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Measurement of rumen dry matter and neutral detergent fiber degradability of feeds by Fourier-transform infrared spectroscopy

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ABSTRACT

This study explored the potential of partial least squares (PLS) and Fourier-transform infrared spectroscopy (FTIR) to predict rumen dry matter (DM) and neutral detergent fiber (NDF) degradation parameters of a wide range of feeds for ruminants, as an alternative to the in situ method. In total, 663 samples comprising 80 different feed types were analyzed. In situ DM and NDF degradabilities were determined as follows: effective degradability (ED), rumen soluble fraction (A), degradable but not soluble fraction (B), rate of degradation of the B fraction (C), and indigestible NDF (iNDF). Infrared spectra of dry samples were collected by attenuated total reflectance from 600 to 4000 cm^{-1}. Feeds were randomly classified into 2 subsets of samples with representation of all feed types; one subset was used to develop regression models using partial least squares, and the second subset was used to conduct an external validation of the models. This study indicated that universal models containing all feed types and specific models containing concentrate feeds could provide only a relatively poor estimation of in situ DM degradation parameters because of compositional heterogeneity. More research, such as a particle size distribution analysis, is required to determine whether this lack of accuracy was due to limitations of the FTIR approach, or simply due to methodological error associated with the in situ method. This latter hypothesis may explain the low accuracy observed in the prediction of degradation rates if there was physical leakage of fine particles from the mesh bags used during in situ studies. In contrast, much better predictions were obtained when models were developed for forage feeds alone. Models for forages led to accurate predictions of DM_A, DM_B, NDF_{ED}, and NDF concentration (R^2 = 0.91, 0.89, 0.85, and 0.79, standard error = 4.34, 5.97, 4.59, and 4.41% of DM, respectively), and could be used for screening of DM_{ED}, NDF_C, and iNDF. These models relied on certain regions of the FTIR spectrum (900–1150 and 1500–1700 cm^{-1}), which are mainly compatible with absorption of plant cell wall components, such as cellulose, pectin, lignin, cutin, and suberin, but also with nonstructural carbohydrates and certain active compounds. In conclusion, FTIR spectroscopy could be considered a low-cost alternative to in situ measurements in feed evaluation.

Key words: Fourier-transform infrared spectroscopy, in situ method, neutral detergent fiber, rumen degradability

INTRODUCTION

Ruminant farmers and nutritionists who need to optimize feed utilization by ruminants have a genuine requirement for a simple, fast, and accurate technique to estimate the nutritional value of feeds for use in animal rations. Current ruminant feeding systems rely on accurate determination of the chemical composition of feedstuffs and the kinetics of their degradation in the rumen (AFRC, 1993; NRC, 2001). This latter factor has considerable influence on the nutritional value of the feed and allows diets to be formulated to match energy and nitrogen (N) requirements, thus synchronizing the availability of nutrients in the rumen and thereby improving microbial protein synthesis and efficiency of diet utilization (Casper et al., 1999). Moreover, feed degradation kinetics can be incorporated into integrated compartmental models that predict rumen fermentation patterns and nutrient supply (López et al., 2000). Although several methods have been proposed to estimate rumen feed degradation (Deaville et al., 1997), the in sacco or in situ technique has often been chosen as the preferred reference method (Verite and Peyraud, 1989; Madsen et al., 1995; Van Duinkerken et al., 2011) because of the close relationship with in vivo measurements (Gosselink et al., 2004). This method measures the progressive disappearance of feed from a polyester bag incubated in the rumen. The in situ method is highly robust but expensive because it requires rumen-cannulated animals and substantial amounts of labor and time because incubations can last

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for more than a week. Throughput is limited by the number of feeds that can be evaluated in parallel in any one animal (1 to 3 in sheep, 4 to 6 in cattle), and the amount of residue remaining after incubation often limits downstream analysis. Because of these limitations, the in situ method is generally not suitable for routine evaluation of feeds used on commercial farms, and the need exists for a cheap, high-throughput, and reliable alternative method.

Moderated and highly variable correlations have recurrently been observed between the chemical composition of feeds and their in situ degradability in terms of CP (R^2 = 0.08 to 0.90), DM (R^2 = 0.18 to 0.90) and NDF fractions (R^2 = 0.01 to 0.86) (Hoffman et al., 1999; Andres et al., 2005a,b). As a result, during the last decade, there have been several reports of multivariate regression models based on near-infrared reflectance spectra (**NIRS**) accurately predicting rumen degradabilities of DM, NDF, and CP in forage samples (Andres et al., 2005a,c; Ohlsson et al., 2007). However, there have been few reports of mid-infrared (**mid-IR**) spectrometry being used for this purpose even though this technique could provide several advantages over NIRS analysis: (1) light absorptivity is greater in the mid-IR (600–4000 cm^{-1}) than in the NIRS (4000–12500 cm^{-1}) range; (2) mid-IR uses broadband source rather than monochromatic, which allows faster data acquisition across the entire wavelength range; and (3) mid-IR spectroscopy provides information on fundamental molecular vibrations, which are stronger and more selective than the harmonic vibrations and overtone absorptions observed in NIRS. As a result, mid-IR generally has greater sensitivity and precision than NIR and therefore could give better insight into the chemical composition of the sample (Griffiths, 1983). In recent years, mid-IR spectroscopy has been revolutionized by the development of cheaper Fourier-transform infrared (**FTIR**) spectrometers (Vandevoort, 1992). Furthermore, this technology is compatible with methods such as attenuated total reflectance that allow spectra to be acquired from samples nondestructively with high throughput in a manner that requires only small amounts of sample and little preparation. Recently, the multivariate analysis of FTIR data has been reported to allow the discrimination of plant species (Huang et al., 2008) and the prediction of several compositional parameters including lignin, ash, C, and N content in several forage grasses (Allison et al., 2009a,b). Nevertheless, FTIR technology has not yet been used to predict the rumen degradation parameters of feed DM and NDF and it is unknown whether FTIR spectroscopy can predict these with a greater accuracy than is achieved with NIRS.

In a previous study (Belanche et al., 2013), we reported on the accuracy of partial least squares (**PLS**) regression models based on FTIR spectra to predict CP content and CP rumen degradability in several feeds used in ruminant nutrition. The present study investigates the use of similar chemometric models based on FTIR spectra to discriminate between feeds according to their NDF concentration and rumen DM and NDF degradabilities. The capacity of FTIR spectrometry to predict the feed degradability using a universal equation valid for many feeds used in ruminant nutrition was evaluated and compared with models developed for specific feed types.

MATERIALS AND METHODS

Sample Composition and Rumen Degradability

A total of 663 samples from 80 different feeds were collected during a 10-yr period in several locations in northern Europe. Table 1 shows the different samples and their botanic or industrial origin. All samples were freeze-dried, ground to pass through a 1.5-mm-diameter sieve, and stored in airtight containers until further analysis.

In situ DM degradability was determined using 3 nonlactating Danish Holstein cannulated cows with an approximate mean BW of 700 kg (Hvelplund and Weisbjerg, 2000). A subset of 111 forages was randomly selected and their ash-free NDF content was determined using the Fiber-Tec system (Foss, Hillerød, Denmark; Mertens, 2002) with sulfite treatment and heat-stable amylase. Similarly, in situ NDF degradability was determined for 82 of those forage samples. All animal experiments complied with the Danish Ministry of Justice Law No. 726 (9 September 1993) concerning experiments with animals and care of experimental animals. Cows were fed at maintenance level according to the NorFoc system (Volden, 2011) with a mixed diet consisting of 2 kg of artificially dried grass-clover hay, 4 kg of barley straw, and 2.8 kg of pelleted concentrate [composition in % of fresh matter: 40% barley grain, 40% oat grain, 10% soybean meal, 3% rapeseed meal, 3% sugar beet molasses, and 4% commercial mineral mixture (containing 6% Ca, 10% P, 12% Mg, 5% Na; Type 3, Vitfoss, Gråsten, Denmark)]. The forage to concentrate ratio in the diet was 67:33 on a DM basis, and CP concentration was 139 g/kg of DM to avoid depressing diet degradability. The daily feed allowance was given in 2 meals of equal size. Polyester Dacron bags (11 × 8.5 cm and 38-μm pore size, Saatifil PES 38/31, Saatitec S.p.A, Como, Italy) containing 1 g of sample were presoaked in tap water at 39°C for 20 min before incubation; 2-g samples were used in bags for 288-h incubation according to the NorFor procedure (Åkerlind et al., 2011). Bags were incubated in the

Table 1. Description of the sample set analyzed by Fourier-transform infrared (FTIR) spectrometry (n = 663); samples are grouped according to botanical or industrial origin

