species and forage mixtures (142 trials) for which OMD and OMVI were measured.

Forage and faeces samples were submitted to NIRS analysis and predictive calibrations were developed from forage spectra, faeces spectra, forage and faeces subtracted spectra, and faeces and forage concatenated spectra. Working on faecal spectra (alone or concatenated) enabled us to develop the best calibration equations for both OMD and OMVI estimation. The coefficient of determination (R^2) was greater than 0.8. The standard error of cross validation (SECV) for OMD and OMVI was 0.021 and 4.51 g/kg $BW^{0.75}$, respectively, and the accuracy was similar to that obtained with other predictive methods. With regard to the faecal spectra (second derivative mode), the fat absorbency at wavelengths of 1730, 2310 and 2350 nm was higher when the corresponding forage was highly digestible and ingestible.

In conclusion, applying NIRS to faeces is a rapid and easy analytical method that could be an interesting tool for managing grazing ruminants and optimising their performance.

Keywords: NIRS; Faecal spectra; Grass quality; Feeding management tool

1. Introduction

The performances of herbivores when grazing depend directly on forage digestibility and intake. Measuring these parameters for herd management at pasture can be difficult, costly, time consuming, labour intensive and not suitable over long periods. The digestibility of ingested grass is usually estimated by chemical analyses of samples collected in the field. This is based on determining the regression between forage parameters defined in laboratory and *in vivo* measurements. Common laboratory analysis methods include that described by Tilley and Terry (1963) and *in vitro* enzymatic digestibilities (Bartiaux-Thill and Oger, 1986; De Boever et al., 1988; Aufrère and Demarquilly, 1989). With these methods, accuracy is generally good, with a residual error of prediction of 0.015–0.030 units of digestibility (Peyraud, 1998). However, the accuracy of such regressions is a function of the method used to collect field samples that are as representative as possible of the ingested diet. To overcome this difficulty, ingested diet can be obtained by using oesophageal-fistulated animals (Ward et al., 1982; Holechek et al., 1982; Forbes and Beattie, 1987; Stuth et al., 1989), but this practice has an adverse effect on animal welfare. In addition, on heterogeneous pasture such sampling methods are not always representative of diets selected by animals. Coates et al. (1987) showed that diet legume percentages of extrusa collected from oesophageal-fistulated steers, non-resident on pasture, were poorly correlated with those ingested by non-fistulated steers resident on pasture ($R^2 = 0.127$). Jones and Lascano (1992) suggested that this difference could be linked to the sampling strategy of extrusa (difference between morning and afternoon extrusa) or to behavioural differences in diet selection between resident and non-resident cattle. For instance, fasted or satiated oesophageal-fistulated cattle introduced in pasture do not have the same diet selection. Other methods have been developed to estimate grass digestibility by measuring such chemical faecal parameters as

nitrogen (Bartiaux-Thill et al., 1985; Peyraud, 1998) or indigestible fibres (Lippke et al., 1986; Sunvold and Cochran, 1991). According to Peyraud (1998), the nitrogen faecal index is fairly accurate for assessing digestibility, but the relationship between faecal nitrogen and digestibility are strongly linked to pasture characteristics in terms of botanical composition or localisation and therefore lack a universal application (Holloway et al., 1981). In addition, on rangeland or with tropical forages, the chemical composition of faeces does not appear to be a good indicator of forage quality because of the diversity of forage species ingested and the occurrence of some anti-nutritional factors, such as tannins and phenolic compounds that precipitate protein and lead to higher nitrogen faecal concentration than that observed in the initial diet (Wofford et al., 1985). More recently, the alkane recovery rates in faeces have been used to assess the digestibility of grazed forage. Dove and Mayes (1991, 1996) and Sandberg et al. (2000) suggest that these methods, based on analysing the natural alkanes in the cuticular waxes of plants and the dosed alkanes, reflect more accurately the digestibility of temperate or tropical herbivore diets.

Voluntary intake quantification requires measuring both digestibility and faecal output obtained by total faeces collection (Holechek et al., 1986) or using indigestible markers such as chromium oxide (Bartiaux-Thill et al., 1988; Compère et al., 1992), and ytterbium (Brandyberry et al., 1991; Galyean, 1993; Mambrini and Peyraud, 1997). The *n*-alkanes method is also used to estimate individual voluntary intake. Mayes et al. (1986), Malossini et al. (1996) and Dove et al. (2000) found that a good estimation of intake could be obtained by using C_{32} as a dosed alkane and C_{33} as a herbage alkane, but, as for digestibility determination, these techniques are difficult to apply under grazing conditions. The main source of variation in these methods remains the collection of a representative herbage sample (Smit et al., 2005). For example, grazing animals can select some plant species or parts of plants in which the *n*-alkane profiles differ from the averaged grass sampled in the field (Dove et al., 1996).

To address this problem, approaches based on near infrared reflectance spectroscopy (NIRS) applied to faeces and/or forage have been developed to analyse the diet quality of grazing animal intake (Stuth et al., 1989; Coleman et al., 1989; Coleman and Murray, 1993; Leite and Stuth, 1995; Lyons et al., 1995; Coates, 2000; Decruyenaere et al., 2002; Stuth et al., 2003). Lyons and Stuth (1992) found that monitoring forage diet quality and intake using NIRS scanning of faecal samples appeared promising. They demonstrated that grass *in vivo* digestibility can be estimated by NIRS applied to faeces with the same accuracy as that obtained with conventional analysis methods. If there are appropriate calibration equations, NIRS is a rapid and non-destructive technology that could predict the digestibility and intake of a large set of similar samples.

The aim of this study is to evaluate the potential of NIRS, applied to forage and/or faeces, for determining the *in vivo* organic matter digestibility (OMD) and the organic matter voluntary intake (OMVI) obtained from *in vivo* feeding trials as reference values.

