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Nutritive evaluation of herbage from permanent meadows by near-infrared reflectance spectroscopy: 2. Prediction of crude protein and dry matter degradability

Sonia Andrés,[1,2]* Alfredo Calleja,[1,2] Secundino López,[1,2] Ángel R Mantecón,[2,3] and F Javier Giráldez[2,3]

[1]Departamento de Producción Animal I, Universidad de León, E-24071 León, Spain
[2]Unidad Asociada al CSIC Grupo de Nutrición-Praticultura del Departamento de Producción Animal I de la Universidad de León a través de la Estación Agricola Experimental de León, León, Spain
[3]Estación Agricola Experimental, CSIC, Apartado 788, E-24080 León, Spain

Abstract: A total of 62 herbage samples, harvested in natural meadows located in the uplands of León (northwestern Spain) and characterised by a diverse botanical composition and different stages of maturity of the plants, were used to evaluate the ability of chemical composition and near-infrared reflectance spectroscopy to predict dry matter (DM) and crude protein (CP) ruminal degradability. Three non-productive Holstein-Friesian cows fitted with rumen cannulae were used to incubate the herbage samples. Once the DM and CP disappearance rates had been calculated, the exponential model of McDonald was fitted to estimate the kinetic parameters, which were used to calculate the potential and effective ruminal degradability at different passage rates. A Bran+Luebbe InfraAlyzer 500 spectrophotometer was used to obtain the near-infrared (NIR) spectra corresponding to the 62 original herbage samples. Prediction equations for the estimation of the DM and CP degradability parameters were generated using the chemical composition data and the NIR spectra as independent variables. The results showed that the kinetic parameters were predicted with less accuracy than the potential or effective degradability of the chemical fractions. When NIR spectra were used as independent variables, the accuracy of the predictions of the potential or effective degradability of DM and CP was higher. Overall, the degradability of CP was predicted less successfully than the degradability of DM, probably owing to errors in the reference method, such as the microbial contamination of the incubation residues.
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Keywords: degradation; ruminant; herbage samples; NIRS

INTRODUCTION

The new ruminant feeding systems[1–3] require information about not only the composition of the feedstuffs but also other attributes, such as degradation kinetics in the rumen, in order to assess the nutrient supply to the animal.[4] For example, the crude protein content of the feed provides little information about the availability of protein to the ruminant, as the protein reaching the duodenum comprises not only the feed protein that escapes ruminal degradation but also the microbial protein synthesised in the rumen.[5]

The nylon bag or *in situ* technique is considered to be a reference method for the estimation of the extent of degradation in the rumen, owing to the close relationship between the results obtained with this procedure and those measured *in vivo*.[6,7] However, to evaluate a large number of samples, the technique is laborious and time-consuming. Also, the number of bags that can be placed in the rumen of a cannulated animal is limited (6–9 bags in a sheep and 25–30 bags in a cow at the same time), and it takes more than a week to evaluate the kinetic parameters of the feed fractions. The *in situ* method is generally not suitable for routine screening of forages, and other procedures have been used, eg the gas production technique,[8] proteolytic enzymes[9,10] and, more recently, near-infrared reflectance spectroscopy (NIRS).[5,11–15]

The different molecular structure of proteins influences the crude protein degradation of forages.[16] Further, it has been observed that the changes in the structure of proteins can be detected using ultraviolet (250 nm)[17] and near-infrared (1100–2500 nm)[18]

* Correspondence to: Sonia Andrés, Departamento de Producción Animal, Universidad de León, E-24071 León, Spain
E-mail: dp1sal@unileon.es
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spectra. Thus NIRS has been suggested as a feasible method to predict accurately the dry matter[11–13] and crude protein[5,13–15] ruminal degradation. There is, however, little information in the literature on the suitability of NIRS for the prediction of *in situ* degradability parameters of forages characterised by a diverse botanical composition and with significant differences in the plant stage of maturity. This study, which is the second of a series[19] dealing with botanically complex herbage samples, was implemented to assess and compare the ability of chemical composition and near-infrared spectroscopy to predict dry matter and crude protein degradability parameters of herbage samples obtained in natural meadows located in the uplands of León (northwestern Spain).