Botanical origin	Industrial origin
Cereal grains (n = 62)	DDGS[1] (n = 34)
29 Barley	28 Corn DDGS
12 Wheat	4 Wheat DDGS
7 Rye	2 Barley DDGS
5 Triticale	Oil by-products (n = 200)
4 Oat	112 Rapeseed
3 Maize	42 Soybean
2 Grain mix	25 Sunflower
Tropical feeds (n = 9)	12 Cottonseed
Maize silage (n = 12)	2 Soypass
10 Maize silage	2 Treated soybean meal
2 Maize silage with pulp	4 Others
Grass-clover forage (n = 76)	Protein products (n = 16)
29 Grass-clover forage	7 Guar meal
16 Grass-clover silage	4 Malt sprouts
11 Grass forage	3 Brewers grains
8 Grass silage	2 Potato protein
7 Artificial-dry grass	Mill by-products (n = 17)
4 Clover forage	7 Maize gluten feed
1 Festulolium forage	3 Maize feed meal
Barley-wheat forage (n = 12)	3 Wheat gluten feed
8 Winter wheat silage	2 Wheat bran
4 Barley whole crop silage	2 Amyfeed
Concentrate mix (n = 127)	Soybean hulls (n = 15)
Maize forage (n = 2)	
Legume forage (n = 26)	
10 Lupinus whole crop	
4 Peas whole-crop silage	
4 Galega forage	
3 Lucerne forage	
2 Field beans whole crop	
2 Artificial dry lucerne	
1 Peas whole-crop forage	
Legume seeds (n = 16)	
6 Peas	
3 Soybean	
3 Toasted soybean	
2 Rapeseed	
2 Lupinus	
Beets (n = 22)	
14 Dry sugar beet pulp	
6 Fodder beets	
2 Beet pulp	
TMR (n = 19)	

[1]DDGS = dried distillers grains with solubles.

ventral rumen sac for 0, 2, 4, 8, 16, 24, 48, and 96 h. Bags for 0 h were not incubated in the rumen but were mechanically washed for estimation of soluble DM content. After incubation, bags were rinsed with tap water, frozen at −20°C for at least 24 h, thawed, and washed with cold water in a washing machine without soap or spinning. For forage samples for DM degradation, bag residues were treated in a stomacher (Seward, Worthing, UK) with 60 mL of demineralized water and then soaked to reduce the microbial contamination attached to the residue. Thereafter, incubation residues were dried overnight at 100°C for further DM determination. Residues for NDF degradation were quantitatively transferred from bags to filter crucibles without drying to avoid overestimation of the NDF residues.

In situ degradability values (expressed in % of DM for all 663 samples) were used to estimate the degradation profiles parameters based on nonlinear regression curve fitting using the least squares method according to the equation described by Ørskov and McDonald, (1979):

$$\text{DM degraded } (t) = \text{DM}_\text{A} + \text{DM}_\text{B} \times (1 - e^{-\text{DMc} \times t}), \quad [1]$$

where $\mathbf{DM_A}$ is the immediately degradable soluble fraction in the rumen, $\mathbf{DM_B}$ is the insoluble degradable fraction in the rumen, $\mathbf{DM_C}$ is the fractional rate of degradation of fraction DM_B (expressed as %/h), and t is the time (h). Potentially degradable DM ($\mathbf{DM_{PD}}$) was calculated as the sum of DM_A and DM_B. Lag time was not considered in our equations because it is dependent on other degradation parameters, especially rate of degradation, and could limit the validity of FTIR models. In the calculations of the degradation parameters for NDF, fraction NDF_A was assumed to be 0 because NDF is not immediately degradable in the rumen. Two approaches were used to determine the NDF degradation rate depending on whether the degradation values at 0 h were considered ($\mathbf{NDF_C}$) or not ($\mathbf{NDF_{C\text{-}UND}}$). This latter degradation rate was calculated to prevent problems in whole-crop cereals as a result of the presence of starch in the residue after 0 h incubation.

Dry matter effective degradability ($\mathbf{DM_{ED}}$) was calculated for each of the 663 samples according to the following equation:

$$\text{DM}_\text{ED} = \text{DM}_\text{A} + \text{DM}_\text{B} \times [\text{DM}_\text{C}/(\text{DM}_\text{C} + \text{DM}_k)], \quad [2]$$

where k is the fractional outflow rate from the rumen (h^{-1}).

Neutral detergent fiber effective degradability ($\mathbf{NDF_{ED}}$) for each of the 82 samples that were analyzed using the in situ technique was calculated as follows:

$$\text{NDF}_\text{ED} = \text{NDF}_\text{B} \times [\text{NDF}_\text{C}/(\text{NDF}_\text{C} + \text{NDF}_k)], \quad [3]$$

where $\mathbf{NDF_B}$ is the insoluble degradable fraction in the rumen, k is the fractional outflow rate from the rumen (h^{-1}). In this paper, the value of k was considered to be fixed and equal to 5%/h for DM_k and 2%/h for NDF_k because 20 h and 50 h are the average rumen retention time for nonfibrous and fibrous feeds, respectively (Hvelplund et al., 2003). Moreover, DM_ED was calculated without correction for particle loss, and NDF_ED was calculated with correction for particle loss.

Finally, a total of 111 forage samples were incubated in the rumen for 288 h to determine the indigestible NDF content (**iNDF**). Sample preparation, incubation, and analysis were identical to those described for NDF degradation analysis except that sample size was 2 g and incubations were in 12-μm pore size bags according to the NorFor standardized method (Åkerlind et al., 2011).

Collection of FTIR Spectra and PLS Regression

Spectroscopic analysis and data analysis were performed as described previously (Belanche et al., 2013). Briefly, infrared spectra were collected from freeze-dried and ground samples by attenuated total reflectance (ATR) from 600 to 4000 cm^{-1} using an Equinox 55 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) fitted with a Golden Gate ATR accessory (Specac Ltd., Slough, UK). All spectra were averaged over 64 scans at a resolution of 2 cm^{-1}. Samples were analyzed in duplicate and absorbance spectra were converted to text files in Opus software (version 4.2, Bruker UK Ltd., Coventry, UK) for subsequent data analysis.

Spectral data were imported in Matlab (version 7.5.0, MathWorks, Cambridge, UK), duplicates were averaged, derivatized to the first or second Savitsky-Golay derivative (15 units of filter width and polynomial order of 2), normalized, and mean center scaled. Regression models considering the whole spectral data were constructed using PLS regression SIMPLS algorithms using the PLS Toolbox (Eigenvector Research Inc., Wenatchee, WA). Additional preprocessing procedures were investigated (e.g., detrending, standard normal variate, or multiplicative scatter correction) to correct for scatter and possible baseline drift errors. Similarly, prediction models based on the fingerprinting region (600–1450 cm^{-1}) were tested; however, they did not lead to significant improvements in the model performance and were therefore not used in the final procedures. Models were trained on a randomly selected subset of spectra (containing between 81 and 86% of the samples) using a "Venetian blinds" cross-validation protocol of 10 data splits. Predictive accuracy was assessed using an independent data set of spectra (14 to 19% of the samples) that had been excluded from the model construction. Outlying spectra were removed from the model based on high Hotelling's T^2 (a statistic used in multivariate quality control charts), high Q residuals (a statistic used to determine lack of fit of the model), or when observed values differed by more than 3 standard deviations with respect to the predicted values. The number of outliers removed represented less than 5% of the samples analyzed. The fit of the model to the data was given by the coefficients of determination $\mathbf{R^2_C}$, $\mathbf{R^2_{CV}}$, and $\mathbf{R^2_P}$, which give the proportion of variance of one variable that is predictable by the other in the calibration, cross-validation, and prediction data sets, respectively. Similarly, the robustness of the model was defined by the root mean square error (**RMSE**), which measures the variability of the difference (d_i) between the predicted and reference values for a set of n samples (**RMSEC** and **RMSECV** for the internal validation and cross-validation data set; and **RMSEP** for the external validation data set) according to the following equation:

$$\text{RMSE} = \sqrt{\left(\Sigma {d_i}^2\right) / n}. \quad [4]$$

The number of latent variables (**LV**) included in the model was selected to minimize the RMSECV and therefore avoid model over-fitting (a description of random error by the model instead of the underlying information). The ratio of performance to deviation (**RPD**: SE of the original data divided by SE of the prediction) and the ratio of the range in the reference (**RER**: range of the reference data divided by SE of the prediction) were determined as a measurement of the ability of the model to predict a feed parameter. To assess the success of a model, RPD values <2 were considered to not give a relevant prediction; values between 2.0 and 2.5 were considered adequate for qualitative feed evaluation or screening purposes; values >2.5 were considered acceptable for quantification (or RER >10); and values >3 were considered to indicate that the equation could be used for highly accurate quantitative analysis (Williams and Sobering, 1996). Finally, the variable importance in projection (**VIP**) scores, which estimate the importance of each variable in the projection used in a PLS model, were used to identify the most important wavenumbers in the models. The greater the VIP score of a variable, the greater its importance is in given model.