2. Materials and methods

The potential of NIRS to estimate *in vivo* organic matter digestibility and organic matter voluntary intake (g/kg $BW^{0.75}$) of fresh grass was evaluated using an important *in vivo*

Table 1
Digestibility and intake reference databases

	Nature	Year	N[a]	Feeding level[b]	OMD range	OMVI range
CRA-W						
1	Rye grass 4n (Meltra)	1992	148	al	0.584–0.841	40.64–64.44
2	Rye grass 2n (Talbot)	1992	148	al	0.526–0.822	44.98–65.65
3	Mixed forage without clover	1993	90	al	0.570–0.763	47.81–76.40
4	Mixed forage with clover	1993	90	al	0.550–0.775	41.38–73.80
5	Mixed forage without clover	1993	104	150 M	0.545–0.841	39.92–55.72
6	Mixed forage with clover	1993	104	150 M	0.542–0.849	43.62–54.21
7	Mixed forage without clover	1993	208	M	0.601–0.842	28.93–38.80
			892			
INRA						
1	Natural pastures		43	al	0.583–0.760	47.08–70.68
2	Cocksfoot		34	al	0.535–0.744	37.43–74.65
3	Tall fescue		9	al	0.570–0.742	50.37–70.63
4	Timothy		5	al	0.677–0.778	57.41–77.94
5	Rye grass		42	al	0.566–0.815	47.10–93.54
6	Lucerne		4	al	0.599–0.763	63.29–74.20
7	Red clover		5	al	0.625–0.802	56.55–87.74
			142			

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake.

[a] N: number of forage and faeces samples based on 6-day moving averages calculated for the CRA-W trials, number of forage samples, as mean of the trial, and faeces samples, as mean of six sheep over the trial, for the INRA trials.

[b] Feeding level: M: maintenance = 23 g OM digestible/kg $BW^{0.75}$; 150 M: 1.5 maintenance; al: *ad libitum*.

database obtained from feeding trials performed at Libramont (49°58′N–5°38′E, 440 m above sea level) in the Farming Systems Section of the Walloon Agricultural Research Centre (CRA-W) in Belgium, and at Clermont-Ferrand in the experimental farm of Theix (45°43′N–3°01′E, 890 m above sea level) of the National Institute of Agricultural Research (INRA) in France. The CRA-W database held data from digestibility trials carried out in 1992 (CRA-W 1–2) and 1993 (CRA-W 3–7) during the plant vegetative growth phase. The INRA database held data from 142 digestibility trials conducted in the early 1980s (Table 1).

2.1. Forage and animal management

2.1.1. CRA-W trials

Seven trials were conducted as a continuous measurement of digestibility and intake during the main grass-growth seasons (spring, summer). The trials lasted 20–60 days in order to cover the widest range of digestibility and intake variations.

The forage tested came from temporary pastures sown in 1990 (CRA-W 1–2) and 1992 (CRA-W 3–7) and harvested in 1992 (CRA-W 1–2) and 1993 (CRA-W 3–7), in their second or first year of production. The sampled grasslands for CRA-W 1–2 consisted of pure ryegrass (diploid and tetraploid), whereas CRA-W 3–7 consisted of two mixed swards. The composition of these mixed swards was determined by manual sorting and was, for

the first sward, ryegrass (625 g/kg DM), timothy (250 g/kg DM) and white clover (125 g/kg DM) and, for the second sward, ryegrass (715 g/kg DM) and timothy (285 g/kg DM).

The fertilisation levels were 88 units of P_2O_5/ha and 176 units of K_2O/ha, with a nitrogen application rate of 80 units/ha after each cut.

To perform the digestibility trials, the forage was supplied fresh, at different feeding levels, to sheep (castrated males weighing 45–60 kg) confined in individual digestibility crates.

The feeding levels were calculated according to the OMD of grass. This parameter was estimated daily by NIRS on a microwave-dried grass sample (Biston et al., 1989). Between two and six sheep were individually fed at maintenance level (23 g digestible organic matter/kg $BW^{0.75}$), at 150% of maintenance level or at *ad libitum*.

In each trial the forage was cut daily at 08.00 h, chopped to a length of 4–5 cm, stored at 6 °C and distributed to sheep the following day. Throughout the trial period the sheep were fed twice daily, at 09.00 and 16.30 h, and had continuous access to water and salt block. The daily forage supply, refusals and faeces were individually weighed and sampled.

2.1.2. INRA trials

A total of 142 averaged samples were obtained from short digestibility trials (each trial lasting 6 days). The forage consisted of a wide range of fresh grass, including natural pastures, pure gramineous species (ryegrass, timothy, cocksfoot, tall fescue) and pure legume species (red clover, lucerne) in their first, second or third growth cycle.

The forage was cut daily at 08.00 h, chopped to a length of 4–5 cm and provided fresh, at *ad libitum*, twice daily (09.00 and 16.30 h) to six sheep (castrated males 45–60 kg) housed in individual digestibility crates. The forage, refusals and faeces were individually weighed and sampled each day.

2.2. Sample management and calculation of reference values

All the forage, refusals and faeces samples collected in the CRA-W and INRA trials were oven dried (65 °C for 36 h), roughly ground in a hammer mill and then ground again in a Cyclotec mill with a 1 mm screen.

The forage samples from INRA were bulked over the trial period and individual faecal samples were bulked daily for the six sheep in the trial to provide averaged samples. Ground samples of forage, refusals and faeces were stored in hermetically sealed plastic boxes until NIRS analysis.

For both the INRA and CRA-W trials, the OMD was calculated according to Demarquilly et al. (1995), whereas the OMVI was calculated according to the difference between organic matter supplied and organic matter refused.

To determine the organic matter content of supplied and refused forage and faeces, ash (g/kg DM) was estimated using NIRS (Table 2). Other chemical characteristics of forage, such as crude protein (CP, g/kg DM), cellulose (CEL, g/kg DM according to the Weende method, Anonyme (1985)) and *in vitro* organic matter digestibility (OMDcel as described by De Boever et al. (1988)), and faeces, such as CP (g/kg DM) and CEL (g/kg DM), were also estimated using NIRS based on calibrations previously developed at CRA-W (Table 2).