EXPERIMENTAL

Forage samples

This work was carried out with 62 herbage samples harvested in 2000 after either the spring primary growth (early or late cutting dates in late May–early June (22 samples) or in late June–early July (22 samples)) or the summer/autumn secondary regrowth (18 samples from this harvest season in mid-September) of permanent meadows located in the uplands of León (northwestern Spain) at an altitude of 900–1450 m. These meadows are plant communities classified within the vegetation type *Arrhenatheretalia*, ie pastures and meadows on well-drained, relatively fertile mineral soils.[20] Predominant forage species were *Alopecurus pratensis* L, *Anthoxanthum odoratum* L, *Arrhenatherum elatius* (L) Beauv ex J & K Presl, *Bromus hordeaceus* L, *Cynosurus cristatus* L, *Dactylis glomerata* L, *Festuca rubra* L, *Holcus lanatus* L, *Lolium perenne* L, *Poa pratensis* L, *Poa trivialis* L, *Trisetum flavescens* (L) Beauv, *Trifolium pratense* L, *Trifolium repens* L, *Bellis perennis* L, *Carum carvi* L, *Centaurea nigra* L, *Cerastium fontanum* Baumg, *Plantago lanceolata* L, *Ranunculus bulbosus* L, *Rumex acetosa* L, *Taraxacum officinale* GH Weber ex Wiggers and *Veronica arvensis* L. The proportion of each species in the herbage samples was highly variable. Owing to the different harvest seasons (spring, summer and autumn) and the various cutting dates within each season, plants were at significantly different stages of maturity. Samples were oven dried at 60 °C for 48 h and then ground to pass a 4 mm screen. The coarse samples were used for the nylon bag technique. A subsample was further ground to pass a 1 mm screen for chemical analyses and NIRS.

Chemical composition

Dry matter (DM), ash and crude protein (CP = N × 6.25, N being the nitrogen content) were determined by the proximate procedures outlined by the AOAC.[21] Neutral detergent fibre (NDF) was determined according to the procedure proposed by Van Soest *et al*,[22] and acid detergent fibre (ADF) and acid detergent lignin (ADL) according to the procedures of Goering and Van Soest.[23] Acid detergent-insoluble nitrogen (ADIN) was determined by measuring the N content of the ADF residue by the macro-Kjeldahl procedure.[23] Finally, hemicellulose (HCEL) and cellulose (CEL) contents were calculated as the difference between NDF and ADF and between ADF and ADL respectively.

Nylon bag technique (*in situ* degradability)

The degradation kinetics of the herbage samples were measured using three non-productive Holstein-Friesian cows fitted with rumen cannulae. The animals received a diet comprising 1.5 kg of alfalfa hay, 1.5 kg of cereal straw and 2 kg of a commercial compound food and were fed twice daily at 08:00 and 17:00. Nylon bags, 10 cm × 15 cm with 46 μm pore size (Saatilon polyamide monofilament 120.38-YPW, SAATI, Cerigrafía Ibérica SA, Almazora, Castellón, Spain), were dried and weighed before being filled with approximately 5 g of DM of each sample. The bags were incubated for 2, 4, 8, 12, 24, 48, 72 and 96 h, using one bag for each sample, incubation time and animal. The incubation period started prior to the morning meal. All nylon bags corresponding to the same time of incubation were inserted and withdrawn from the rumen simultaneously. Upon removal, bags were soaked in cold water for 15 min to stop the microbial activity, then frozen at −30 °C for 24 h to remove any microbial cells adhering to the herbage particles. The bags were defrosted and washed in an automatic washing machine with cold water and no spinning. Three bags not previously incubated in the rumen were washed using the same procedure to determine the washout fraction (disappearance at time zero). All bags were oven dried at 60 °C for 48 h and weighed. Finally, the residues corresponding to the same forage and incubation time period were pooled, ground through a 1 mm screen and then analysed for CP content. The exponential model proposed by McDonald[24] was fitted to the observed DM and CP disappearance rates:

$$P = a \quad \text{for } t \le L \tag{1}$$

$$P = a + b(1 - e^{-c(t-L))} \quad \text{for } t > L \tag{2}$$

where P is the disappearance rate at time t, a is the intercept at time 0 and represents the fraction that is rapidly washed out of the bag, b is the difference between the asymptote and the intercept (a) and represents the insoluble but potentially degradable fraction which is degraded by the micro-organisms according to first-order kinetics, c is the fractional rate of degradation, L is the lag time and $1 - (a + b)$ is the undegradable fraction.