Developing Feed-Specific PLS Regressions

Due to the high spectral variability observed between feed types (Figure 1), the predictive accuracy of PLS universal models was compared with that obtained from more specific models developed for particular feed types. To develop models for sample classification, 663 averaged FTIR spectra were derivatized to the first Savitsky-Golay derivative to smooth baseline noise and improve spectral resolution using a 13-point window and mean center normalized (mean = 0, SD = 1; Allison et al., 2009a). Underlying structures in the spectra correlating with differences between the feed types were

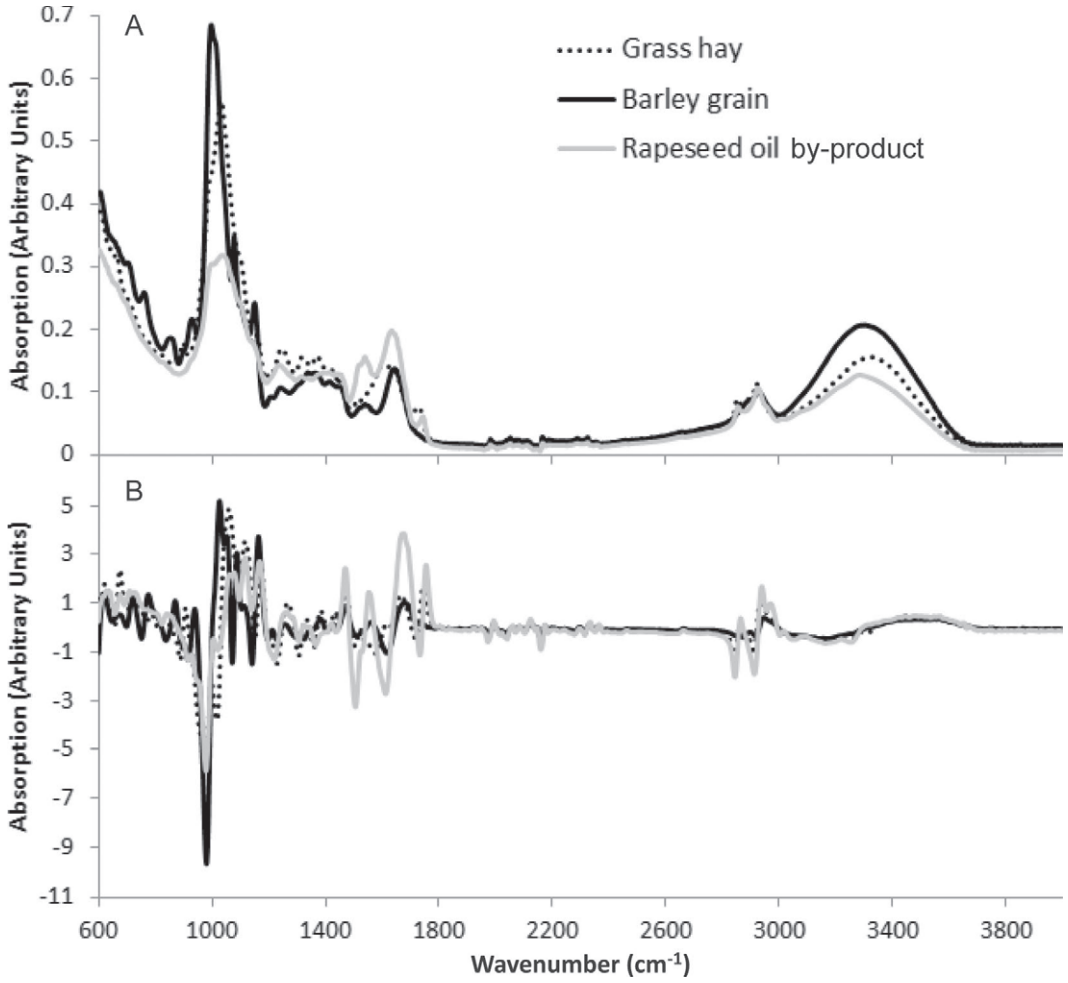

Figure 1. (A) Example of Fourier-transform infrared row spectra of 3 different feeds: grass hay, barley grain, and rapeseed oil by-product; (B) the same spectra after derivatization to the first Savitsky-Golay derivative and vector normalization.

investigated by principal components analysis (PCA) and significant differences between groups of samples were detected by multivariate ANOVA (MANOVA, using Wilk's lambda as a multivariate test) and canonical variate analysis (CVA) with a level of statistical significance set at 95% confidence using GenStat (version 10, VSN International Ltd., Hemel Hempstead, UK).

RESULTS AND DISCUSSION

Universal Models to Predict Feed DM Degradability

The in situ technique revealed considerable diversity in rumen DM degradation kinetics between the 663 samples analyzed (Table 2). For example, cereal grains had the greatest DM_{ED}, DM_A, and DM_C, but the lowest DM_B as a result of their high starch content. The opposite was true (low DM_{ED}, DM_A, and DM_C but high DM_B) for soybean hulls because of their high content of structural carbohydrates. These differences are a consequence of the diverse botanical origin, state of plant maturity at harvest, feed processing, and factory and preservation methods used.

Table 3 shows the abilities of universal models to predict the rumen DM degradability of the feeds used in this study, which represent many of those used by the ruminant production industry in Europe and beyond. All of these models had a RER >10, probably due to the high range of variation between feeds, but all RPD values were <2.5, with the R^2_C values being <0.77, indicating that these universal models were not accurate enough for quantitative purposes. In particular, the fractions DM_{PD} and DM_C were poorly predicted, whereas fractions DM_A, DM_B, and DM_{ED} were predicted with a slightly better accuracy (R^2_{CV} = 0.74, 0.69, and 0.64, respectively) and may be suitable for conducting coarse screening of the feeds according to rumen DM degradation pattern.

Table 2. In situ DM degradabilities[1] of the different feeds (in % of DM, unless otherwise stated) in each feed sample set: forages (FOR), energy-rich concentrates (ERC), and protein-rich concentrates (PRC)

		DM_{ED}		DM_A		DM_B		DM_C (%/h)		DM_{PD}	
Item[2]	No.	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
FOR											
Barley/wheat forage	12	52–70	62 ± 6	41–59	52 ± 5	27–38	31 ± 3	1.2–3.4	2.4 ± 0.6	79–86	83 ± 3
Maize forage	12	58–69	63 ± 3	36–57	48 ± 7	30–54	39 ± 8	2.1–4.4	3.1 ± 0.8	85–90	87 ± 2
Total mixed ration	19	63–77	70 ± 4	39–54	45 ± 5	39–54	47 ± 5	3.7–10	5.7 ± 1.8	91–95	93 ± 1
Legume forage	26	49–83	62 ± 10	27–72	39 ± 11	20–55	41 ± 8	4–15	6.3 ± 2.6	70–93	81 ± 6
Grass/clover forage	76	33–82	66 ± 11	12–62	38 ± 10	29–69	53 ± 7	1.4–15	6.1 ± 2.5	73–98	91 ± 6
Soybean hulls	15	43–58	50 ± 4	8–15	12 ± 2	79–92	86 ± 4	3.2–5.2	3.9 ± 0.6	90–100	98 ± 3
Beet byproducts	22	52–92	71 ± 13	1–81	34 ± 29	14–100	64 ± 30	5.0–18	7.9 ± 3.1	89–100	98 ± 3
ERC											
Cereal grains	62	61–97	83 ± 6	36–92	56 ± 12	8–61	36 ± 12	3.9–38	20 ± 9.0	72–100	92 ± 5
Mill byproducts	17	53–82	69 ± 7	22–66	45 ± 11	27–75	48 ± 14	3.5–9.3	5.6 ± 1.8	69–100	93 ± 8
Tropical feeds	9	52–68	62 ± 6	31–47	38 ± 5	15–60	42 ± 13	4.5–12	6.9 ± 2.3	59–95	80 ± 11
Concentrate mix	127	49–83	71 ± 6	15–64	44 ± 8	26–72	49 ± 9	3.3–16	6.5 ± 2.2	82–100	93 ± 4
PRC											
Dried distillers grains with solubles	34	45–83	62 ± 8	15–69	39 ± 11	26–77	50 ± 12	2.7–5.8	4.4 ± 0.9	75–100	89 ± 6
Legume seeds	16	71–89	78 ± 6	31–71	49 ± 13	20–69	49 ± 14	4.2–12	7.3 ± 2.0	91–100	99 ± 3
Protein products	16	41–77	65 ± 11	16–45	33 ± 8	37–84	60 ± 12	2.1–8.2	6.2 ± 1.9	75–100	93 ± 10
Oil byproducts	200	38–83	62 ± 7	8–72	31 ± 8	23–92	59 ± 11	2.1–14	6.0 ± 2.2	74–100	90 ± 6
Partial least squares sample sets											
Universal calibration set	573	26–97	67 ± 10	1.2–92	39 ± 14	8.0–100	52 ± 15	1.2–38	7.2 ± 5.3	47–100	91 ± 6.9
Universal validation set	90	28–93	67 ± 10	2.9–85	39 ± 13	10–95	51 ± 14	2.0–35	7.3 ± 5.9	51–100	90 ± 6.1
FOR calibration set	150	33–92	65 ± 11	1.2–81	38 ± 15	14–100	52 ± 18	1.2–18	5.7 ± 2.8	70–100	90 ± 7.4
FOR validation set	30	35–86	64.0 ± 11	1.4–75	37 ± 16	16–99	53 ± 18	1.3–12	5.5 ± 2.1	72–100	90 ± 7.2
ERC calibration set	180	26–98	74 ± 9.0	15–92	47 ± 11	8.0–75	45 ± 11	2.4–36	10 ± 7.8	47–100	92 ± 6.1
ERC validation set	35	28–93	73 ± 10	19–87	48 ± 12	10–68	43 ± 13	2.9–38	10 ± 8.8	49–100	91 ± 7.3
PRC calibration set	222	38–89	63 ± 8.2	7.9–72	33 ± 9.5	20–92	58 ± 11	2.1–14	5.9 ± 2.2	75–100	91 ± 6.7
PRC validation set	44	38–86	64 ± 9.1	9.0–71	37 ± 12	22–76	54 ± 12	2.9–10	5.7 ± 1.9	74–100	90 ± 6.6

[1]DM_{ED} = DM effective degradability; DM_A = immediately degradable soluble fraction in the rumen; DM_B = insoluble degradable fraction in the rumen; DM_C = fractional rate of degradation of fraction DM_B; DM_{PD} = potentially degradable DM (DM_A + DM_B).