Table 2
NIRS calibrations to estimate the chemical composition of forage and faecal samples

Parameters	*N*	Mean	S.D.	SEC	R^2	SECV
Forage						
Ash (g/kg DM)	2468	96.9	25.7	9.7	0.86	9.9
CP (g/kg DM)	2765	147.7	59.0	8.6	0.98	8.6
CEL (g/kg DM)	2494	266.6	54.1	13.3	0.94	13.5
OMDcel	1598	0.771	0.102	0.022	0.95	0.022
Faeces						
Ash (g/kg DM)	115	196.0	91.0	9.9	0.99	11.5
CP (g/kg DM)	78	166.0	22.9	7.9	0.88	10.3
CEL (g/kg DM)	57	147.6	23.9	6.6	0.92	9.8

S.D.: standard deviation of the reference database; R^2: coefficient of determination of NIRS equations; SEC: standard error of calibration; SECV: standard error of cross validation; DM: dry matter; OMDcel: *in vitro* organic matter digestibility as described by De Boever et al. (1988); CP: crude protein; CEL: cellulose.

2.3. NIRS measurements, spectral treatments and calibrations

Faecal and forage samples from the CRA-W and INRA trials were submitted to NIRS scanning (NIRS system monochromator 5000–1100 to 2498 nm of wavelength by 2 nm steps) at the Farming Systems Section in 1989 (INRA), 1992 (CRA-W 1–2) and 1993 (CRA-W 3–7). The absorbency data were expressed as log 1/*R*.

For the CRA-W trials, in order to compare CRA-W and INRA databases, a moving average over 6 days was calculated for the reference values and the corresponding forage and faecal daily spectra, wavelength by wavelength. Thus, the day i (d_i) value equalled the 6-day mean from d_{i-2} to d_{i+3}.

For the whole database (CRA-W and INRA), a concatenation of faeces and forage spectra was made to extend the spectral information. This involved juxtaposing faeces and forage spectra by merging data in the same file that doubled the number of absorbency values. Similarly, the differences between forage and faeces spectra were calculated. These differences were expected to give a better representation of the digestive utilisation of the forage.

The NIRS calibrations were developed to estimate the OMD and OMVI (g/kg $BW^{0.75}$) from faeces spectra, forage spectra, concatenated spectra and spectra obtained by calculating the differences. The NIRS models were set up with a modified partial least square (PLS) procedure with cross validation in WINISI® 1.50 software (Naes et al., 2002). For each parameter tested, 64 calibration equations varying in terms of derivative, gap, smooth and scatter correction were performed. The best predictive model was obtained using the second derivative mode spectrum with scatter correction using standard normal variate and detrend (SNV-D). The population boundaries for calibration were set with a maximum standardized *H* (distance between a sample and the centroid of the group) value of 3.0 (Shenk and Westerhaus, 1991).

To identify the wavelengths highly correlated to OMD and OMVI, the CRA-W and INRA databases were merged, sorted initially by ascending OMD and then by ascending OMVI. These databases were then divided into four equal groups per parameter. Each

group was averaged to provide one NIRS spectrum associated with the corresponding reference values. The four averaged spectra corresponding to the ascending OMD or OMVI were visually compared using the Plot Spectra and Score procedure in WINISI® 1.50 software.

2.4. Statistical analysis

The performance of the NIRS calibration equations was expressed in terms of coefficient of determination (R^2), standard error of calibration (SEC) and standard error of cross validation (SECV). The RPD calculated as the ratio of the standard deviation of the original data to the standard error of cross validation (Williams, 2004), was also used to evaluate calibration performance.

In order to estimate the OMD and OMVI, a multiple regression analysis was performed (GLM procedure – Statistica 1999). The independent variables were the CP (g/kg DM) and CEL (g/kg DM) content of forage and faeces.

The number of days elapsed since 1 January were tested only for the CRA-W databases to evaluate the OMD and OMVI evolution during the vegetative growth period.

3. Results

3.1. Forage characteristics

The CRA-W forages tested in 1992 (CRA-W 1–2) were quite different from that tested in 1993 (CRA-W 3–7) and from the INRA forages, especially for CP and ash which were lower for 1992 forages (CP = 71.2 g/kg *vs* 132.5 g/kg and 149.6 g/kg DM and ashes = 80.7 g/kg *vs* 104.8 g/kg and 107.4 g/kg DM, in average, respectively, for the CRA-W 1–2, CRA-W 3–7 and INRA databases). The CEL content was similar over years and between databases, whereas the OMDcel was higher for forages tested in 1992 (Table 3).

With regard to plant growth, the OMD from the CRA-W trials decreased linearly from the first week of May until last week of June (OMD = −0.00454 × day since 1 January + 1.41; R^2 = 0.79; S.E. = 0.0257). Thus, in order to maintain the defined level of intake, the OMVI increased throughout the measurement period from 29.4 to 35.6 g/kg $BW^{0.75}$ for sheep fed at

Table 3
Average and range of the chemical composition and enzymatic *in vitro* digestibility of CRA-W and INRA forage, estimated by NIRS

	CRA-W 1–2	CRA-W 3–7	INRA
OMDcel	0.780 (0.647–0.923)	0.746 (0.642–0.860)	0.721 (0.528–0.873)
CP (g/kg DM)	71.2 (44.8–107.8)	132.5 (102.1–185.9)	149.6 (62.0–242.0)
CEL (g/kg DM)	259.1 (181.3–327.6)	286.7 (230.0–338.7)	266.7 (168.0–355.0)
Ash (g/kg DM)	80.7 (73.5–90.7)	104.8 (98.3–118.3)	107.4 (75.3–142.7)

DM: dry matter; OMDcel: *in vitro* organic matter digestibility as described by De Boever et al. (1988); CP: crude protein; CEL: cellulose.

