animal

Prediction of rumen degradability parameters of a wide range of forages and non-forages by NIRS

A. Foskolos[1], S. Calsamiglia[1], M. Chrenková[2], M. R. Weisbjerg[3] and E. Albanell[4†]

[1]*Animal Nutrition, Management and Welfare Research Group, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain;* [2]*National Agricultural and Food Centre, Research Institute for Animal Production, Nitra, 951 41 Lužianky, Slovakia;* [3]*Department of Animal Science, AU Foulum, Aarhus University, 8830 Tjele, Denmark;* [4]*Group of Ruminant Research, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*

Kinetics of nutrient degradation in the rumen is an important component of feed evaluation systems for ruminants. The in situ *technique is commonly used to obtain such dynamic parameters, but it requires cannulated animals and incubations last several days limiting its application in practice. On the other hand, feed industry relies strongly on NIRS to predict chemical composition of feeds and it has been used to predict nutrient degradability parameters. However, most of these studies were feedstuff specific, predicting degradability parameters of a particular feedstuff or category of feedstuffs, mainly forages or compound feeds and not grains and byproducts. Our objective was to evaluate the potential of NIRS to predict degradability parameters and effective degradation utilizing a wide range of feedstuffs commonly used in ruminant nutrition. A database of 809 feedstuffs was created. Feedstuffs were grouped as forages (FF;* n *= 256), non-forages (NF;* n *= 539) and of animal origin (*n *= 14).* In situ *degradability data for dry matter (DM;* n *= 665), CP (*n *= 682) and NDF (*n *= 100) were collected. Degradability was described in terms of washable fraction (*a*), slowly degradable fraction (*b*) and its rate of degradation (*c*). All samples were scanned from 1100 to 2500 nm using an NIRSystems 5000 scanning in reflectance mode. Calibrations were developed for all samples (ALL), FF and NF. Equations were validated with an external validation set of 20% of total samples. NIRS equations to predict the effective degradability and fractions* a *and* b *of DM, CP and NDF could be evaluated from being adequate for screening (*$r^2 > 0.77$*; ratio of performance to deviation (RPD) = 2.0 to 2.9) to suitable for quantitative purposes (*$r^2 > 0.84$*; RPD = 3.1 to 4.7), and some predictions were improved by group separation reducing the standard error of prediction. Similarly, the rate of degradation of CP (*CP_c*) and DM (*DM_c*) was predicted for screening purposes (RPD ⩾2 and 2.5 for* CP_c *and* DM_c*, respectively). However, the rate of degradation of NDF was not predicted accurately (*NDF_c*:* $r^2 < 0.75$*; RDP < 2).*

Implications

NIRS is an alternative technology used widely to assess feedstuff chemical composition, but most feed evaluation systems also need values to model kinetics of nutrient degradation. For the current study, a large set of samples of feedstuffs commonly used in ruminant nutrition was used and it became possible to predict degradability parameters of dry matter and CP. However, for some degradability parameters of NDF our data set was not sufficiently large to obtain robust predictions.

Introduction

Kinetics of nutrient degradation in the reticulo-rumen is one major determinant of feedstuffs evaluation for ruminants.

Most current feed evaluation systems are based on the kinetics of nutrient degradation (e.g. Cornell Net Carbohydrate and Protein System (Sniffen *et al.*, 1992); Molly (Baldwin, 1995); Dairy NRC (National Research Council, 2001); NorFor (Volden, 2011)), which usually are obtained with the *in situ* method. The *in situ* is a well-established technique for the determination of the degradation kinetic parameters and the effective degradation (ED) of nutrients (Huhtanen *et al.*, 2006). However, it is an expensive and tedious method because it requires rumen-cannulated animals and rumen incubations may last several days. These factors limit its use for routine evaluation of feeds by the feed industry and in practice tabulated values are used to estimate ruminal degradability of nutrients and to formulate diets for ruminants. A considerable variability has been documented around these tabulated values (von Keyserlingk *et al.*, 1996;

† E-mail: Elena.Albanell@uab.cat

Hvelplund and Weisbjerg, 2000) that may lead to imbalances in diet formulation at farm level.

Several biological and chemical methods have been tested as alternatives. López *et al.* (1998) compared the *in situ* method with the gas production technique and enzymatic assays using hay samples and reported considerable differences depending on the botanical composition of hay. Moreover, Huhtanen *et al.* (2008) utilized the *in vitro* gas production technique to estimate degradability parameters of NDF in forages and reported accurate estimation of the intrinsic degradation rate, although incubations with rumen fluid for up to 60 h are still needed. On the other hand, feed industry relies strongly on IR reflectance spectroscopy as an alternative to wet chemistry (Roberts *et al.*, 2004; Workman and Shenk, 2004). IR reflectance spectroscopy is a fast, reliable and cost-effective way to predict feedstuffs' chemical composition (Andrés *et al.*, 2005a) and it is used in combination with mathematical models to formulate diets for cattle (Roberts *et al.*, 2004). Recently, Belanche *et al.* (2013 and 2014) used Fourier transformation mid-IR spectroscopy (FTIR) to estimate degradability parameters of CP, dry matter (DM) and NDF, and results suggested that mid-IR can be used for screening purposes but not for estimating dynamic parameters of degradability. Similarly, several efforts using NIRS to predict rumen degradation parameters have been reported (Todorov *et al.*, 1994; De Boever *et al.*, 2003; Andrés *et al.*, 2005b; Ohlsson *et al.*, 2007). However, most of these studies are feedstuff specific, predicting degradation parameters of a particular feedstuff or a specific category of feedstuffs, mainly forages or compound feeds and not byproducts or grains.

Our objective was to evaluate the potential of the NIRS technique to predict degradability parameters and ED of feed samples utilizing a wide range of feedstuffs commonly used in ruminant nutrition.