The effective ruminal degradability (ED) of the DM or CP was estimated according to France *et al*[25] and

Dhanoa *et al*:[26]

$$ED_k = a + \frac{bc}{c+k}e^{-kL} \quad (3)$$

where a, b, c and L are the parameters of eqn (1) and k represents the rumen passage rate. In the present study, two different rumen passage rates were used: $0.02\,h^{-1}$, characteristic of a low level of intake (maintenance), and $0.06\,h^{-1}$, representing a situation where the forage would be consumed at a higher level of intake.[1]

Near-infrared technology

Original herbage samples were scanned at 2 nm intervals over the near-infrared (NIR) spectral range (1100–2500 nm) using an InfraAlyzer 500 spectrophotometer (Bran + Luebbe GmbH, Norderstedt, Germany). Herbage samples were scanned twice in duplicate repacking using two different cells (four spectra per sample) and the absorbance data recorded as $\log(1/R)$, where R is the reflectance. The mean spectrum for each sample was calculated.

Prediction equations

Partial least squares regression (PLSR) was used to develop NIR prediction equations for *in situ* degradability parameters. During calibration development, SESAME software (version 2.1, Bran + Luebbe, New York, NY, USA) was used when first- or second-order derivatives were applied to the spectra, and the UNSCRAMBLER program (version 5.03, Camo, Trondheim, Norway) when multiplicative scatter correction (MSC) was applied. In both cases, full cross-validation was performed to avoid overfitting the PLSR equations.

When the chemical data were used to predict *in situ* degradability parameters, independent variables were selected using the stepwise multiple linear regression (MLR) procedure in SAS,[27] with cross-validation performed using the UNSCRAMBLER program. This allowed comparison with the standard error of cross-validation (SE_{CV}) for the NIR equations. In fact, the optimal equation for the prediction of each parameter was selected on the basis of the lowest SE_{CV}.

RESULTS AND DISCUSSION

The mean value, range and standard deviation (SD) of the chemical composition data and the *in situ* degradability parameters of DM and CP are summarised in Table 1.

NIR spectra and different combinations of chemical composition data were used as independent variables

Table 1. Range, mean and standard deviation of chemical data (ash, CP, NDF, ADF, HCEL, CEL, ADL and ADIN) and *in situ* DM and CP degradability parameters

	Sample set ($n = 62$)			
	Range	Mean	SD	CV
Chemical parameters (g kg^{-1}DM)				
Ash	61–201	93	20.5	22.0
CP	52–179	118	28.1	23.8
NDF	359–684	518	77.9	15.0
ADF	210–383	290	40.6	14.0
HCEL	148–346	228	42.3	18.6
CEL	170–359	260	45.5	17.5
ADL	11–55	31	10.2	33.0
ADIN	0.5–4.4	2.2	1.11	50.5
In situ DM degradability parameters (% of DM unless otherwise stated)				
a_{DM}	18.1–41.1	30.2	5.18	17.2
b_{DM}	42.5–60.6	50.2	4.23	8.4
$c_{DM}(h^{-1})$	0.024–0.107	0.061	0.0208	34.1
L_{DM}(h)	0.0–3.1	0.9	0.82	91.1
$a + b_{DM}$	67.5–90.3	80.5	6.30	7.8
$EDDM_{0.02}$	49.2–78.6	66.6	8.20	12.3
$EDDM_{0.06}$	36.5–67.8	53.5	8.37	15.6
In situ CP degradability parameters (% of CP unless otherwise stated)				
a_{CP}	18.6–48.1	32.9	6.73	20.5
b_{CP}	30.3–76.9	55.0	9.91	18.0
$c_{CP}(h^{-1})$	0.036–0.147	0.083	0.0240	29.0
L_{CP}(h)	0.0–3.5	1.3	1.17	90.0
$a + b_{CP}$	74.6–95.5	87.9	4.50	5.1
$EDCP_{0.02}$	60.9–85.2	75.6	4.56	6.0
$EDCP_{0.06}$	46.3–72.8	61.8	4.71	7.6

EDDM = effective degradability of dry matter at different rumen passage rates (0.02 and $0.06\,h^{-1}$); EDCP = effective degradability of crude protein at different rumen passage rates (0.02 and $0.06\,h^{-1}$).