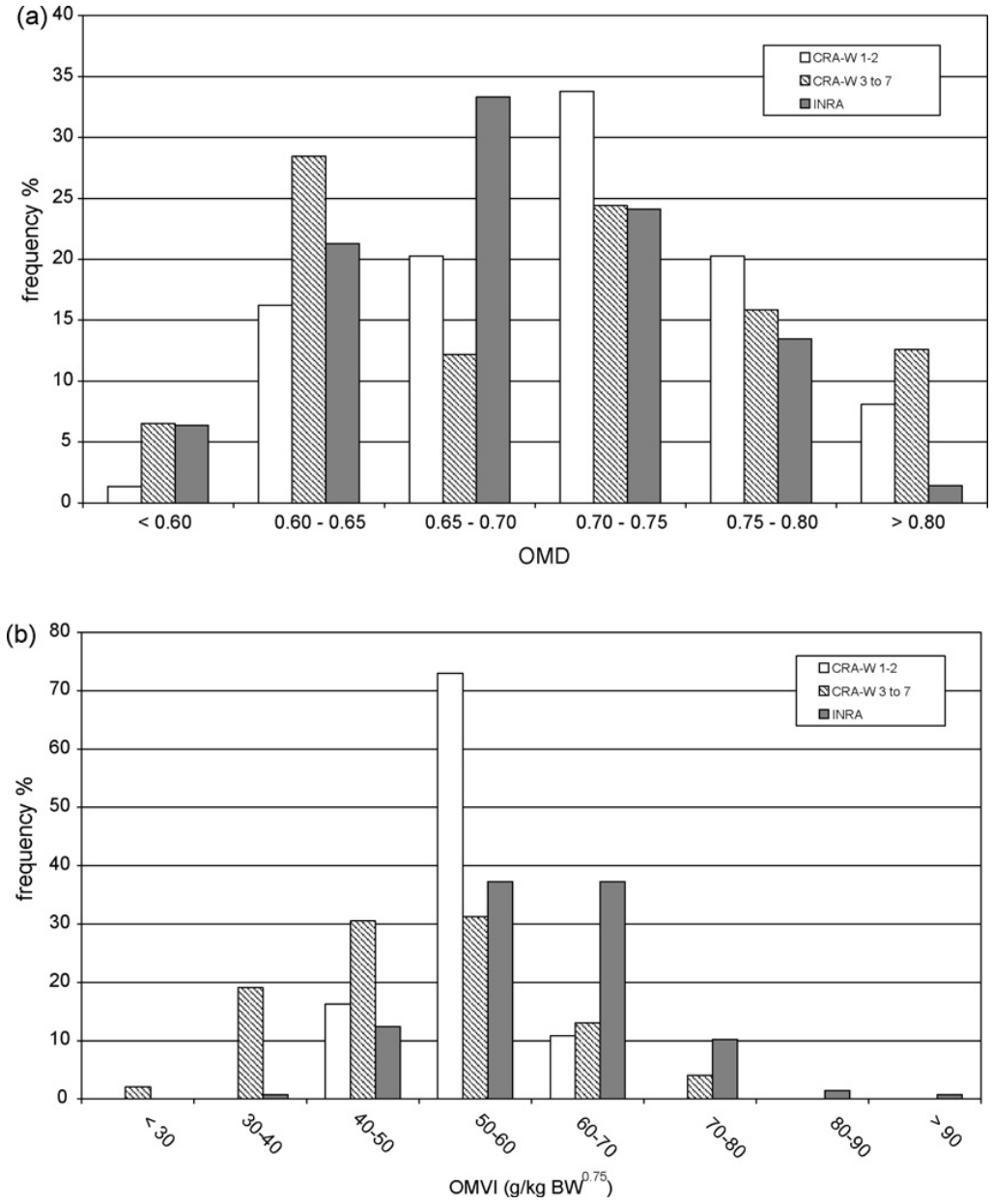

Fig. 1. Frequency distribution of forage OMD (a) and OMVI (b) for the INRA and CRA-W databases.

maintenance level and from 41.9 to 52.0 g/kg $BW^{0.75}$ for sheep fed at 150% of maintenance level. For sheep fed *ad libitum*, the OMVI decreased linearly from 65.3 at the beginning of the trial period to 46.6 g/kg $BW^{0.75}$ at the end of this period (OMVI = −0.5556 × day since 1 January + 143.14; $R^2 = 0.66$; S.E. = 4.17 g/kg $BW^{0.75}$).

For both databases, more than 65% of the OMD reference values were between 0.6 and 0.75 (CRA-W 1–2: 70%; CRA-W 3–7: 65%; INRA: 78%) (Fig. 1a). More than 70% of the INRA OMVI values were between 50 and 70 g/kg $BW^{0.75}$ whereas 60% of the CRA-W 3–7 OMVI values were between 40 and 60 g/kg $BW^{0.75}$. Again, CRA-W 1–2 differed, with more than 70% of the population in only one class of intake (Fig. 1b).

Table 4
Relationship between OMD or OMVI and forage or faeces CP and CEL

Database	Parameter	Relation	R^2	N	S.E.	F	P
CRA-W	OMD	0.916 + 0.0081 CPforage-0.0099 CELforage	0.76	236	0.0272	(2,233) 380.1	<0.001
	OMVI	72.18 − 0.72 CPforage-0.45 CELforage	0.06	236	10.42	(2,233) 9.3	<0.001
	OMD	1.294 − 0.0137 CPfaeces-0.0169 CELfaeces	0.78	236	0.0265	(2,233) 409.5	<0.001
	OMVI	−129.06 + 6.72 CPfaeces + 3.95 CELfaeces	0.25	236	9.37	(2,233) 39.0	<0.001
INRA	OMD	0.952 + 0.0010 CPforage − 0.0105 CELforage	0.65	141	0.0344	(2,138) 131.7	<0.001
	OMVI	59.77 + 0.98 CPforage − 0.51 CELforage	0.34	137	7.18	(2,134) 35.9	<0.001
	OMD	0.608 + 0.0118 CPfaeces − 0.0052 CELfaeces	0.81	141	0.0253	(2,138) 300.9	<0.001
	OMVI	16.74 + 2.42 CPfaeces + 0.47 CELfaeces	0.31	137	7.33	(2,134) 31.7	<0.001

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; CP (g/kg DM): crude protein; CEL (g/kg DM): cellulose.