Material and methods

Database

A database of 809 feedstuffs frequently used in ruminant nutrition were collected over the course of a 10-year period at AU Foulum (Aarhus University, Denmark) and used in this study. All samples were freeze-dried, ground to pass through a 1.5-mm diameter sieve and stored in airtight containers at −20°C until further analysis. Feedstuffs were classified, according to their use in ruminant nutrition, as forages (FF; $n = 256$), non-forages (NF; $n = 539$) and of animal origin ($n = 14$). Table 1 shows the wide variety of feedstuffs. The FF group included hays, straws, whole crops, grass pellets and silages. The NF group included concentrates ($n = 234$) and byproducts ($n = 305$). Concentrates included different mixed feeds, seeds and grains. Byproducts included those of distillery and oil production. Moreover, samples of animal origin ($n = 14$) were included for calibrations with all feedstuff (ALL).

Not all feeds in the database were analyzed for each nutrient component. In total, 665 feedstuffs were analyzed

for DM degradability parameters and 682 for CP degradability parameters. The reported DM and CP values do not necessarily relate to the same feedstuff. For NDF, a total of 100 samples were available and the majority of feedstuffs were grouped as FF. Thus, for NDF predictions separation in groups included only FF. The exact number of feedstuffs per group and nutrient is presented in Tables 2 and 3.

In situ *analyses*

In situ analyses were performed according to the NorFor procedure (Madsen *et al.*, 1995; Åkerlind *et al.*, 2011). Feed samples were incubated in the rumen of three dry Holstein–Friesian cows in 11×8.5 cm (10×7.5 effective size) Dacron bags with 38 µm pore size (PES material 38/31 with 31% open bag area, Saatifil PES 38/31; Saatitech S.p.A., Veniano, Como, Italy). Cows used for incubations were fed at maintenance level a ration containing (kg/day) 2.0 spring barley straw, 4.0 artificially dried grass hay, 0.15 vitamin-mix and 2.8 concentrate (concentrate composition (g/kg): 400 barley, 400 oats, 100 soybean meal, 30 rapeseed meal, 30 beet molasses and 40 mineral mixture). Chemical composition of ration was (g/kg DM) 139 CP, 465 NDF and 137 starch. Feed samples were ground to pass a 1.5-mm screen and 1 g was weighed into each bag. Bags were mounted with plastic strips on rubber stoppers. The rubber stoppers (with hooks) were mounted on a plastic tube fitted with rings. The plastic tube had a sink (weight 200 g) in one end and strings with a length of 40 cm at both ends to ensure its mobility in relation to the rumen cannula. Bags were incubated in the rumen for 0, 2, 4, 8, 16, 24, 48, 96, 126 and 168 h. Maximum incubation time for protein degradation was 48 h for concentrates and 96 h for forages; and for NDF degradation was 126 h for concentrates and 168 h for forages. After rumen incubation, bags on the rubber stopper were rinsed with cold tap water and machine-washed (AEG, Fredericia, Denmark) twice for 5 min with 22 l of water at 25°C. Subsequently, bags were frozen at −20°C until analysis. To remove adhering microbes, forage residues were transferred to a plastic bag with 60 ml demineralized water, and treated for 5 min in the stomacher before returned to the Dacron bag and washed thoroughly with demineralized water. Residues in bags for DM and CP were transferred to tarred nitrogen-free filter paper, dried at 103°C to determine the DM residue, then analyzed for nitrogen content using an automated Foss-Kjeldahl apparatus (Fisher Scientific, Pittsburgh, PA, USA) and CP concentration was calculated as $N \times 6.25$ (Association of Official Analytical Chemists, 1990). Residues in bags for NDF were transferred directly to porosity 2 filter crucibles and ash-free NDF residue was determined in a Fibertec system (FOSS, Hillerød, Denmark) using a heat stable amylase and sodium sulfite according to the study by Mertens (2002).

Degradability parameters were fitted using PROC NLIN in SAS (version 9.2; SAS Institute Inc., Cary, NC, USA) according to the model of Ørskov and McDonald (1979). ED of DM, CP and NDF (DM_{ED}, CP_{ED} and NDF_{ED}, respectively) was

Table 1 *Feedstuffs (ALL) classified in the database as forages (FF) and non-forages (NF) for the prediction of ruminal degradability parameters*

FF	n	NF	n	NF	n
Forages	205	Byproducts	305	Concentrates	234
Grass clover	36	Rapeseed byproduct	107	Mix feeds	123
Maize whole crop	34	Soybean byproduct	39	Barley grain	29
Grass	20	Corn distillers	28	Total mixed ration	19
Lucerne	17	Sunflower byproduct	24	Wheat grain	12
Barley whole crop	14	Soybean hulls	16	Peas	11
Lupin whole crop	12	Maize gluten meal	14	Soybeans	10
Red clover	12	Cottonseed byproduct	13	Rye grain	8
Wheat whole crop	10	Dry sugar–beet pulp	13	Maize grain	6
Tropical forages	9	Treated soybean meal	8	Triticale grain	5
White clover	8	Guarmeal	7	Oat grain	4
Grass hay	7	Fodder beet roots	6	Grain mix	2
Grass pellets	6	Wheat distillers	5	Lupin grain	2
Peas whole crop	5	Wheat gluten feed	5	Rapeseed	2
Beans whole crop	4	Malt sprouts	4	Field beans	1
Galega	4	Brewers grains	3		
Barley straw	2	Citrus pulp	2		
Ryegrass straw	2	Potato protein	2		
Festulolium	1	Wheat bran	2		
Peas straw	1	Coconut cake	1		
Red fescue straw	1	Elipe cake	1		
		Grain distillers	1		
Silages	51	Malt dust	1	Animal origin	14
Grass clover	17	Palm kernel cake	1	Hair meal	10
Maize	8	Simsim cake	1	Fishmeal	3
Winter wheat	8	Wheat–barley distillers	1	Feather meal	1
Ryegrass	7				
Barley whole crop	4				
Peas whole crop	4				
Maize pulp mix	2				
Pea lucerne	1				
Total FF	256	Total NF	539	Total ALL	809

Table 2 *Population statistics of calibration and validation data set of all feedstuffs (ALL)*