[2]Rumen DM degradability was determined in situ considering a rumen fractional passage rate of 5%/h.

Table 3. Universal partial least squares (PLS) models developed using Fourier-transform infrared (FTIR) spectroscopy to predict rumen DM degradation parameters (in % of DM, unless otherwise stated)[1]

			Calibration (n = 573)		Cross validation		Prediction (n = 90)			
Item[2]	SG	LV	R^2_C	RMSEC	R^2_{CV}	RMSECV	R^2_P	RMSEP	RPD	RER
DM_{ED}	2	7	0.69	5.62	0.64	6.10	0.64	6.14	1.71	10.4
DM_A	1	6	0.77	6.17	0.74	6.58	0.65	7.62	1.78	11.9
DM_B	1	7	0.73	7.41	0.69	7.96	0.63	8.79	2.25	10.6
DM_{PD}	2	6	0.53	4.30	0.46	4.61	0.51	4.04	1.69	10.3
DM_C (%/h)	1	6	0.50	2.58	0.43	2.75	0.51	2.39	2.25	15.3

[1]R^2_C, R^2_{CV}, and R^2_P = determination coefficient of calibration, cross-validation, and prediction, respectively, and RMSEC, RMSECV, and RMSEP = their respective root mean square errors; RPD = ratio of performance to deviation; RER = range in reference ratio (ratio of the SD of the original data to RMSEP).

[2]Rumen DM degradability was determined in situ considering a rumen fractional passage rate of 5%/h. Details are given of the Savitsky-Golay (SG) derivative and the number of latent variables (LV) used in the model. DM_{ED} = DM effective degradability; DM_A = immediately degradable soluble fraction in the rumen; DM_B = insoluble degradable fraction in the rumen; DM_C = fractional rate of degradation of fraction DM_B; DM_{PD} = potentially degradable DM (DM_A + DM_B).

The low level of accuracy of universal models may be partially explained by external factors, such as the differences in the rumen conditions of the different animals used for the in situ measurements, but may also be partly explained by feed-dependent factors. In this experiment, a fixed rumen retention time was assumed, although this can be modified by the animal's physiology (i.e., intake and rumination) and composition of the feed (e.g., CP concentration, cell wall fibers, nonstructural carbohydrates, minerals, tannins, saponins), and these can therefore modify the in situ DM degradability of a given feed (Hvelplund et al., 2003). Moreover, in a recent paper, Krämer et al. (2013) demonstrated that it is difficult to compare rumen DM degradability using the in situ method when samples differ widely in their physicochemical structure (i.e., forages vs. concentrates) due to differential physical leakage of fine particles (<38 μm) from the polyester bags. This leakage is mainly governed by the physical structure of the feed, which is not assessed by FTIR analysis and therefore represents a source of error in our universal models in which all different feeds are considered. These methodological limitations may help explain the current lack of robust universal models based on NIRS analysis of feeds containing samples of forages and nonforages. To minimize these analytical limitations in the current study, we investigated whether the use of PLS predictions for particular feed types could improve the accuracy found with the universal models.

Feeds Classified According to Their FTIR Spectra

Principal components analysis of spectral data from the entire spectral range (600–4000 cm^{-1}) indicated that 90% of the total variance was captured in the first 10 components and these were therefore used for the subsequent canonical variate analysis (Figure 2). Analysis of the FTIR spectra showed that samples could be classified into 3 major groups according to their botanical or industrial origin: (1) Forages (**FOR**): this group comprised samples of grass and clover, whole-crop cereals (maize, barley, and wheat) and legumes. Moreover, samples of beet byproducts, soybean hulls, and TMR were also classified as forages because of the similarity of their spectral patterns to those of conventional forages. (2) Energy-rich concentrates (**ERC**): comprising samples of cereal grains, concentrate mixes, mill byproducts, and tropical feeds. (3) Protein-rich concentrates (**PRC**): comprising feeds generally having a CP concentration of more than 30%, such as legume seeds, dried distillers grains with solubles (**DDGS**), oil byproducts, and protein products.

A scatter plot of first and second canonical scores (Figure 1A) showed clear separation of multivariate means for FTIR spectra derived from FOR, ERC, and PRC, with highly significant differences being detected between these 3 groups (multivariate ANOVA Wilk's lambda = 0.069; $P < 0.001$). Individual analysis of each of these 3 feed types (Figure 1B, 1C, and 1D) revealed differences in the FTIR profile between feeds, but those differences were always smaller than those observed between the 3 main feed types. In agreement with our observations, previous studies have reported differences in FTIR spectra among different types of grasses (Schmidt and Skidmore, 2001; Allison et al., 2009a), varieties of tea (He et al., 2007), and parts of the plant (Allison et al., 2009b). This discrimination capacity of FTIR spectroscopy could be used to achieve quick and easy identification of sample origin even when only small quantities of substrate are available.

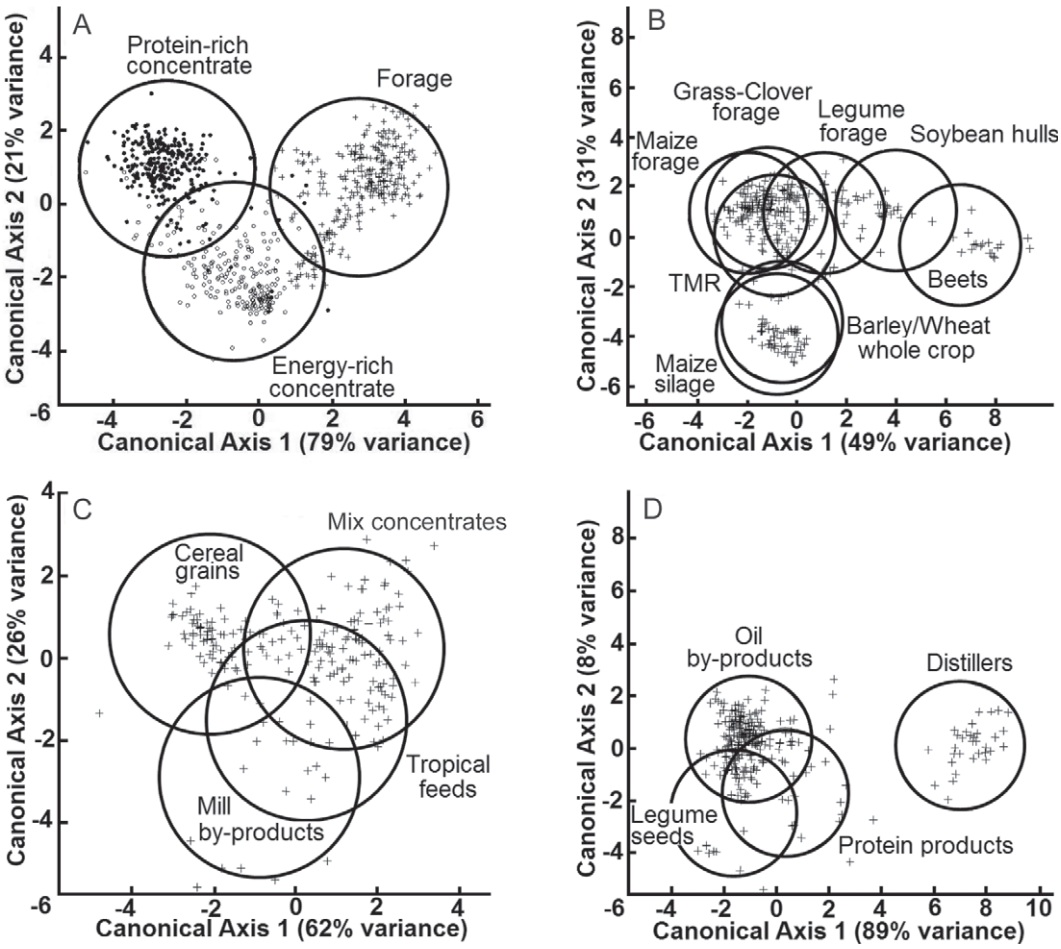

Figure 2. Canonical variate analysis score plot of feeds Fourier-transform infrared spectra according to (A) their pertinence to the 3 main categories (n = 663); (B) the type of forages (n = 180); (C) the type of energy-rich concentrates (n = 215); and (D) the type of protein-rich concentrates (n = 266). Circles indicate the 95% CI of the populations considering the first 10 principal components (PC).