3.2. Estimation of OMD and OMVI from the chemical characteristics of forage and faeces

Table 4 shows the correlations between the CP and CEL content of forage or faeces and the OMD and OMVI. For the entire CRA-W database, the OMD averaged across sheep was well correlated to the CP and CEL content of forage or faeces ($R^2 = 0.76$; S.E. = 0.027 and $R^2 = 0.78$; S.E. = 0.026). It seemed impossible to estimate the OMVI, respectively, from forages or faeces chemical characteristics ($R^2 = 0.06$; S.E. = 10.42 and $R^2 = 0.25$; S.E. = 9.37 g/kg $BW^{0.75}$).

The same results were observed for the INRA trials. The OMD was well correlated with the grass CP and CEL ($R^2 = 0.65$; S.E. = 0.034). Similarly, the grass CP and CEL explained only 34% of the OMVI variability ($R^2 = 0.34$; S.E. = 7.18 g/kg $BW^{0.75}$). The relationship between OMD and OMVI and faeces composition was very highly significant ($R^2 = 0.81$; S.E. = 0.0253 and $R^2 = 0.31$; S.E. = 7.33 g/kg $BW^{0.75}$, respectively, for OMD and OMVI) but, again, only the OMD could be estimated with sufficient accuracy.

3.3. NIRS calibrations to estimate OMD and OMVI

For each database viewed separately (Table 5), the estimations of OMD by NIRS applied to forage or faeces were relatively good. The R^2 were greater than 0.85 and SECV ranged between 0.020 and 0.018 for CRA-W forage or faeces calibration. For the INRA forage, the NIRS models developed from forage or faeces spectra showed similar accuracy (SECV = 0.021). For both the CRA-W and INRA databases, the best NIRS model for estimating OMD was obtained with concatenated spectra ($R^2 = 0.95$; SECV = 0.016 and $R^2 = 0.91$; SECV = 0.019, respectively, for CRA-W and INRA). Working with sub-

Table 5
NIRS calibration performance in relation to the nature of the spectra (forage or faeces) and the origin of samples (INRA or CRA-W databases)

NIRS spectra	Parameters	*N*	Mean value	S.D.	SEC	R^2	SECV	RPD
CRA-W database								
Forage	OMD	190	0.733	0.0541	0.0197	0.87	0.0198	2.73
	OMVI	180	53.70	7.17	6.27	0.23	7.00	1.03
Faeces	OMD	886	0707	0.0711	0.0171	0.94	0.0177	4.03
	OMVI	884	49.01	10.39	3.46	0.89	3.58	2.90
Subtracted	OMD	887	0.707	0.0713	0.0185	0.93	0.0191	3.73
	OMVI	887	49.09	10.47	3.82	0.87	3.95	2.65
Concatenated	OMD	817	0.714	0.0708	0.0154	0.95	0.0159	4.45
	OMVI	806	49.78	9.97	3.54	0.87	3.60	2.77
INRA database								
Forage	OMD	138	0.685	0.058	0.0186	0.90	0.0214	2.70
	OMVI	132	60.29	8.01	5.56	0.52	6.24	1.28
Faeces	OMD	140	0.687	0.0575	0.0195	0.88	0.0213	2.70
	OMVI	137	60.68	8.83	5.15	0.66	6.05	1.46
Subtracted	OMD	140	0.687	0.0575	0.024	0.83	0.0290	1.98
	OMVI	136	60.68	8.87	5.09	0.67	6.34	1.40
Concatenated	OMD	140	0.687	0.0575	0.0172	0.91	0.0191	3.00
	OMVI	137	60.68	8.83	4.96	0.68	6.15	1.44

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; R^2: coefficient of determination of NIRS equations; SEC: standard error of calibration; SECV: standard error of cross validation; S.D.: standard deviation of the reference database; RPD: S.D./SECV.

tracted spectra led to less accurate NIRS models only for the INRA database ($R^2 = 0.93$; SECV = 0.019 and $R^2 = 0.83$; SECV = 0.029, respectively, for CRA-W and INRA).

With the CRA-W forage samples, it was not possible to develop a robust calibration to measure OMVI ($R^2 = 0.23$; SECV = 7.00 g/kg $BW^{0.75}$). The OMVI estimations from faecal NIRS calibration were more efficient ($R^2 = 0.89$; SECV = 3.58 g/kg $BW^{0.75}$).

The NIRS equation statistics obtained with the INRA forage database to estimate OMVI ($R^2 = 0.52$; SECV = 6.24 g/kg $BW^{0.75}$) showed performances similar to those obtained from faecal spectra ($R^2 = 0.66$; SECV = 6.05 g/kg $BW^{0.75}$). The use of subtraction between forage and faeces spectra did not improve the NIRS equation statistics for the OMVI, as illustrated by the higher SECV values. Similarly, developing an NIRS equation with the concatenated spectra did not really improve the accuracy of the model (Table 5).

As mentioned earlier, the databases studied differed in terms of forage species and chemical composition. Fig. 2 illustrates spectral variability of the databases according to the first two axes of a PCA analysis of the faecal spectra. Ryegrass diploid and tetraploid cut in 1992 (CRA-W 1–2) had lower CP and ash content and higher OMDcel, and on a faecal basis they were completely different from the CRA-W 3–7 and INRA databases. This observation was confirmed by standardized *H* distances between the databases (Table 6). CRA-W 1–2 differed considerably from INRA ($H = 24.73$) and from CRA-W 3–7 ($H = 22.33$). The INRA database was far more variable than CRA-W 3–7 ($H = 14.35$). In contrast, CRA-W 3–7 appeared to be well integrated into the INRA database, with *H* lower than 3 ($H = 1.91$).