Degradability Parameters	Calibration set					Validation set				
	N	Min	Max	Mean	CV	n	Min	Max	Mean	CV
DM_a	554	0.01	0.81	0.39	35.4	111	0.01	0.72	0.37	34.6
DM_b	554	0.06	1.00	0.51	30.0	111	0.13	0.99	0.54	27.2
DM_c	554	0.001	0.377	0.072	76.4	111	0.023	0.298	0.069	65.8
DM_{ED}	554	0.11	0.93	0.66	18.8	111	0.15	0.90	0.66	16.2
DM_{PD}	554	0.17	1.00	0.90	11.9	111	0.21	1.00	0.91	9.9
CP (% DM)	569	5.6	94.1	27.2	57.1	115	7.8	90.0	27.5	53.2
CP_a	569	0.02	0.92	0.39	51.5	113	0.01	0.90	0.39	50.3
CP_b	569	0.04	0.98	0.56	38.9	113	0.05	0.99	0.57	37.2
CP_c	568	0.004	0.372	0.073	67.5	113	0.011	0.303	0.068	62.5
CP_{ED}	569	0.12	0.95	0.69	22.0	113	0.13	0.93	0.69	23.2
CP_{PD}	569	0.21	1.00	0.95	10.4	113	0.19	1.00	0.96	9.2
NDF (% DM)	84	17.5	83.1	41.1	31.4	16	23.3	81.5	42.7	29.9
NDF_b	84	0.43	1.00	0.76	19.2	16	0.44	1.00	0.78	22.2
NDF_c	84	0.009	0.417	0.055	87.5	16	0.015	0.128	0.050	63.4
NDF_{ED}	84	0.23	0.78	0.51	27.3	16	0.28	0.82	0.52	31.2

N = number of samples for calibration; n = number of samples for validation; min = minimum value of the data set; max = maximum value of the data set; mean = the mean of the data set; CV (%) = coefficient of variation (s.d./mean × 100); DM_a and CP_a = washable fraction of dry matter (DM) and CP; DM_b, CP_b and NDF_b = slowly degradable fraction of DM, CP and NDF; DM_c, CP_c and NDF_c = degradation rate of DM_b, CP_b and NDF_b; DM_{ED}, CP_{ED} and NDF_{ED} = effective degradation of DM, CP and NDF; DM_{PD}, CP_{PD} and NDF_{PD} = potentially degradable DM, CP and NDF ($a + b$).

Table 3 *Population statistics of calibration and validation data set of feedstuffs classified as forages (FF) and non-forages (NF)*

Degradability Parameters	Calibration set					Validation set				
	N	Min	Max	Mean	CV	n	Min	Max	Mean	CV
FF										
DM_a	112	0.12	0.62	0.41	24.4	23	0.28	0.72	0.42	24.0
DM_b	112	0.15	0.69	0.47	23.4	23	0.20	0.60	0.46	21.5
DM_c	112	0.012	0.150	0.056	45.0	23	0.022	0.140	0.069	41.7
DM_{ED}	112	0.33	0.82	0.64	15.3	23	0.50	0.83	0.66	13.0
DM_{PD}	112	0.59	0.98	0.88	8.5	23	0.70	0.99	0.88	9.4
CP (% DM)	120	6.6	30.6	17.0	31.8	23	9.2	27.6	17.0	29.6
CP_a	120	0.17	0.92	0.55	37.5	23	0.23	0.90	0.58	37.2
CP_b	120	0.04	0.74	0.40	54.5	23	0.05	0.76	0.36	61.4
CP_c	120	0.019	0.372	0.082	70.0	23	0.030	0.166	0.076	45.0
CP_{ED}	120	0.46	0.95	0.78	12.3	23	0.60	0.93	0.80	11.1
CP_{PD}	120	0.79	1.00	0.94	4.1	23	0.88	0.99	0.94	3.5
NDF (% DM)	67	22.9	83.1	41.9	29.4	13	23.3	81.5	44.0	31.0
NDF_b	67	0.43	1.00	0.76	18.0	13	0.44	0.94	0.77	19.9
NDF_c	67	0.009	0.120	0.048	54.0	13	0.015	0.128	0.049	70.0
NDF_{ED}	67	0.23	0.78	0.50	28.2	13	0.28	0.79	0.50	30.4
NF										
DM_a	427	0.01	0.81	0.39	35.9	89	0.01	0.67	0.35	36.6
DM_b	427	0.13	1.00	0.53	28.3	89	0.26	0.99	0.56	26.4
DM_c	427	0.021	0.377	0.077	73.8	89	0.022	0.297	0.072	71.4
DM_{ED}	427	0.41	0.93	0.68	14.9	89	0.46	0.90	0.65	15.5
DM_{PD}	427	0.74	1.00	0.92	6.2	89	0.69	1.00	0.92	7.0
CP (% DM)	427	5.6	82.3	28.4	46.5	88	7.8	70.4	29.1	44.3
CP_a	436	0.02	0.82	0.36	48.9	89	0.01	0.80	0.36	46.4
CP_b	436	0.15	0.98	0.61	31.6	89	0.20	0.99	0.62	29.0
CP_c	436	0.009	0.370	0.071	67.1	89	0.010	0.303	0.066	62.9
CP_{ED}	436	0.26	0.94	0.68	19.7	89	0.27	0.93	0.67	22.4
CP_{PD}	436	0.53	1.00	0.97	6.2	89	0.59	1.00	0.97	5.5

N = number of samples for calibration; n = number of samples for validation; min = minimum value of the data set; max = maximum value of the data set; mean = the mean of the data set; CV (%) = coefficient of variation (s.d./mean $\times$ 100); DM_a and CP_a = washable fraction of dry matter (DM) and CP; DM_b, CP_b and NDF_b = slowly degradable fraction of DM, CP and NDF; DM_c, CP_c and NDF_c = degradation rate of DM_b, CP_b and NDF_b; DM_{ED}, CP_{ED} and NDF_{ED} = effective degradation of DM, CP and NDF; DM_{PD}, CP_{PD} and NDF_{PD} = potentially degradable DM, CP and NDF ($a + b$).

calculated according to the following equation:

$$ED = a + b\,(c/(c+k))$$

where a is the washable fraction (DM_a and CP_a for DM and CP, respectively; not included for NDF), b the slowly degradable fraction (DM_b, CP_b and NDF_b for DM, CP and NDF, respectively), c the rate of degradation (DM_c, CP_c and NDF_c for DM, CP and NDF, respectively) and k the fractional outflow rate from the rumen (5%/h for CP and DM; 2%/h for NDF). Then, the potentially degradable (PD) DM and CP (DM_{PD} and CP_{PD}, respectively) was calculated as the sum of fractions a and b. Degradation parameters of NDF did not include fraction a because NDF degradation at 0 h was considered to be zero.