Table 2. Prediction of *in situ* DM degradability parameters corresponding to 62 herbage samples

Y	*X* variables		R^2	SEC	SE_{CV}	RPD
a_{DM}	Chemical data	NDF	0.765	2.49	2.58	2.00
	NIRS technology	NIR spectra (MSC + 2D), $p = 4$	0.897	1.65	2.15	2.41
b_{DM}	Chemical data	HCEL, CP, CEL	0.309	3.48	3.75	1.13
	NIRS technology	NIR spectra (2,20,10), $p = 5$	0.614	2.74	3.03	1.39
c_{DM}	Chemical data	NDF, ADIN	0.609	0.0129	0.0135	1.54
	NIRS technology	NIR spectra (MSC), $p = 2$	0.571	0.0135	0.0142	1.46
L_{DM}	Chemical data	ADF, ash	0.188	0.730	0.796	1.03
	NIRS technology	NIR spectra (MSC + 2D), $p = 1$	0.153	0.746	0.779	1.05
$a + b_{DM}$	Chemical data	ADF, ash	0.789	2.87	3.01	2.10
	NIRS technology	NIR spectra (1,4,4), $p = 6$	0.860	2.48	2.83	2.22
$EDDM_{0.02}$	Chemical data	NDF, CP, ADIN, HCEL	0.904	2.52	2.78	2.95
	NIRS technology	NIR spectra (MSC + 2D), $p = 3$	0.932	2.12	2.41	3.40
$EDDM_{0.06}$	Chemical data	NDF, ADIN, ADL	0.900	2.65	2.84	2.96
	NIRS technology	NIR spectra (MSC + 2D), $p = 3$	0.920	2.35	2.70	3.10

EDDM = effective degradability of dry matter (% DM) at different rumen passage rates (0.02 and 0.06 h^{-1}); NIR spectra (−, −, −) = pre-treatment of the NIR spectra, where the first number is the derivative order, the second number is the gap between points used to calculate the difference, and the last one is the number of data points used to smooth the data; p = number of terms in the equation; MSC = multiplicative scatter correction; 2D = second-order derivative; R^2 = coefficient of determination; SEC = standard error of calibration; SE_{CV} = standard error of cross-validation; RPD = ratio performance deviation calculated as $SD_{reference\ data}/SE_{CV}$.

for predicting the *in situ* DM and CP degradability parameters. Statistics commonly used to assess the accuracy of prediction of each DM degradation parameter are shown in Table 2.

The results presented in Table 2 demonstrate that neither chemical data nor NIR spectra produced accurate estimations for some *in situ* DM degradability parameters, in particular b_{DM}, c_{DM} and L_{DM}, which showed poor coefficients of determination ($R^2 < 0.65$). Also, the RPD statistic, which is calculated as the ratio of the SD of the reference values to the SE_{CV} and gives an indication of the usefulness of the calibration, was very low for these parameters (RPD < 1.6). In this sense, Williams and Sobering[28] suggested that a value of at least 2.5 had to be achieved for an equation to be acceptable.

As reported by other authors,[11–13,15] the a_{DM} fraction was predicted better by NIRS ($R^2 = 0.897$) than the b_{DM} fraction ($R^2 = 0.614$), probably because the former is better correlated with the chemical composition than the latter.

The fractional rate of degradation (c_{DM}) and the lag time (L_{DM}) were poorly predicted by NIRS technology ($R^2 = 0.571$ and 0.153 respectively). Similar results have been published previously for barley forage,[11] barley straw,[12] diverse forages,[13] dried grass or lucerne forage,[14] fresh grass, grass silage, maize silage[29] and fresh herbage.[30] The low degree of precision of the nylon bag technique at early incubation times due to the differences in rumen liquid of the animals could have resulted in poor fitting of the exponential model proposed by McDonald[24] to the DM disappearance rates. This may have had a negative influence on the estimation of the c_{DM} and L_{DM} parameters and subsequent prediction using NIR spectra. Conversely, the potential degradability of DM ($a + b_{DM}$) and the effective degradability of DM at different rumen passage rates ($EDDM_{0.02}$ and $EDDM_{0.06}$) were predicted with a higher degree of accuracy (Table 2) for both chemical data and NIR spectra.