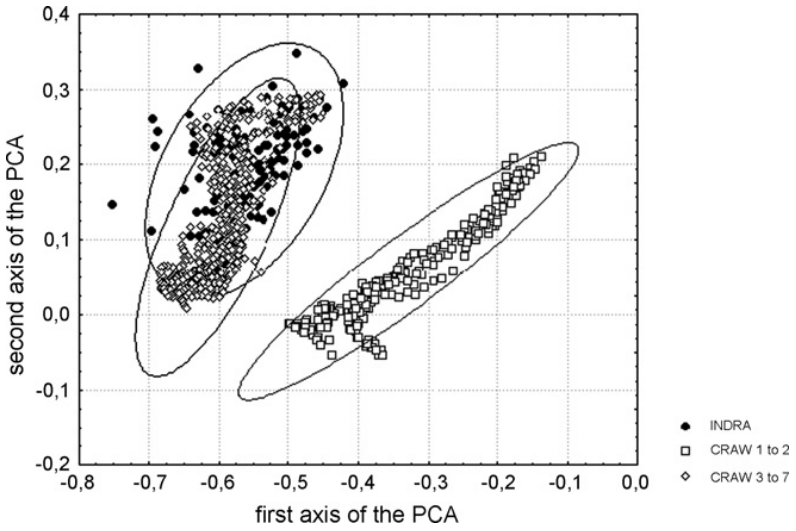

Fig. 2. Spectral variability of the databases in relation to the first two axes of a PCA analysis of NIRS faecal spectra.

Table 6
Standardized *H* distance between the INRA, CRA-W 1–2 and CRA-W 3–7 databases, on a faecal spectra basis

Spectra files/PCA files	INRA	CRA-W 1–2	CRA-W 3–7
INRA	–	49.80	14.35
CRA-W 1–2	24.73	–	22.33
CRA-W 3–7	1.91	17.59	–

These results suggested it was not possible to estimate the OMD or OMVI of INRA faecal samples with CRA-W faecal NIRS equations. In order to increase the variability, the databases were merged to develop new global NIRS calibrations (Table 7). The global calibration equations developed using faeces spectra had an intermediate accuracy (SECV = 0.021 and 4.53 g/kg $BW^{0.75}$, respectively, for OMD and OMVI) compared with the calibrations obtained with the individual databases (Table 5). However, the accuracy

Table 7
NIRS calibration results from the merged database (INRA and CRA-W)

NIRS spectra	Parameters	*N*	Mean value	S.D.	SEC	R^2	SECV	RPD
Forage	OMD	328	0.713	0.0606	0.0226	0.86	0.0231	2.62
	OMVI	323	57.06	8.73	7.29	0.30	7.47	1.16
Faeces	OMD	951	0.710	0.0698	0.0200	0.92	0.0207	3.35
	OMVI	936	51.27	10.46	4.28	0.83	4.53	2.31
Subtracted	OMD	943	0.710	0.0694	0.0210	0.90	0.0224	3.10
	OMVI	925	51.21	10.43	4.13	0.84	4.40	2.37
Concatenated	OMD	953	0.709	0.0701	0.0174	0.94	0.0185	3.77
	OMVI	942	51.35	10.42	3.85	0.86	4.13	2.52

OMD: *in vivo* organic matter digestibility; OMVI (g/kg $BW^{0.75}$): organic matter voluntary intake; SEC: standard error of calibration; SECV: standard error of cross validation; S.D.: standard deviation of the reference database; RPD: S.D./SECV.

of the faecal NIRS models was higher than that observed for regressions developed on the basis of the CP and CEL forage or faeces content, as reported in Table 4. As observed for the separate databases, it was not possible to estimate the OMVI from forage NIRS analysis ($R^2 = 0.30$; SECV = 7.74). Working with concatenated spectra improved the calibration performances slightly for both OMD and OMVI. Compared with the other faecal NIRS equations, OMD estimation from forage–faeces subtracted spectra appeared less accurate as illustrated by higher SECV value.

3.4. Relevant NIR wavelengths

The visual comparison of the four averaged faecal spectra (second derivative mode) indicated a strong absorption in the wavelength region characteristics of fat ($\lambda = 1730$; $\lambda = 1764$; $\lambda = 2310$; $\lambda = 2350$), fibres ($\lambda = 2078$–2110; $\lambda = 2268$) and protein ($\lambda = 2058$; $\lambda = 2166$), as defined by Bertrand (2002). For fat and protein wavelengths, the NIRS absorbencies decreased with OMD values, as illustrated in Fig. 3. Conversely, the absorbencies were higher in the region of fibres for faecal samples obtained after the intake of low digestibility forage. Wavelengths characteristic of fat content were very important for quantifying forage OMVI on the basis of the corresponding faecal spectra. The CP and CEL wavelengths did not appear to be so relevant.

4. Discussion

The efficiency of NIRS equations can be evaluated using various statistical parameters, such as R^2, SECV and the RPD ratio. To be acceptable, the NIRS equations must have an R^2 higher than 0.80, a SECV close to the SEC and an RPD ratio higher than 3 (Williams, 2004).

Calibration equations developed to estimate OMD gave an excellent R^2 ($R^2 > 0.90$) and appeared sufficiently robust, with an RPD higher than 3 for all faecal databases (faeces spectra alone: RPD = 3.35; subtracted spectra: RPD = 3.10; concatenated spectra: RPD = 3.77). With the SECV varying from 0.021 to 0.018 for faecal and concatenated databases, respectively, the faecal NIRS appeared to be a good tool to estimate the OMD of temperate forage. The efficiency of NIRS applied to forage or faeces to assess diet quality had also been confirmed by earlier studies.