NIRS analyses
All samples were scanned in the same month from 1100 to 2500 nm using an NIRSystems 5000 scanning mono-chromator (FOSS). Reflectance was recorded in 2 nm steps, which gave 692 data points for each sample, as log ($1/R$),

where R represents reflected energy. Samples, ground at 1.5-mm screen, were scanned twice in duplicate (four scans per sample) using closed ring cup cells and the mean spectrum was calculated for each sample.

NIRS calibrations for CP, NDF and degradability parameters of DM, CP and NDF were performed using the WinISI III (version 1.6) software. The modified partial least squares (MPLS) regression method was used for calibration development. Separate MPLS calibrations were performed for each parameter in the calibration set. Multiple scatter correction, standard normal variate (SNV), detrend (D), and SNV and D (SNV-D) algorithms were used to remove or reduce the effects of scatter. In addition, six derivative mathematical treatments were tested in the development of NIRS calibrations: 1, 4, 4, 1; 1, 10, 10, 1; 2, 4, 4, 1; 2, 10, 10, 1; 3, 4, 4, 1 and 3, 10, 10, 1, where the first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing. Hence, in all cases, 24 regression

Table 4 *Calibration and validation statistics for estimation of dry matter (DM) degradability parameters by near-IR analysis on feedstuffs*

| Parameter | Group | Scatter correction | Calibration | | Cross-validation | | Validation | | | |
			R^2	SEC	r_{cv}^2	SECV	r^2	SEP	RPD	RER
DM_a	ALL	D	0.89	0.05	0.84	0.05	0.80	0.06	2.3	12.7
	FF	SNV-D	0.95	0.02	0.91	0.03	0.84	0.03	3.1	13.9
	NF	D	0.92	0.04	0.87	0.05	0.87	0.05	2.7	13.7
DM_b	ALL	D	0.88	0.05	0.84	0.06	0.78	0.07	2.2	12.7
	FF	D	0.96	0.02	0.90	0.03	0.77	0.04	2.4	9.7
	NF	D	0.92	0.04	0.87	0.05	0.84	0.06	2.5	12.4
DM_c	ALL	MSC	0.72	0.016	0.68	0.017	0.64	0.016	2.8	17.2
	FF	MSC	0.86	0.008	0.79	0.010	0.73	0.010	2.5	11.8
	NF	SNV	0.78	0.018	0.73	0.020	0.71	0.019	2.6	14.5
DM_{ED}	ALL	SNV-D	0.94	0.03	0.92	0.04	0.87	0.04	2.9	20.4
	FF	SNV-D	0.94	0.03	0.89	0.03	0.80	0.03	2.5	9.4
	NF	SNV-D	0.90	0.03	0.87	0.04	0.83	0.04	2.5	11.1
DM_{PD}	ALL	SNV-D	0.90	0.03	0.85	0.04	0.90	0.03	3.1	27.1
	FF	SNV-D	0.97	0.01	0.92	0.02	0.84	0.03	3.1	10.9
	NF	SNV-D	0.83	0.02	0.76	0.03	0.66	0.03	2.0	9.6

R^2 = coefficient of determination for calibration; SEC = standard error of calibration; r_{cv}^2 = coefficient of determination for cross-validation; SECV = standard error of cross-validation; r^2 = coefficient of determination for external validation; SEP = standard error of prediction; RPD = ratio of performance to deviation (s.d./SEP); RER = range error ratio (range/SEP); DM_a = washable fraction of DM; DM_b = slowly degradable fraction of DM; DM_c = rate of DM_b degradation; DM_{ED} = effective degradation of DM; DM_{PD} = potentially degradable DM $(a+b)$; ALL = all samples; FF = group of forages; NF = group of non-forages; D = detrend; SNV = standard normal variate; MSC = multiple scatter correction.

equations per parameter were developed by combining six spectral derivative math treatments and four scatter correction methods.

Cross-validation was applied to optimize calibration models, determine the optimal number of terms for the calibration equation and to identify chemical and spectral outliers. In addition to cross-validation, an external validation was performed using a set of 20% of total samples (Tables 2 and 3). Samples in the validation set were selected randomly from the total matrix and were balanced according to the previously mentioned grouping of feedstuffs and year of selection to represent a wide range of composition. Samples in the validation set were not used in the calibration set or vice versa. The optimum calibration model was selected on the basis of minimum standard error of calibration and standard error of prediction (SEP), and of greatest coefficient of determination of calibration (R^2), cross-validation (r_{cv}^2) and validation (r^2). These coefficients were used as indicators of precision. Further, performance of calibrations was evaluated using the ratio of performance to deviation (RPD) described as the ratio of standard deviation for the validation samples to the SEP, and the range error ratio (RER) described as the ratio of the range in the reference data (validation set) to the SEP. With an RPD $\leqslant 1.9$ calibration is considered to be not suitable; values between 2.0 and 2.4 are considered poor and only adequate for rough screening purposes, values between 2.5 and 2.9 are providing a fair prediction that can be used for screening and values $\geqslant 3.0$ (or RER >10) indicate good prediction and can be used for quantitative analysis (Williams, 2014). Equations were obtained using all available feedstuffs (ALL) and the two groups (FF and NF).