In general, the NIR predictions were better than those achieved with chemical data, as indicated by lower standard error of calibration (SEC) and higher R^2 (Table 2). NIR spectra contain information associated with all chemical entities of a sample, and as rumen degradation parameters are related to all chemical components, an improved prediction would be expected with NIR spectra compared with individual chemical data. In addition to the chemical components, NIR spectra contain information relating to the physical properties of a sample.[31] These properties may have a significant influence on the extent of DM degradation in the rumen. For example, the fibre content increases as a plant matures owing to a higher stem-to-leaf ratio, greater degree of lignification of the secondary cell wall and development of the cuticular layer.[32] In general, these changes are more pronounced in grass than in legume species and make plant tissues more resistant to the activity of the microbial enzymes, leading to a noticeable decline in their ruminal degradability.[32,33]

The calibration and cross-validation statistics for the prediction of CP degradability parameters are shown in Table 3.

As can be observed (Table 3), a_{CP}, c_{CP} and L_{CP} were not predicted accurately ($R^2 < 0.8$, RPD < 2.5) using either chemical data or NIR spectra as independent variables. However, as observed with *in situ* DM degradability, the predictions were more accurate using NIR spectra rather than chemical data as independent variables. Similar results have been observed by other authors.[34]

The b_{CP} fraction was predicted better by NIRS ($R^2 = 0.810$) than the a_{CP} fraction ($R^2 = 0.720$) (Table 3). This could be due to the fact that components of the b_{CP} fraction, including cytoplasm

Table 3. Prediction of *in situ* CP degradability parameters corresponding to 62 herbage samples

Y	*X* variables		R^2	SEC	SE_{CV}	RPD
a_{CP}	Chemical data	CEL, HCEL	0.441	4.98	5.26	1.27
	NIRS technology	NIR spectra (2,5,5), $p = 3$	0.720	3.59	3.94	1.68
b_{CP}	Chemical data	ADF	0.568	6.46	6.73	1.47
	NIRS technology	NIR spectra (2,15,5), $p = 2$	0.810	4.33	4.54	2.15
c_{CP}	Chemical data	ADF	0.258	0.021	0.021	1.14
	NIRS technology	NIR spectra (2,2,20), $p = 6$	0.513	0.018	0.020	1.20
L_{CP}	Chemical data	Ash	0.103	1.10	1.15	1.02
	NIRS technology	NIR spectra (log(1/*R*)), $p = 2$	0.180	1.04	1.07	1.05
$a + b_{CP}$	Chemical data	CP, NDF, LIG	0.735	2.29	2.52	1.78
	NIRS technology	NIR spectra (MSC + 1D), $p = 3$	0.837	1.80	2.08	2.16
$EDCP_{0.02}$	Chemical data	CP, HCEL, ADL	0.620	2.38	2.61	1.76
	NIRS technology	NIR spectra (MSC + 2D), $p = 2$	0.773	2.16	2.35	1.94
$EDCP_{0.06}$	Chemical data	HCEL, LIG	0.327	3.85	4.03	1.17
	NIRS technology	NIR spectra (MSC + 2D), $p = 3$	0.645	2.78	3.29	1.43

EDCP = effective degradability of crude protein (% CP) at different rumen passage rates (0.02 and 0.06 h^{-1}); NIR spectra (−, −, −) = pre-treatment of the NIR spectra, where the first number is the derivative order, the second number is the gap between points used to calculate the difference, and the last one is the number of data points used to smooth the data; p = number of terms in the equation; MSC = multiplicative scatter correction; 1D = first-order derivative; 2D = second-order derivative; LIG = degree of lignification of the cell wall calculated as ADL/NDF; R^2 = coefficient of determination; SEC = standard error of calibration; SE_{CV} = standard error of cross-validation; RPD = ratio performance deviation calculated as $SD_{reference\ data}/SE_{CV}$.

and chloroplast proteins of the cell contents (which precipitate and become insoluble with the moderate heat of drying), proteins associated with the cell wall (extensins) and nucleoproteins, all show a characteristic secondary structure which can be detected by NIR spectra.[18] In contrast, the a_{CP} fraction may contain a high proportion of non-protein nitrogen compounds (peptides or amino acids)[32] lacking any secondary structure, thus resulting in a less-defined spectrum. The physical loss of particulate matter from the bag may also contribute to lower precision in the estimation of the a_{CP} fraction by the *in situ* methodology,[35] explaining in part the reduced accuracy by NIRS. Other studies, eg those by Todorov *et al*[13] and De la Roza *et al*[15], also found that the soluble CP fraction could not be predicted accurately in diverse forages and silages respectively.