Prediction of Rumen DM Degradability in Different Types of Feeds

Forages typically represent a substantial proportion of a ruminant's diet, and an accurate determination of their DM degradability pattern in the rumen is therefore imperative if diets are to be formulated to optimize rumen function and the efficiency of diet utilization (Casper et al., 1999). The studied FOR feeds covered a wide range of values (Table 2) in terms of DM_{ED} (from 33 to 92%), fraction DM_A (1.2 to 81%) and DM_B (14 to 100%), DM_C and DM_{PD} (1.2 to 18.5%/h and 70 to 100%, respectively). Our findings indicated that substantial improvements in the model accuracy to predict the in situ DM degradability were achieved when FOR-specific models were used compared with the universal models.

Fraction DM_A was the parameter best predicted in FOR samples (R^2_{CV} = 0.91, RPD = 3.64), and DM_B was also predicted with a high accuracy (R^2_{CV} = 0.89, RPD = 3.01). Higher accuracies in the prediction of DM_A than DM_B have previously been reported in the literature using NIRS (Mathison et al., 1999; Andres et al., 2005a; Ohlsson et al., 2007), probably because the former is better correlated with the chemical structure than the latter. Moreover, the expected errors in these estimations were 3.28 and 2.82 units lower than observed for DM_A and DM_B in the universal models, respectively. Although the use of universal models containing a broad range of feeds might improve the accuracy of the models, our findings indicated that better evaluation of the forages' degradability using PLS models developed on FTIR spectra was achieved by using forage samples exclusively. As a result, these models can be used satisfactorily for forage evaluation. Our prediction models, possibly because a broader range of forages was considered, were substantially better than models developed on NIRS analysis to predict the

rumen DM degradation kinetics in meadow herbages (R^2_{CV} = 0.90 and 0.61, RPD = 2.41 and 1.39 for DM_A and DM_B, respectively; Andres et al., 2005a), grasses (R^2_{CV} = 0.86 and 0.75, RPD = 2.8 and 2.0; Ohlsson et al., 2007), and barley straw (R^2_{CV} = 0.84 and 0.69, RPD = 2.50 and 1.77; Mathison et al., 1999).

Forage DM_{ED} values were predicted with reasonable accuracy (R^2_{CV} = 0.75, RPD = 2.07) compared with the universal model. Although this accuracy cannot be considered high enough for formal quantification, it may be useful for screening purposes (Williams and Sobering, 1996). These values are in agreement with those reported for NIRS predictions of DM_{ED} in silage (R^2_{CV} between 0.55 and 0.77; Liu et al., 2008) and barley straw (R^2_{CV} = 0.75; Mathison et al., 1999). However, slightly better predictions have been described for meadow herbages (R^2_{CV} = 0.89; Andres et al., 2005c) and TMR (R^2_{CV} = 0.85; Mentink et al., 2006) when DM_{ED} was determined in vitro. These better estimations could be explained by the greater homogeneity in the data in these latter experiments and also by the acknowledged underestimation of in vitro DM_{ED} values compared with those measured in situ (Chaudhry and Mohamed, 2011). Similarly, FOR-specific models that used NIRS to predict in vivo DM digestibility (Andueza et al., 2011) showed slightly higher coefficients of determination (R^2_{CV} = 0.85) than those observed in our study for in situ DM_{ED}. The smaller methodological error of the in vivo DM digestibility measurements used previously, compared with the in situ DM degradability measurements used in this study, may explain these differences.

The fraction DM_{PD} and fractional degradation rates were predicted with a poor accuracy in FOR feeds, which has also been reported for models based on NIRS spectra (R^2_{CV} between 0.18 and 0.51; Mathison et al., 1999; Andres et al., 2005a; Ohlsson et al., 2007). Two reasons might explain the low predictive accuracy of these fractions using IR spectrometry (Ohlsson et al., 2007): (1) these fractions are determined more by the physical structure of the feed (association of different types of structural carbohydrates and lignin) than by the chemical composition, and (2) the low degree of precision of the in situ technique at early incubation times because of differences in the density and compositing of the rumen liquid between animals and because of physical leakage of the feed from the bags (Krämer et al., 2013).

Using NIRS-based models to predict in vivo OM digestibility in sheep, Andueza et al. (2011) reported a substantial improvement in the standard error of prediction when calibrations for particular forage species were used instead of a global equation in which all forages were included. This suggests that the subdivision of our FOR database according to botanical origin may similarly improve the accuracy of our model but with the penalty of the model being less useful when forage identity is unknown.

With regard to ERC feeds (Table 4), in situ DM degradability parameters were poorly predicted by FTIR spectroscopy (R^2_{CV} <0.67 and RPD <2.08) and therefore cannot be considered sufficiently accurate for feed evaluation.

Models including PRC were, however, more accurate than those developed for ERC feeds, and had an error rate similar to that observed for FOR (RMSEP ≈ 5% of DM for DM_{ED}, DM_A, DM_B, and DM_{PD} and 1.14% of DM/h for DM_C). This could be considered an artifact resulting from the PRC feeds being more homogeneous than the FOR samples, and could explain why indicators of the model accuracy (R^2_{CV}, RPD, and RER) were lower for the PRC group than were found for the FOR models. Consequentially, only the model for DM_B can be considered sufficiently robust for screening of PRC feeds. This low predictive accuracy for in situ DM degradability of concentrate feeds may be due to excessive physical leakage of fine material out of the bags, which substantially increases the error of the in situ method when concentrate feeds are analyzed, compared with FOR measurements. A particle size distribution analysis could help to understand this differential leakage of fine particles between FOR and concentrate feeds. If this is the case, the lack of accuracy is actually due to the underlying analytical data, which may explain the abundance of studies that describe the use of IR spectrometry and chemometric models to evaluate the rumen degradation pattern of forages (Mathison et al., 1999; Andres et al., 2005a; Ohlsson et al., 2007) but the lack of studies of concentrates.

Prediction of Rumen NDF Degradability in Forages

Forages have a high concentration of NDF, which represents the insoluble fraction of the cell wall. As a result, NDF content greatly influences the voluntary intake of DM and is an important parameter in most ruminant feeding systems (AFRC, 1993; NRC, 2001). A group of FOR samples were analyzed for NDF concentration (n = 111) and in situ NDF degradation parameters (n = 82). This subset of FOR samples was mainly composed of cereal and legume forages, with beet and soybean hulls also being included because of their FOR-like spectral similarity. Therefore, this data set varied widely with respect to NDF concentration and rumen NDF degradabilities (Table 5).

A PLS model developed with these spectral data allowed accurate prediction of the NDF concentration of FOR samples (Table 6; R^2_{CV} = 0.79, RPD =

Table 4. Partial least squares models developed using Fourier-transform infrared (FTIR) spectroscopy to predict rumen DM degradation parameters (in % of DM, unless otherwise stated) for forages (FOR), energy-rich concentrates (ERC), and protein-rich concentrates (PRC)[1]

			Calibration		Cross validation		Prediction			
Item[2]	SG	LV	R^2_C	RMSEC	R^2_{CV}	RMSECV	R^2_P	RMSEP	RPD	RER
FOR				(n = 150)				(n = 30)		
DM_{ED}	2	8	0.87	3.92	0.75	5.44	0.77	5.26	2.07	11.2
DM_A	1	8	0.94	3.61	0.91	4.55	0.93	4.34	3.64	18.5
DM_B	2	8	0.94	4.45	0.89	5.85	0.89	5.97	3.01	14.4
DM_{PD}	2	6	0.81	2.96	0.68	3.84	0.61	4.99	1.49	6.13
DM_C (%/h)	1	5	0.75	1.24	0.67	1.42	0.52	1.38	1.96	12.6
ERC				(n = 180)				(n = 35)		
DM_{ED}	2	8	0.84	3.23	0.67	4.75	0.70	5.20	1.78	9.31
DM_A	1	7	0.59	6.59	0.42	7.95	0.67	7.36	1.53	10.4
DM_B	1	7	0.63	6.48	0.47	7.94	0.62	7.76	1.53	8.68
DM_{PD}	2	5	0.58	2.68	0.40	3.23	0.44	3.06	2.08	13.6
DM_C (%/h)	1	7	0.69	3.72	0.60	4.29	0.68	4.54	1.78	7.69
PRC				(n = 222)				(n = 44)		
DM_{ED}	2	8	0.75	3.73	0.61	4.66	0.66	4.91	1.71	10.4
DM_A	1	6	0.70	4.31	0.62	4.90	0.65	5.51	1.85	11.7
DM_B	1	7	0.78	4.51	0.70	5.33	0.75	5.09	2.38	14.8
DM_{PD}	2	5	0.85	2.49	0.80	2.84	0.71	3.59	1.93	7.51
DM_C (%/h)	1	8	0.70	1.20	0.53	1.52	0.67	1.14	1.93	10.7

[1]R^2_C, R^2_{CV}, and R^2_P = determination coefficient of calibration, cross-validation, and prediction, respectively, and RMSEC, RMSECV, and RMSEP = their respective root mean square errors; RPD = ratio of performance to deviation; RER = range in reference ratio (ratio of the SD of the original data to RMSEP).