Based on forage analysis, De Boever et al. (1996) reported that the *in vivo* OMD of 36 grass silages had a better correlation with NIR-estimated OMD ($r = 0.89$) than with *in vitro* enzymatic OMD ($r = 0.83$), rumen fluid OMD ($r = 0.81$) or ADL content ($r = -0.73$). Similarly, De La Roza et al. (2000) reported that the correlation between *in vivo* OMD and *in vitro* OMD was poor ($R^2 = 0.51$ and S.E. = 0.050) compared with NIRS performance in quantifying the *in vivo* OMD ($R^2 = 0.86$ and SECV = 0.028). They concluded that NIRS spectra provided more information than *in vitro* enzymatic digestibility.

Norris et al. (1976) and Lippke et al. (1989) also showed that digestibility could be quantified from the NIRS analyses of forage harvested in the field or obtained from oesophageal fistula, with the SEC varying between 0.032 and 0.036. Compared with these results, the NIR model developed in the merged forage database (CRA-W and INRA) gave a better

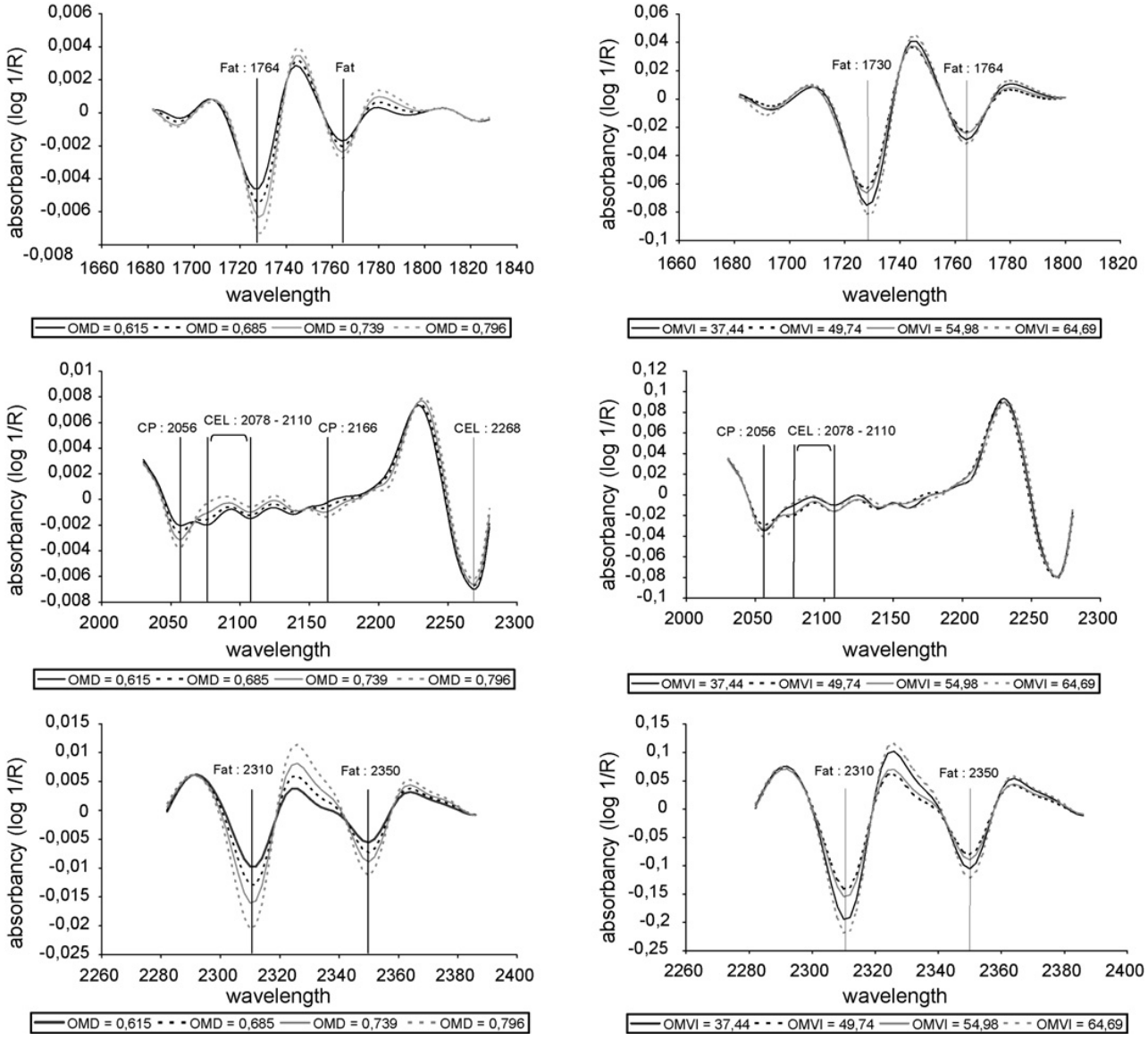

Fig. 3. Second derivative mode of the faecal spectral region in relation to four ascending OMD and OMVI (g/kg $BW^{0.75}$) values.

SEC (0.023). The use of faecal NIRS to estimate OMD tended to improve the performances of the models, as illustrated by our results and some earlier studies. Coleman et al. (1989) confirmed that faecal NIRS equations obtained from a wide range of forage species or forage mixtures to assess dry matter digestibility were precise enough to manage the nutrition of grazing herds. Similarly, Stuth et al. (1989) showed that the NIRS analyses of faeces could estimate the OMD of rangeland grazing ruminant diets with better accuracy than calibration equations developed from oesophageal extrusa (SEC = 0.033 and 0.051 for NIRS equations developed from faeces and oesophageal extrusa, respectively).

The accuracy of faecal NIRS models in estimating OMD was similar to or better than that obtained using other predictive methods, as reported by our results and several other studies. For instance, the faecal nitrogen index (faecal N) commonly used in

linear, quadratic or hyperbolic functions could estimate the OMD of grazed temperate or tropical forage with a similar accuracy (Greenhalgh and Corbett, 1960; Bartiaux-Thill and Oger, 1986; Comeron and Peyraud, 1993; Boval et al., 1996; Bouazizi and Majdoub, 1999). Although the *n*-alkanes ratio appeared to be one of the best methods for estimating OMD at pasture, the results obtained using these techniques could be highly variable due to the lack of precision of the analytical procedure and to the partial digestibility of some *n*-alkane chains (Sandberg et al., 2000; Moshtaghi Nia and Wittenberg, 2002).