Results

Calibration and validation matrices
Table 2 presents the descriptive statistics of the calibration and validation data set for ALL, including the mean, minimum and maximum values of each parameter, the standard deviation, the CV and the total number of samples used. All parameters were well represented in both calibration and validation matrices covering similar ranges. The degradability parameters database included samples where the degradation at time 0 was 80.2% of total DM content and others where the degradation after 48 h was only 16.5%, representing the existing variability in samples. The CP concentration of feedstuffs included in the calibration matrix ranged from 5.6% to 94.1% on DM basis with a CP_{ED} ranging from 12% to 95%. The NDF content ranged from 17.5% to 83.1% on DM basis in the calibration matrix. The *in situ* parameter with the greater CV was the degradation rate, ranging from 67.5% for CP_c to as high as 87.5% for NDF_c of ALL in the calibration data set (Table 2). Compared with ALL, grouping samples in two major groups (FF and NF) reduced variation mainly for FF; however, a wide variation within parameters was still present (Table 3). Similar to ALL, fraction *c* was the parameter with the greatest CV, and was lower for both the calibration and validation set of DM_c for FF compared with NF and ALL.

Degradability parameters of DM
Table 4 presents calibration and validation statistics of the equations used to predict the degradability parameters of DM including ALL, FF and NF groups. The mathematical treatment that fitted best for most degradability parameters

Table 5 *Calibration and validation statistics for estimation of CP degradability parameters by near-IR analysis on feedstuffs*

Parameter	Group	Scatter correction	Calibration		Cross-validation		Validation			
			R^2	SEC	r^2_{cv}	SECV	r^2	SEP	RPD	RER
CP (% DM)	ALL	SNV-D	0.99	0.9	0.99	1.2	0.98	2.1	7.0	39.3
	FF	SNV-D	0.98	0.8	0.97	1.0	0.94	1.3	3.9	14.4
	NF	SNV-D	0.99	0.9	0.99	1.1	0.97	2.1	6.1	29.6
CP_a	ALL	SNV-D	0.89	0.07	0.85	0.08	0.84	0.08	2.5	11.3
	FF	SNV-D	0.97	0.04	0.94	0.05	0.95	0.05	4.4	13.7
	NF	D	0.90	0.05	0.85	0.07	0.80	0.07	2.5	11.6
CP_b	ALL	SNV-D	0.86	0.08	0.82	0.09	0.77	0.10	2.1	9.4
	FF	SNV	0.97	0.04	0.94	0.06	0.96	0.05	4.7	15.1
	NF	MSC	0.88	0.07	0.80	0.08	0.79	0.08	2.2	9.6
CP_c	ALL	SNV	0.59	0.020	0.50	0.022	0.58	0.021	2.1	13.9
	FF	D	0.82	0.018	0.71	0.024	0.82	0.016	2.3	8.8
	NF	SNV-D	0.70	0.018	0.57	0.022	0.52	0.022	2.0	13.2
CP_{ED}	ALL	SNV-D	0.89	0.05	0.84	0.06	0.81	0.06	2.7	13.6
	FF	SNV-D	0.89	0.03	0.84	0.04	0.86	0.03	2.7	10.0
	NF	SNV-D	0.88	0.05	0.82	0.05	0.81	0.05	2.8	12.2
CP_{PD}	ALL	SNV-D	0.89	0.02	0.84	0.03	0.85	0.03	2.8	26.1
	FF	SNV-D	0.94	0.01	0.87	0.01	0.89	0.01	2.8	9.2
	NF	SNV-D	0.48	0.02	0.33	0.02	0.34	0.02	2.5	19.5

R^2 = coefficient of determination for calibration; SEC = standard error of calibration; r^2_{cv} = coefficient of determination for cross-validation; SECV = standard error of cross-validation; r^2 = coefficient of determination for external validation; SEP = standard error of prediction; RPD = ratio of performance to deviation (s.d./SEP); RER = range error ratio (range/SEP); CP (% DM) = CP concentration as % of dry matter (DM); CP_a = washable fraction of CP; CP_b = slowly degradable fraction of CP; CP_c = rate of CP_b degradation; CP_{ED} = effective degradation of CP; CP_{PD} = potentially degradable CP $(a+b)$; ALL = all samples; FF = group of forages; NF = group of non-forages; SNV = standard normal variate; D = detrend; MSC = multiple scatter correction.

and groups of feedstuffs was the 2, 4, 4, 1, whereas the best scatter correction method differed among parameters and groups.

For ALL, the calibrations for DM_a ($r^2 = 0.80$; RPD = 2.3) and DM_b ($r^2 = 0.78$; RPD = 2.2) were good for screening, whereas those for DM_{ED} ($r^2 = 0.87$; RPD = 2.9) and DM_{PD} ($r^2 = 0.90$; RPD = 3.1) can be used for quantitative purposes. Predictions for DM_a and DM_b were improved by separating samples into groups, reducing the SEP (0.06 *v.* 0.03 and 0.05 for DM_a and 0.07 *v.* 0.04 and 0.06 for DM_b of ALL *v.* FF and NF, respectively). However, group separation did not improve predictions for DM_{ED} and DM_{PD}, even though SEP was lower for FF. In addition, RPD of DM_{ED} decreased for both FF and NF, but it remained >2.5. The rate of degradation (DM_c) was predicted for quantitative purposes when all samples were included ($r^2 = 0.64$; RPD = 2.81), whereas separating into groups reduced the SEP for FF (0.010 *v.* 0.016 for FF and ALL, respectively).

Degradability parameters of CP
Table 5 summarizes the statistics of calibration and validation of the equations used to predict the degradability parameters of CP. The concentration of CP was predicted with high accuracy by using SNV-D transformation and second derivative treatment of the spectra ($r^2 = 0.98$; RPD = 7.0). Most of the degradability parameters were best predicted by 2, 4, 4, 1 and like in degradability parameters of DM, there was not a unique scatter correction that gave the best predictions of degradability parameters.

Similar to degradability parameters of DM, NIRS predicted CP_a and CP_b for screening purposes and CP_{ED} for quantitative purposes. Separating in groups provided equations with lower SEP for both FF and NF. The coefficient of determination of validation of CP_c was low when all samples were included ($r^2 = 0.58$), and prediction was improved for FF ($r^2 = 0.82$) but not for NF ($r^2 = 0.52$). Further, RPD was >2 in all cases (2.1, 2.3 and 2.0 for ALL, FF and NF, respectively) and group separation reduced SEP for FF (0.016) but not for NF (0.022) compared with ALL (0.021). Moreover, CP_{PD} was precisely predicted when all samples were included ($r^2 = 0.85$; RPD = 2.8), and predictions were improved for FF by group separation as indicated by the reduced SEP (0.01) and increased r^2 (0.89). However, prediction for NF group was below acceptable limits ($r^2 = 0.34$), even though RPD remained >2.5 and SEP was reduced (0.02 *v.* 0.03 for NF and ALL, respectively).