[2]Rumen DM degradability was determined in situ considering a rumen fractional passage rate of 5%/h. Details are given of the Savitsky-Golay (SG) derivative and the number of latent variables (LV) used in the model. DM_{ED} = DM effective degradability; DM_A = immediately degradable soluble fraction in the rumen; DM_B = insoluble degradable fraction in the rumen; DM_C = fractional rate of degradation of fraction DM_B; DM_{PD} = potentially degradable DM (DM_A + DM_B).

2.66). These findings are in line with previous reports in which FTIR spectroscopy was used to predict the concentrations of lignin, ferulic, and coumaric acids, N, and alkali index in forage samples (Allison et al., 2009a,b). Models based on NIRS have been reported as being slightly more accurate for prediction of the NDF concentration (R^2_{CV} about 0.90) for TMR (Mentink et al., 2006), grasses (Ohlsson et al., 2007), meadow herbage (Andres et al., 2005c), and barley straw (Mathison et al., 1999), but this could be sample-specific and our results demonstrate that PLS models based on FTIR spectra were able to predict the NDF content in a diverse group of feeds.

The NDF_{ED} content of FOR is an important parameter in ruminant nutrition because it determines the amount of energy that is available for the rumen microbial population and, to some extent, rumen retention time. Our results indicate that NDF_{ED} can be predicted accurately using FTIR regression models (R^2_{CV} = 0.85, RPD = 3.19) and are therefore of potential utility for feed evaluation (Williams and Sobering, 1996). Moreover, this model performed substantially better than models based on NIRS data (R^2_{CV} between 0.60 and 0.70) for in situ (Andres et al., 2005b; Ohlsson et al., 2007) and in vitro NDF degradabilities (Mentink et al., 2006; Liu et al., 2008) respectively.

Fraction NDF_B in the feeds was poorly predicted, however, and differences between the R^2_{CV} and R^2_P indicate that not all of the variability in the feeds may have been present in the calibration set. The fine feed grinding used (1.5 mm) could explain the high variability and potential overestimation of fraction NDF_B observed in this experiment. In contrast, NDF_C was predicted with a fair accuracy (R^2_{CV} = 0.69, RPD = 2.52) when 0-h values were included in the degradation parameter estimation. Models based on NIRS developed to predict NDF_C in different herbages samples have reported similar accuracy to ours (R^2_{CV} = 0.66; Andres et al., 2005b), whereas NIRS models developed just for grass samples were less successful (R^2_{CV} = 0.34; Ohlsson et al., 2007). Moreover, our predictions improved substantially when data from the initial incubation time were excluded from the model ($NDF_{C\text{-}UND}$; R^2_{CV} = 0.68, RPD = 3.05). This can be explained by the low degree of precision of the in situ method at early incubation times because of the poor exchange of soluble particles (Andres et al., 2005b), as well as the other sources of error discussed previously.

Finally, iNDF, which was calculated after 288 h of incubation, was predicted with similar accuracy to NDF_C (R^2_{CV} = 0.73, RPD = 2.71). This finding is consistent with previous observations (Mentink et al.,

Table 5. Neutral detergent fiber content and in situ degradability for the different type of feeds (in % of DM, unless otherwise stated)[1]

Item	NDF			NDF_{ED}			NDF_B		NDF_C (%/h)		$NDF_{C\text{-}UND}$ (%/h)		iNDF		
	No.	Range	Mean ± SD	No.[2]	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	No.	Range	Mean ± SD
Barley-wheat forage	26	37–82	45 ± 11	26	32–65	42 ± 7	57–93	74 ± 9	1.4–5.1	2.8 ± 1.0	1.1–5.1	2.5 ± 1.0	6	8–43	25 ± 13
Maize forage	15	38–52	43 ± 4	3	34–39	38 ± 3	95–100	98 ± 3	1.1–1.4	1.2 ± 0.2	1.0–1.4	1.2 ± 0.2	42	17–31	23 ± 3
Legume forage	13	30–74	41 ± 12	13	26–62	40 ± 10	43–74	55 ± 9	3.1–10	5.4 ± 1.9	3.1–10.2	5 ± 1.9	12	35–59	45 ± 7
Grass-clover forage	55	23–83	41 ± 12	37	23–79	59 ± 14	59–95	82 ± 10	0.9–13	6.2 ± 2.9	0.9–12.8	6 ± 2.9	35	5–44	19 ± 10
Soybean hulls	2	69–72	71 ± 2	2	58–60	59 ± 1	99–100	100 ± 0.4	2.8–3.0	2.9 ± 0.1	2.8–3.0	2.9 ± 0.1	2	2.6–2.9	2.7 ± 0.2
Beet byproducts	2	21–22	21 ± 0.5	2	75–77	76 ± 1	93–95	94 ± 1	8.3–8.7	8.5 ± 0.2	8.3–8.7	8.5 ± 0.2	2	10–11	11 ± 0
Partial least squares sample sets															
Calibration set	91	23–83	43 ± 12	65	23–79	51 ± 15	43–100	76 ± 14	0.9–13	4.9 ± 2.9	0.9–13	4.7 ± 2.9	79	2.9–59	24 ± 11
Validation set	20	23–82	41 ± 10	17	24–77	48 ± 10	45–100	77 ± 14	1.1–11	4.9 ± 1.8	1.1–11	4.6 ± 1.7	20	2.6–59	24 ± 12

[1]Rumen NDF degradability was determined in situ considering a rumen fractional passage rate of 2%/h. NDF_{ED} = NDF effective degradability; NDF_B = insoluble degradable fraction in the rumen; NDF_C = fractional rate of degradation of fraction NDF_B; iNDF = indigestible NDF; $NDF_{C\text{-}UND}$ was calculated omitting 0-h incubations and iNDF after 288 h of incubation.

[2]Number of observations for NDF_{ED}, NDF_B, NDF_C, and $NDF_{C\text{-}UND}$.

2006), underlying the accuracy of infrared spectroscopy to measure recalcitrant compounds from the cell wall such as lignin (Allison et al., 2009b).

Therefore, this study has demonstrated that PLS models based on FTIR spectra offer not only a quick and inexpensive procedure, but one that is sufficiently accurate to quantify the concentration of NDF and NDF_{ED} in FOR samples and to allow screening according to their iNDF concentration and NDF_C. These findings suggest that this technology could provide substantial improvements in feed evaluation that is applicable to on-farm conditions.

Analysis of VIP Scores

Nonstructural carbohydrates (e.g., simple sugars, starch, and fructans) are quickly degraded in the rumen; structural carbohydrates (e.g., cellulose, hemicelluloses and pectin) are degraded more slowly, whereas lignin degradation in the rumen is negligible. Therefore, an underlying hypothesis of this study was that rumen DM and NDF degradation patterns were determined by the proportions of different types of carbohydrate. The IR absorption spectra of even pure compounds often have more peaks than would be expected from the number of fundamental vibrations, as additional peaks often arise due to the combination of fundamental vibrations or harmonic overcomes (Allison et al., 2009b). Structural interpretation of spectra from complex samples, such as different feed types, is therefore particularly difficult. However, analysis of model VIP scores gives a good indication of the most relevant wavenumbers and may indicate which types of components are paramount to model function.

Our findings (Table 7) indicated that FTIR models that described the feed DM and NDF degradabilities in the rumen had prominent VIP scores that corresponded with plant carbohydrate absorption regions (Socrates, 1994; Stewart, 1994; Lammers et al., 2009). Most of these wavenumbers were placed within the fingerprint region (600–1450 cm^{-1}) and, although the complexity of infrared spectra in this region makes it difficult to assign the absorption, these vibrations are likely associated with C–O, C–O–C, and COOH stretching. More specifically, most of our models showed prominent VIP scores (above 30 units) between 976 and 1086 cm^{-1}, which corresponds with the IR absorbance by sugars, starch, cellulose, lignin, cutin, suberin, fatty acids, and aldehydes. In addition, several important vibrations were observed between the spectrum regions of 1450 to 4000 cm^{-1}, which is due to the stretching vibrations of diatomic units. Within this latter region, vibrations compatible with lignin, pectins, tannins, and polyconjugated and

Table 6. Partial least squares models developed using Fourier-transform infrared (FTIR) spectroscopy to predict rumen NDF degradation parameters for forages (in % of DM, unless otherwise stated)[1]

			Calibration (n = 91)		Cross-validation		Prediction (n = 20)			
Item[2]	SG	LV	R^2_C	RMSEC	R^2_{CV}	RMSECV	R^2_P	RMSEP	RPD	RER
NDF	2	6	0.91	3.52	0.79	5.29	0.83	4.41	2.66	14.0
NDF_{ED}	2	7	0.96	3.15	0.85	5.91	0.84	4.59	3.19	13.0
NDF_B	2	6	0.86	5.43	0.50	10.42	0.33	11.98	1.26	4.83
NDF_C (%/h)	2	8	0.96	0.51	0.69	1.47	0.67	1.11	2.52	11.0
$NDF_{C\text{-}UND}$ (%/h)	2	7	0.93	0.69	0.68	1.51	0.77	0.94	3.05	13.4
iNDF	2	7	0.85	4.33	0.73	8.67	0.83	4.39	2.71	13.1

[1]R^2_C, R^2_{CV}, and R^2_P = determination coefficient of calibration, cross-validation, and prediction, respectively, and RMSEC, RMSECV, and RMSEP = their respective root mean square errors; RPD = ratio of performance to deviation; RER = range in reference ratio (ratio of the SD of the original data to RMSEP).