It was not possible to estimate the OMVI from the NIRS measurements of forage with sufficient accuracy ($R^2 = 0.30$; SEC = 7.29 g/kg $BW^{0.75}$; SECV = 7.47 g/kg $BW^{0.75}$). Comparable results were obtained by Norris et al. (1976). Minson et al. (1983) reported that voluntary dry matter intake could be measured by NIRS analyses of forage samples, with the SEC between 7 and 9 of g/kg $BW^{0.75}$. Ward et al. (1982) estimated the OMVI with similar accuracy (SEC = 9.6 g/kg $BW^{0.75}$). Working on faecal spectra (alone, subtracted or concatenated) improved the statistics of the NIRS models. The faecal calibration equations developed to analyse the OMVI had an R^2 between 0.80 and 0.90, and an SECV lower than 5 g/kg $BW^{0.75}$, and led to RPD values between 2.31 and 2.52.

The NIRS equations developed from concatenated databases seemed more suitable for estimating the OMVI (SEC = 4.13 g/kg $BW^{0.75}$). Stuth et al. (1989) estimated the dry matter intake with an accuracy of 17.3 g/kg $BW^{0.75}$. The calibration equations developed in our study from faeces and from concatenated spectra were more accurate, with an SEC three times smaller. More recently, Boval et al. (2004) and Landau et al. (2004) confirmed the potential of faecal NIRS to characterise the diet attributes of ruminants (cattle and goats) grazing tropical grasslands.

Compared with other methods, faecal NIRS appeared to be accurate enough for estimating the OMVI. For instance, the *n*-alkanes ratio technique was as accurate as faecal NIRS for estimating the intake of different ruminants, such as sheep, cattle and goats (Mayes and Dove, 2000). However, it was difficult to use it for long periods because *n*-alkane dosing needs to be regular.

One explanation of the relevance of faecal NIRS for estimating OMD and OMVI was that these parameters also depended on physiologic and metabolic parameters, such as the digestion rate in the rumen (Illius and Jessop, 1996), plant characteristics (Jung and Allen, 1995; Allen, 1996) and animal behaviour (Faverdin, 1999; Provenza et al., 2003). Linked to animals, these factors were difficult or impossible to quantify only by analysing forage samples (Coelho et al., 1988). Faeces reflect biological and chemical characteristics of the forage consumed by animals as well as the physiological status of the herbivore. This chemical composition can be detected by NIRS and successfully correlated to the OMD and OMVI. This could explain why the NIRS analysis of faeces was as efficient, or more efficient, than the NIRS analysis of forage for assessing diet characteristics.

In our study, on the second derivative spectra of faeces from forage with low digestibility and a low intake level, there were higher peaks in the wavelength region of fibres (2078–2110 nm, 2268 nm). As confirmed by Leite and Stuth (1995), this peak was higher in faeces when the supplied forage was old. Similarly, Coleman and Murray (1993) showed that faecal spectra were negatively correlated with digestibility at 2100 nm. This peak was

characteristic of the OH and CO groups such as sugar, starch and cellulose (Bertrand, 2002). It could be explained by the accumulation of more fibre residues in faeces when digestibility decreased. The relevant wavelength regions related to the OMD and OMVI were also similar to those selected on the forage spectra by Norris et al. (1976) and Lippke and Barton (1988).

With regard to faeces spectra, the negative peak centred at 1730, 1764, 2310, 2350 nm could be associated with the presence of fat. Peaks in these spectral regions were higher when plant digestibility and plant intake were high (Fig. 3). This observation was confirmed by Leite and Stuth (1995) who showed, with goat faeces, the highest absorption at 2301 nm for high quality forage. One explanation of the relevance of fat peaks in faeces could be related to the presence of endogenous residues linked to microbial activity in the rumen. According to Lecomte (1995), microbial contamination of forage nylon bag residues (measurement of *in situ* degradability) could be successfully estimated using NIRS. On these samples the absorbency peaks appeared clearly at 1722 and 2306 nm, characteristic wavelengths of the O–H link, representative of fatty acid. Moreover, Lecomte et al. (1994) have shown that rumen microbes contained a high proportion of stearic acid (532 g/kg DM). The relevance of fat wavelength to estimate OMD and OMVI could be linked to higher microbial growth in the rumen in relation to the high forage quality, as well as to a higher proportion of microbes linked to the faecal forage residues. Similarly, when grass came to maturity, the balance between protein and energy nutrients available for the rumen micro-organisms became negative. This led to a decrease in the cellulolytic activities of rumen bacteria, a decrease in digestibility and finally a decrease in bacterial contamination of forage residues. With such unbalanced diets, the retention time of the forage in the rumen was longer and the level of intake lower. Another explanation of the fat wavelengths relevance could be linked to the presence in faeces of cuticular wax with a plant origin, such as *n*-alkanes commonly used to estimate digestibility and intake (Coleman and Murray, 1993). Cortes et al. (2005) reported that the total *n*-alkane concentration of ryegrass and tall fescue decreased during the plant growth. This could explain the importance of fat peaks in faecal spectra when young forage was consumed.

5. Conclusion

This work underline the high potentialities of NIRS applied to faeces or faeces and forage, in this case on concatenated spectra, to estimate grazed grass digestibility. The accuracy of the NIR model to estimate OMVI is similar to or better than the accuracy of the others methods of estimation. We suggest that the accuracy achieved is acceptable in view of the difficulty to obtain this dietary parameter.

However, NIRS analysis of faeces can provide estimates of both OMD and OMVI only if the database variability, used to develop the calibrations, is high enough to include the diversity of field conditions. Future work will involve the validation of the performances of the faecal calibrations on independent data sets, under diverse grazing management schemes and its mobilisation to develop decision support system aiming to improve grazing management.

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