Degradability parameters of NDF
Table 6 summarizes calibration and validation statistics of the equations used to predict the degradability parameters of NDF. In this case, the number of feedstuffs was lower (100 in total; Table 2) and the need for an external validation matrix reduced it further (*N* calibration = 84; *n* validation = 16). Similar to degradability parameters of DM and CP, most of the parameters were best predicted using the second derivative pre-treatment.

The NDF content for ALL could be predicted with high precision ($r^2 = 0.96$; RPD = 4.8; SEP = 2.6), whereas the

Table 6 *Calibration and validation statistics for estimation of NDF degradability parameters by near-IR analysis on feedstuffs*

Parameter	Group	Scatter correction	Calibration		Cross-validation		Validation			
			R^2	SEC	r^2_{cv}	SECV	r^2	SEP	RPD	RER
NDF (% DM)	ALL	MSC	0.97	2.1	0.94	3.0	0.96	2.6	4.8	22.0
	FF	SNV-D	0.98	1.6	0.96	2.3	0.98	2.2	6.3	26.9
NDF_b	ALL	SNV	0.85	0.06	0.65	0.08	0.77	0.09	2.0	6.4
	FF	D	0.91	0.04	0.73	0.07	0.78	0.07	2.1	7.0
NDF_c	ALL	SNV-D	0.69	0.015	0.55	0.018	0.53	0.019	1.7	6.0
	FF	SNV-D	0.82	0.011	0.75	0.013	0.50	0.015	1.9	7.5
NDF_{ED}	ALL	SNV-D	0.86	0.05	0.80	0.06	0.88	0.06	2.4	9.3
	FF	SNV-D	0.96	0.03	0.90	0.04	0.89	0.05	2.9	9.5

R^2 = coefficient of determination for calibration; SEC = standard error of calibration; r^2_{cv} = coefficient of determination for cross-validation; SECV = standard error of cross-validation; r^2 = coefficient of determination for external validation; SEP = standard error of prediction; RPD = ratio of performance to deviation (s.d./SEP); RER = range error ratio (range/SEP); NDF (% DM) = concentration of NDF as % of dry matter (DM); NDF_b = degradable fraction of NDF; NDF_c = rate of NDF degradation; NDF_{ED} = effective degradation of NDF; ALL = all samples; FF = group of forages; MSC = multiple scatter correction; SNV = standard normal variate; D = detrend.

equation for NDF_{ED} was suitable for screening purposes ($r^2 = 0.88$; RPD = 2.3; SEP = 0.06). Group separation improved predictions increasing RPD and lowering SEP in both cases. For ALL, NDF_b and NDF_c were not precisely predicted providing RPD values <2, but performing analysis for FF increased RPD of NDF_b. Owing to the low number of samples in the validation set, RPD was calculated for cross-validation as well and was as follows: 2.1 and 2.3 for NDF_b of ALL and FF, respectively and 1.7 and 2.2 for NDF_c of ALL and FF, respectively.

Discussion

The broad range of feeds represented in the database with different botanical origins, states of maturity, feed processing and preservation method resulted in a wide range of values for all parameters. As a consequence, minimum or maximum values reflected particular feedstuffs. For example, minimum value of CP_a for ALL is equal to 2% (Table 2) because in the data set soypass samples (a commercially prepared rumen escape soybean product) were included (average CP_a = 6%, with minimum and maximum value of 2% and 8%, respectively; descriptive statistics per ingredient not shown). Similar values of CP_a for soybean-treated products have been reported in the literature (Harstad and Prestløkken, 2000; Sadeghi *et al.*, 2006). Similarly, minimum value of CP_b for ALL is equal to 4% because winter wheat silages were included into the database (average CP_b = 6%, with minimum and maximum value of 4% and 7%, respectively; descriptive statistics per ingredient not shown).

The main strength of the current work is the number and diversity of available feed samples incorporated into the database and its analysis with 24 spectral models. Four scatter correction techniques and six mathematical treatments were tested to remove or reduce disturbing effects not related to the chemical absorption of light. Results suggested the use of a different spectral model for each parameter instead of the use of a unique model for all parameters.

The second derivative treatment performed best for most parameters. The first and second derivatives are the most common forms in which spectra of agricultural products are displayed, third-order derivative are possible but are rarely used to interpret spectra (Shenk *et al.*, 1992).

Equations for ALL provided acceptable estimations either for screening or quantifying purposes of ED, PD and fractions *a* and *b* of DM and CP and NDF_{ED}, whereas predictions were improved in most cases by group separation, especially for FF group. A key component to explain this improvement is the variance of the data sets. We measured variance with the CV and in accordance with the literature (von Keyserlingk *et al.*, 1996; Hvelplund and Weisbjerg, 2000; Hackmann *et al.*, 2010), the variance of the current data set was high for all degradability parameters. However, group separation reduced the CV in most parameters in FF and some parameters in NF compared with ALL. This resulted in more homogeneous data, but still maintained a high range of values in each parameter tested. For FF, the inclusion of silages provided another factor. Silages provided more accurate predictions of DM and CP degradability parameters (de La Roza *et al.*, 1998) than other forage sources (Todorov *et al.*, 1994; Mathison *et al.*, 1999). However, when silages were included to forage sources predictions were improved (Hsu *et al.*, 1998). Silages have a different degradation pattern than other forages. Fraction CP_a is higher in silages compared with other forages (75% *v.* 43% for silages *v.* other forages, respectively; data not shown), making CP_b lower in silages than in forages (18% *v.* 51% for silages *v.* other forages, respectively; data not shown). Even though differences are not that strong for degradability parameters of DM, they still remained statistically significant. Thus, including silages in FF increased the range of values of degradability parameters improving overall prediction by NIRS.