[2]Rumen NDF degradability was determined in situ considering a rumen fractional passage rate of 2%/h. NDF_{ED} = NDF effective degradability; NDF_B = insoluble degradable fraction in the rumen; NDF_C = fractional rate of degradation of fraction NDF_B; iNDF = indigestible NDF; $NDF_{C\text{-}UND}$ was calculated omitting 0-h incubations and iNDF after 288 h of incubation. Details are given of the Savitsky-Golay (SG) derivative and the number of latent variables (LV) used in the model.

aliphatic compounds were observed (Socrates, 1994; Stewart, 1994; Lammers et al., 2009).

More specifically, the PLS model constructed to quantify DM_A relied heavily on absorbance at 978 cm^{-1} (VIP = 49), which is compatible with pyranose ring vibration (Lammers et al., 2009). This functional group is present in simple sugars, such as glucose, galactose, and mannose, which are immediately solubilized in the rumen. The models for DM_B, however, mainly relied on absorbance at 989 cm^{-1} (VIP = 43), which is compatible with vibrations originated by starch (C-O stretching). Nevertheless, this latter parameter also showed 5 prominent VIP scores in the range 1050–1150 cm^{-1}, which is compatible with vibrations originating from cellulose (C–O stretching), lignin (C–O–C ether vibration), and tannins (C–O stretching). These findings agree with the lower rumen degradation rate observed in cellulosic and lignified forages and in feeds with a high tannin content (Getachew et al., 2000). In addition, pectins are polysaccharides that can make up a substantial proportion of the plant cell wall. Although pectins are not part of the NDF fraction due to their high degradability, hydrated pectin gels might decrease DM degradation within the polyester bag. This would be likely to result in a methodological artifact and might explain the high relevance of pectin-compatible wavenumbers (1605–1630 cm^{-1}) in models describing rumen DM degradation.

The concentration of NDF and the NDF_{ED} in forage samples were also predicted with high accuracy by our PLS models. The VIP scores for the NDF model showed 12 wavenumbers with VIP scores >5, indicating that NDF is a complex fraction comprising several different components, in particular, cellulose, hemicellulose and lignin, although small quantities of fiber-bound proteins, tannins, and certain types of waxes, such as cutin and suberin, are also represented (Van Soest et al., 1991). Our analysis detected high VIP scores between 900 and 1200 cm^{-1}, which is consistent with the vibrations of these cell wall components (Socrates, 1994). Specifically, the highest VIP score was observed at 1032 cm^{-1}, which is compatible with the cellulose vibration (C–O stretching; Stewart, 1994), suggesting that NDF concentration in the forages used in this study is mainly determined by the quantity of cellulose present in the sample.

Models developed to predict NDF rumen degradation parameters showed large numbers of major VIP scores (19, 16, and 20 major VIP scores for the NDF_{ED}, NDF_C, and iNDF models, respectively), indicating that these models rely on many vibrational features and, consequently, plant compounds. In particular, vibrational frequencies 990 and 1064 cm^{-1} had the greatest importance in both NDF_{ED} and NDF_C models (VIP >24), indicating that slowly degraded carbohydrates (cellulose, hemicellulose, pectin) and poorly digestible substrates (lignin, tannin, and waxes) may have special relevance (Lammers et al., 2009). Finally, 20 peaks with VIP scores >5 were detected in the iNDF model, indicating that this fraction is composed of a complex mixture of several poorly degradable compounds, such as lignin, lignin derivatives, cellulose, and alkanes (Lammers et al., 2009).

In addition, several VIP scores that cannot be associated with vibrational features derived from known compounds were detected in most of our PLS models. The heterogeneity of the sample set leads to the presence of VIP scores that are collinear with feed degradability by virtue of them being in some feed components and absent or less abundant in others. These VIP scores are poor indicators of feed degradability per se but serve to enhance the models and make them more accurate.

Table 7. Variable importance in projection (VIP) scores of different partial least squares models showing the most important wavenumbers for the prediction of rumen DM and NDF degradabilities[1] using Fourier-transform infrared (FTIR) spectroscopy

Functional group (compound)[2]			Wave (cm^{-1})	Universal		PRC	Forages							
				DM_A	DM_B	DM_B	DM_{ED}	DM_A	DM_B	NDF	NDF_{ED}	NDF_C	$NDF_{C\text{-}UND}$	iNDF
			608–628		7	9					6			6
			777–779				8				8		6	
			818								*12*			*10*
			868–876				6	6			5			8
C–H deformation (fatty acids and aldhehydes)	Pyranose ring (glucose, galactose and mannose)		897–901				*15*	9		**25**	7	12	*11*	
			914–924				*19*			*11*	*10*	8	7	
			931–941	*13*	9			*15*	5				6	9
			955–957				8		*16*		8	8	*10*	7
			976–978	**38**	**33**	6		**49**						
			986–991				**25**		**43**	*13*	**25**	**28**	**39**	
			1001–1011	5	6		*10*	*12*	6	*14*	7	9	*10*	7
			1020–1028	**25**	**21**		*19*		8				5	
			1032–1036			5		8		**28**	9	*10*	*10*	*13*
		C–O (starch and cellulose)	1043–1053	*10*	9	7	9	9	*17*					*12*
	C–O–C ether (lignin) and C–O (tannin)		1063–1073	5	7		*11*		**24**	*10*	**24**	**30**	**40**	8
			1078–1086			*10*	6	6	*16*		*16*	*16*	**24**	5
			1096–1101						*10*		5	5	8	
C–O and C–O–C (cutin and suberin)			1109–1113	7		7		8		*10*	*11*			*16*
		COOH and C–O–C (mixed polysaccharides)	1122–1132			6	8		*19*	*11*	6	*12*	*12*	*17*
			1138–1142							*11*		*10*	8	5
	β-1,4 C–O–C (cellulose)		1153–1163				8			*17*	5	7	7	
			1182–1184							8	8		5	9
			1195–1205			6					6			6
C–O and CH deformation (cutin and suberin)			1395–1398							6		6		6
Aromatic and CH vibrations (lignin)			1498–1508			6	6				*15*			*15*
			1580–1587			7	*11*		6					*10*
C=O and COOH (pectins)		C–O (tannins)	1605–1607	9	7	7		*11*						
			1626–1632	6		**24**	7							5
C=O and COOH (lignin) and C=C (polyconjugated)			1697–1707			6								*11*
			2851				5		7					
C–H (aliphatic compounds)		CH_2 def. (tannins)[3]	2920–2937			5	7		8					

[1]Only models with R^2 >0.7 and VIP >5 are shown. Scores are formatted according to the VIP value; bold values have the highest VIP; italic values are intermediate, and values in roman (normal) font are lowest. DM_{ED} = DM effective degradability; DM_A = immediately degradable soluble fraction in the rumen; DM_B = insoluble degradable fraction in the rumen; NDF_{ED} = NDF effective degradability; NDF_C and $NDF_{C\text{-}UND}$ = fractional rate of degradation considering and omitting the 0-h degradation values, respectively; iNDF = indigestible NDF; PRC = protein-rich concentrates.

[2]Vibration frequencies of plant carbohydrates described by Lammers et al. (2009). All vibrational features of functional groups are stretching, unless stated.

[3]CH_2 deformation (tannins).

These findings provided evidence that the DM and NDF degradation pattern is mostly determined by the amount of the main plant carbohydrates but also certain minority compounds. Compositional differences are therefore the key factor determining feed nutritional value.

CONCLUSIONS

In situ DM degradability cannot be predicted accurately using a universal PLS model or specific PLS models for concentrates. However, PLS models developed to evaluate forages predicted most in situ degradability parameters (i.e., DM_{ED}, DM_A, and DM_B) accurately. Moreover, NDF concentration and in situ NDF degradation data were also predicted accurately for forages. Our models achieved accuracies similar to those reported for NIRS, and FTIR spectroscopy should therefore be considered as an alternative analytical approach for use in feed evaluation. Further research is needed to determine whether the limitations observed in this study are a measure of methodological limitations or are due to inaccuracies of the in situ method.