Dyer (2004) reviewed the utilization of NIRS for oilseeds and coarse grains and reported that several studies predicted contents of DM, CP, NDF or concentration of particular parameters, such as gossypol in cottonseed, fatty acids, or

even total digestibility and energy estimation. Moreover, De Boever *et al.* (2003) used NIRS to predict degradability parameters of compound feeds. However, to our knowledge, no studies examined the potential to predict degradability parameters of grains or byproducts. The current data set incorporated 539 byproducts and concentrates and results for NF group suggested NIRS can provide fair predictions of DM and CP degradability parameters (RPD > 2.5 and 2.0 for degradability parameters of DM (Table 4) and CP (Table 5), respectively).

Several studies reported the inability of NIRS to predict the rate of degradation of nutrients (Andrés *et al.*, 2005b; Nordheim *et al.*, 2007; Ohlsson *et al.*, 2007). Herrero *et al.* (1997) related this difficulty to the exponential nature of the models used in parameterization of the degradation rate. In the current study, DM_c and CP_c were predicted for screening purposes (RPD between 2.0 and 2.8; Tables 4 and 5), but prediction of NDF_c was not reliable (RPD $\leqslant$ 1.9; Table 6). Moreover, RPD of DM_c was higher than that of DM_b, indicating that the mathematical issue was not the limiting factor. As a secondary procedure, NIRS is not independent of the disadvantages arising from the reference method used for calibration. As mentioned earlier, the variance of the current data set was high for all degradability parameters. In addition and similar to the literature (von Keyserlingk *et al.*, 1996; Hvelplund and Weisbjerg, 2000), the rate of degradation had the greatest CV among degradability parameters. Moreover, for FF the CV of NDF_c was greater than that of DM_c and CP_c (Table 3). Vanzant *et al.* (1998) analyzed variance of the *in situ* and suggested that bags, animals and days contribute to it. Several standard procedures have been proposed to increase the precision of the *in situ* (Nocek, 1988; Vanzant *et al.*, 1998). For the current study, all *in situ* were conducted under the procedure described in the study by Madsen *et al.* (1995), which is the basis for the NorFor procedure (Åkerlind *et al.*, 2011). Dry cows at maintenance level were used to minimize potential effects of intake level on *in situ* disappearance. However, incubation time for NDF was longer than that of DM or CP (maximum incubation time of 168 *v.* 96 h for NDF and CP of forages). This may explain why variance of NDF_c was greater than that of DM_c and CP_c.

Additional variance was introduced by our approach of obtaining universal equations for each category (ALL, FF and NF) owing to the incorporation of within-feed variation. Murray and Cowe (2004) discussed the effect of reference analysis on sample size and NIRS performance and suggested that a good calibration model can be obtained from relatively imprecise data if the data set is sufficiently large. In our data set, the number of available samples for degradability parameters of NDF was much lower compared with DM or CP (100 *v.* 665 or 682 for NDF and DM or CP, respectively). It is possible that a larger data set may improve NDF_c prediction.

Interestingly, provided equations for most degradability parameters with NIRS resulted in higher r^2 and RPD compared with those reported with FTIR for CP (Belanche *et al.*, 2013) and DM and NDF (Belanche *et al.*, 2014) using a

similar data set. The main difference between mid-IR (400 to 4000 cm^{-1} or 2500 to 25 000 m) and NIRS (4000 to 14 000 cm^{-1} or 750 to 2500 nm) ranges is that absorption in mid-IR corresponds to fundamental bands of molecular vibrations, whereas absorptions in NIR correspond to overtones and combinations of these fundamental bands (Williams and Norris, 1987). Ferreira *et al.* (2014) determined quality parameters of soybean samples with both technologies (NIR and FTIR) and reported similar results for the two methods, even though the R^2 of NIRS was greater than FTIR.

Current results suggest that NIRS technology can be used to predict effective and potential degradabilities either utilizing universal equations or by separating feeds into groups. Moreover, using NIRS it is possible to predict DM_c and CP_c for screening purposes, but a larger data set is needed to provide equations for NDF_c.

References

Åkerlind M, Weisbjerg MR, Eriksson T, Thøgersen R, Uden P, Olafsson BL, Harstad OM and Volden H 2011. Feed analysis and digestion methods. In NorFor – the Nordic feed evaluation system (ed. H Volden), pp. 41–54. Wageningen Academic Publishers, Wageningen, The Netherlands.

Andrés S, Murray I, Calleja A and Giráldez F 2005a. Review: nutritive evaluation of forages by near infrared reflectance spectroscopy. Journal of Near Infrared Spectroscopy 13, 301–311.

Andrés S, Giráldez FJ, González JS, Peláez R, Prieto N and Calleja A 2005b. Prediction of aspects of neutral detergent fibre digestion of forages by chemical composition and near infrared reflectance spectroscopy. Australian Journal of Agricultural Research 56, 187–193.

Association of Official Analytical Chemists 1990. Official methods of analysis, 15th edition. AOAC, Arlington, VA, USA.

Baldwin RL 1995. Modeling ruminant digestion and metabolism. Chapman & Hall, London, UK.

Belanche A, Weisbjerg MR, Allison GG, Newbold CJ and Moorby JM 2013. Estimation of feed crude protein concentration and rumen degradability by Fourier-transform infrared spectroscopy. Journal of Dairy Science 96, 7867–7880.

Belanche A, Weisbjerg MR, Allison GG, Newbold CJ and Moorby JM 2014. Measurement of rumen dry matter and neutral detergent fiber degradability of feeds by Fourier-transform infrared spectroscopy. Journal of Dairy Science 97, 2361–2375.

De Boever JL, Vanacker JM and De Brabander DL 2003. Rumen degradation characteristics of nutrients in compound feeds and the evaluation of tables, laboratory methods and NIRS as predictors. Animal Feed Science and Technology 107, 29–43.

de La Roza B, Martínez A, Santos B, González J and Gómez G 1998. The estimation of crude protein and dry matter degradability of maize and grass silages by near infrared spectroscopy. Journal of Near Infrared Spectroscopy 6, 145–151.

Dyer DJ 2004. Analysis of oilseeds and coarse grains. In Near-infrared spectroscopy in agriculture (ed. CA Roberts, JJ Workman and JB Reeves), pp. 321–344. American Society of Agronomy Inc., Madison, WI, USA.

Ferreira DS, Galão OF, Pallone JAL and Poppi RJ 2014. Comparison and application of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for determination of quality parameters in soybean samples. Food Control 35, 227–232.

Hackmann TJ, Sampson JD and Spain JN 2010. Variability in in situ ruminal degradation parameters causes imprecision in estimated ruminal digestibility. Journal of Dairy Science 93, 1074–1085.

Harstad OM and Prestløkken E 2000. Effective rumen degradability and intestinal indigestibility of individual amino acids in solvent-extracted soybean meal (SBM) and xylose-treated SBM (SoyPass®) determined in situ. Animal Feed Science and Technology 83, 31–47.

Herrero M, Jessop NS, Fawcett RH, Murray I and Dent JB 1997. Prediction of the in vitro gas production dynamics of kikuyu grass by near-infrared reflectance spectroscopy using spectrally-structured sample populations. Animal Feed Science and Technology 69, 281–287.

Hsu H, McNeil A, Okine E, Mathison G and Soofi-Siawash R 1998. Near infrared spectroscopy for measuring in situ degradability in barley forages. Journal of Near Infrared Spectroscopy 6, 129–143.

Huhtanen P, Seppälä A, Ots M, Ahvenjärvi S and Rinne M 2008. In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. Journal of Animal Science 86, 651–659.

Huhtanen S, Ahvenjärvi S, Weisbjerg MR and Norgaard P 2006. Digestion and passage of fibre in ruminants. In Ruminant physiology: digestion, metabolism and impact of nutrition on gene expression, immunology and stress (ed. K Sejrsen, T Hvelplund and MO Nielsen), pp. 87–135. Wageningen Academic Publishers, Wageningen, The Netherlands.

Hvelplund T and Weisbjerg MR 2000. In situ techniques for the estimation of protein degradability and postrumen availability. In Forage evaluation in ruminant nutrition (ed. DI Givens), pp. 233–258. CABI, Wallingford, UK.

López S, Carro MD, González JS and Ovejero FJ 1998. Comparison of different in vitro and in situ methods to estimate the extent and rate of degradation of hays in the rumen. Animal Feed Science and Technology 73, 99–113.

Madsen J, Hvelplund T, Weisbjerg MR, Bertilsson J, Olsson I, Spörndly R, Harstad OM, Volden H, Tuori M, Varvikko T, Huhtanen P and Olafsson BL 1995. The AAT/PBV protein evaluation system for ruminants. Norwegian Journal of Agricultural Sciences (suppl. 19), 1–37.

Mathison GW, Hsu H, Soofi-Siawash R, Recinos-Diaz G, Okine EK, Helm J and Juskiw P 1999. Prediction of composition and ruminal degradability characteristics of barley straw by near infrared reflectance spectroscopy. Canadian Journal of Animal Science 79, 519–523.

Mertens DR 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 85, 1217–1240.

Murray I and Cowe I 2004. Sample preparation. In Near-infrared spectroscopy in agriculture (ed. CA Roberts, JJ Workman and JB Reeves), pp. 75–112. American Society of Agronomy Inc., Madison, WI, USA.

National Research Council 2001. Nutrient requirements of dairy cattle, 7th edition. NRC, Washington, DC, USA.

Nocek JE 1988. In situ and other methods to estimate ruminal protein and energy digestibility: a review. Journal of Dairy Science 71, 2051–2069.

Nordheim H, Volden H, Fystro G and Lunnan T 2007. Prediction of in situ degradation characteristics of neutral detergent fibre (aNDF) in temperate grasses and red clover using near-infrared reflectance spectroscopy (NIRS). Animal Feed Science and Technology 139, 92–108.

Ohlsson C, Houmøller LP, Weisbjerg MR, Lund P and Hvelplund T 2007. Effective rumen degradation of dry matter, crude protein and neutral detergent fibre in forage determined by near infrared reflectance spectroscopy. Journal of Animal Physiology and Animal Nutrition 91, 498–507.

Ørskov ER and McDonald I 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92, 499–503.

Roberts CA, Stuth J and Flinn P 2004. Analysis of forages and feedstuffs. In Near-infrared spectroscopy in agriculture (ed. CA Roberts, JJ Workman and JB Reeves), pp. 231–267. American Society of Agronomy Inc., Madison, WI, USA.

Sadeghi AA, Nikkhah A, Shawrang P and Shahrebabak MM 2006. Protein degradation kinetics of untreated and treated soybean meal using SDS-PAGE. Animal Feed Science and Technology 126, 121–133.

Shenk J, Workman J and Westerhaus M 1992. Application of NIR spectroscopy to agricultural products. In Handbook of near-infrared analysis (ed. D Burns and E Ciurczak), pp. 383–431. Marcel Dekker Inc., New York, NY, USA.

Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG and Russell JB 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70, 3562–3577.

Todorov N, Atanassova S, Pavlov D and Grigorova R 1994. Prediction of dry matter and protein degradability of forages by near infrared spectroscopy. Livestock Production Science 39, 89–91.

Vanzant ES, Cochran RC and Titgemeyer EC 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. Journal of Animal Science 76, 2717–2729.

Volden H 2011. NorFor – the Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, The Netherlands.

von Keyserlingk MAG, Swift ML, Puchala R and Shelford JA 1996. Degradability characteristics of dry matter and crude protein of forages in ruminants. Animal Feed Science and Technology 57, 291–311.

Williams P 2014. Tutorial: the RPD statistic: a tutorial note. NIR News 25, 22–26.

Williams P and Norris K 1987. Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists Inc., St. Paul, MN, USA.

Workman JJ and Shenk J 2004. Understanding and using near-infrared spectrum as an analytical method. In Near-infrared spectroscopy in agriculture (ed. CA Roberts, JJ Workman and JB Reeves), pp. 3–10. American Society of Agronomy Inc., Madison, WI, USA.