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REFERENCES

AFRC (Agricultural and Food Research Council). 1993. Agricultural and Food Research Council: Energy and protein requirements of ruminants. An advisory manual prepared by the ARC Technical Committee on response to nutrients. CAB International, Wallingford, UK.

Åkerlind, M., M. R. Weisbjerg, T. Eriksson, R. Thøgersen, P. Uden, B. L. Olafsson, O. M. Harstad, and H. Volden. 2011. Feed analysis and digestion methods. Pages 41–54 in Norfor—The Nordic Feed Evaluation System. EAAP publication. Vol. 130. H. Volden, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.

Allison, G. G., C. Morris, E. Hodgson, J. Jones, M. Kubacki, T. Barraclough, N. Yates, I. Shield, A. V. Bridgwater, and I. S. Donnison. 2009a. Measurement of key compositional parameters in two species of energy grass by Fourier transform infrared spectroscopy. Bioresour. Technol. 100:6428–6433.

Allison, G. G., S. C. Thain, P. Morris, C. Morris, S. Hawkins, B. Hauck, T. Barraclough, N. Yates, I. Shield, A. V. Bridgwater, and L. S. Donnison. 2009b. Quantification of hydroxycinnamic acids and lignin in perennial forage and energy grasses by Fourier-transform infrared spectroscopy and partial least squares regression. Bioresour. Technol. 100:1252–1261.

Andres, S., A. Calleja, S. Lopez, A. R. Mantecon, and F. J. Giraldez. 2005a. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 2. Prediction of crude protein and dry matter degradability. J. Sci. Food Agric. 85:1572–1579.

Andres, S., F. J. Giraldez, J. S. Gonzalez, R. Pelaez, N. Prieto, and A. Calleja. 2005b. Prediction of aspects of neutral detergent fibre digestion of forages by chemical composition and near infrared reflectance spectroscopy. Aust. J. Agric. Res. 56:187–193.

Andres, S., F. J. Giraldez, S. Lopez, A. R. Mantecon, and A. Calleja. 2005c. Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 1. Prediction of chemical composition and in vitro digestibility. J. Sci. Food Agric. 85:1564–1571.

Andueza, D., F. Picard, M. Jestin, J. Andrieu, and R. Baumont. 2011. NIRS prediction of the feed value of temperate forages: Efficacy of four calibration strategies. Animal 5:1002–1013.

Belanche, A., M. R. Weisbjerg, G. G. Allison, C. J. Newbold, and J. M. Moorby. 2013. Estimation of feed crude protein concentration and rumen degradability by Fourier-transform infrared spectroscopy. J. Dairy Sci. 96:7867–7880.

Casper, D. P., H. A. Maiga, M. J. Brouk, and D. J. Schingoethe. 1999. Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J. Dairy Sci. 82:1779–1790.

Chaudhry, A. S., and R. A. I. Mohamed. 2011. Using fistulated sheep to compare in sacco and in vitro rumen degradation of selected feeds. Anim. Prod. Sci. 51:1015–1024.

Deaville, E. R., E. Owen, A. T. Adesogan, C. Rymer, J. A. Huntington, and T. L. J. Lawrence. 1997. In Vitro Techniques for Measuring Nutrient Supply to Ruminants. Occas. Pub. No. 22, Br. Soc. Anim. Sci. Proc., University of Reading, UK.

Getachew, G., H. P. Makkar, and K. Becker. 2000. Effect of polyethylene glycol on in vitro degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. Br. J. Nutr. 84:73–83.

Gosselink, J. M. J., J. P. Dulphy, C. Poncet, S. Tamminga, and J. W. Cone. 2004. A comparison of in situ and in vitro methods to estimate in vivo fermentable organic matter of forages in ruminants. Wageningen J. Life Sci. 52:29–45.

Griffiths, P. R. 1983. Fourier-transform infrared spectrometry. Science 222:297–302.

He, Y., X. Li, and X. Deng. 2007. Discrimination of varieties of tea using near infrared spectroscopy by principal component analysis and BP model. J. Food Eng. 79:1238–1242.

Hoffman, P. C., N. M. Brehm, L. M. Bauman, J. B. Peters, and D. J. Undersander. 1999. Prediction of laboratory and *in situ* protein fractions in legume and grass silages using near-infrared reflectance spectroscopy. J. Dairy Sci. 82:764–770.

Huang, A., Q. Zhou, J. Liu, B. Fei, and S. Sun. 2008. Distinction of three wood species by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. J. Mol. Struct. 883:160–166.

Hvelplund, T., J. Madsen, and M. R. Weisbjerg. 2003. Protein evaluation. Proteinvurdering. Pages 583–602. Vol. 53. DJF Rapport, Husdyrbrug, Danmarks JordbrugsForskning, Foulum, Denmark.

Hvelplund, T., and M. R. Weisbjerg. 2000. In situ techniques for the estimation of protein degradability and postrumen availability. Pages 233–258 in Forage Evaluation in Ruminant Nutrition. D. I. Givens, E. Owen, R. F. E. Axford, and H. M. Omed, ed. CAB International, Wallingford, UK.

Krämer, M., P. Norgaard, P. Lund, and M. R. Weisbjerg. 2013. Particle size alterations of feedstuffs during *in situ* neutral detergent fiber incubation. J. Dairy Sci. 96:4601–4614.

Lammers, K., G. Arbuckle-Keil, and J. Dighton. 2009. FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning. Soil Biol. Biochem. 41:340–347.

Liu, X., L. Han, Z. Yang, and C. Xu. 2008. Prediction of silage digestibility by near infrared reflectance spectroscopy. J. Anim. Feed Sci. 17:631–639.

López, S., J. Dijkstra, and J. France. 2000. Prediction of energy supply in ruminants, with emphasis on forages. Pages 63–94 in Forage Evaluation in Ruminant Nutrition. D. I. Givens, E. Owen, R. F. E. Axford, and H. M. Omed, ed. CAB International, Wallingford, UK.

Madsen, J., T. Hvelplund, M. R. Weisbjerg, J. Bertilsson, I. Olsson, R. Sprndly, O. M. Harstad, H. Volden, M. Tuori, T. Varvikko, P. Huhtanen, and B. L. Olafsson. 1995. The AAT/PBV protein

evaluating system for ruminants. A revision. Nor. J. Agric. Sci. 19(Suppl.):1–37.
Mathison, G. W., H. Hsu, R. Soofi-Siawash, G. Recinos-Diaz, E. K. Okine, J. Helm, and P. Juskiw. 1999. Prediction of composition and ruminal degradability characteristics of barley straw by near infrared reflectance spectroscopy. Can. J. Anim. Sci. 79:519–523.
Mentink, R. L., P. C. Hoffman, and L. M. Bauman. 2006. Utility of near-infrared reflectance spectroscopy to predict nutrient composition and in vitro digestibility of total mixed rations. J. Dairy Sci. 89:2320–2326.
Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds using refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85:1217–1240.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academic Press, Washington, DC.
Ohlsson, C., L. P. Houmoller, M. R. Weisbjerg, P. Lund, and T. Hvelplund. 2007. Effective rumen degradation of dry matter, crude protein and neutral detergent fibre in forage determined by near infrared reflectance spectroscopy. J. Anim. Physiol. Anim. Nutr. (Berl.) 91:498–507.
Ørskov, E. R., and I. McDonald. 1979. Estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499–503.
Schmidt, K. S., and A. K. Skidmore. 2001. Exploring spectral discrimination of grass species in African rangelands. Int. J. Remote Sens. 22:3421–3434.
Socrates, G. 1994. Infrared Characteristic Group Frequencies. 2nd ed. John Wiley & Sons, Chichester, UK.
Stewart, D. 1994. Fourier-transform infrared microspectroscopy of plant tissues. Appl. Spectrosc. 50:357–365.
Van Duinkerken, G., M. C. Blok, A. Bannink, J. W. Cone, J. Dijkstra, A. M. Van Vuuren, and S. Tamminga. 2011. Update of the Dutch protein evaluation system for ruminants: The DVE/OEB (2010) system. J. Agric. Sci. 149:351–367.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.
Vandevoort, F. R. 1992. Fourier transform infrared spectroscopy applied to food analysis. Food Res. Int. 25:397–403.
Verite, R., and J. L. Peyraud. 1989. Protein: The PDI system. Pages 33–48 in in Ruminant Nutrition: Recommended Allowances and Feed Tables. R. Jarrige, ed. John Libbey Eurotext, Paris, France.
Volden, H. 2011. NorFor—The Nordic Feed Evaluation System. In EAAP Publication. Vol. 130. Wageningen Academic Publishers, Wageningen, the Netherlands.
Williams, P. C., and D. C. Sobering. 1996. How we do it: A brief summary of the methods we use in developing near infrared calibrations. Pages 185–188 in Near Infrared Spectroscopy: The Future Waves. A. M. C. Davies and P. C. Williams, ed. NIR Publications, Chichester, UK.