It is known that the different molecular structure of proteins greatly influences the CP degradation of forages,[16] so, by using NIR spectra, this factor could be taken into account, resulting in improved prediction of potential ($a + b_{CP}$) or effective (EDCP) CP degradability. Compared with the prediction of DM degradability, $a + b_{CP}$ was predicted more successfully ($R^2 = 0.837$) than EDCP at different rumen passage rates ($R^2 = 0.773$ and 0.645 for $EDCP_{0.02}$ and $EDCP_{0.06}$ respectively). This was possibly be due to the b_{CP} fraction being more influential in the calculation of $a + b_{CP}$, whereas a_{CP} represents a higher proportion of EDCP, particularly at higher passage rates. Similar results have been published by other authors, showing that the ruminal non-degraded protein content could be predicted successfully using NIRS in roasted soybeans ($R^2 = 0.90$, SE = 2.41% of CP)[36] and in legume and grass silages ($R^2 = 0.84$, SE = 1.55% of CP).[37] Using NIRS, Halgerson *et al*[5] predicted accurately ($R^2 = 0.95$, SE = 1.01% of CP) the CP remaining in alfalfa after 24 h of incubation.

Overall, CP degradability parameters were poorly predicted using NIRS compared with the predictions achieved for the DM degradability. It has been suggested that this could be related to the microbial contamination of the incubation residues.[13,15,38] Further, the inherent limitations of the *in situ* method together with the analytical errors associated with the determination of Kjeldahl N in the incubation residues might have contributed to increase the variability of the reference data. All these sources of error are cumulative and could have affected the accuracy of the NIRS predictions, especially those of the CP degradation parameters.

As expected, calibration statistics for the prediction of chemical composition in similar forages using NIRS[39] were superior to those achieved in this study for the prediction of nutritional attributes such as DM and CP degradation parameters. This is in accordance with the results reported by other authors for barley straw.[12] In fact, NIRS is based on the relationship between spectral characteristics (absorbance at certain wavelengths) and chemical and physical attributes of the sample scanned.[40] Consequently, a high accuracy of prediction is expected for single chemical entities that can be determined with high precision (such as protein),[39] while a lower accuracy can be anticipated for analytical fractions that are not a well-defined compound (such as lignin)[39] or for indicators of the nutritive value that are measured using biological methods (such as digestibility or degradability).[11,13,15,29,30,41,42] In this latter case the relationship between reference and spectral data is complex and has to be attributed to the association between the NIR spectra and several different chemical entities and physical properties that determine the

extent of degradation of that feed in the rumen. These attributes can vary in NIR spectra, altering the relationship between reference methods and the absorbance data. In addition, animal response as measured by biological methods is subject to increased variability from different sources of experimental and sampling errors (differences between animals, days, incubation runs, replicates, etc) which will affect the performance of the NIRS predictions. NIRS is a predictive method and, as such, is highly dependent upon the errors associated with the reference method[43] and will therefore inherit these errors, in addition to those arising from the photometric technique, such as instrument noise, operator, packing and sampling errors.[44] In a previous study looking at the errors associated with reference methods, Coates[45] concluded that the most accurate predictions were achieved with the most accurate reference values. In the present study, taking into account the limitations of the *in situ* technique, the variation between replicates was considerably larger than that observed with the chemical constituents. Hence a higher level of tolerance might be applied when evaluating the prediction statistics of the rumen degradation parameters using NIR spectral data.

The calibration performance attained in the present study was not as robust as that observed in previous studies which used NIRS technology to predict rumen degradation parameters of less complex forage mixtures (barley,[11,12] lucerne[14] or maize silage[15]). Thus, with the types of forages included in the present study, the prediction of rumen degradation parameters using NIR spectra could be accepted as satisfactory (Fig 1) even though the statistics are not particularly outstanding.

CONCLUSIONS

NIRS technology seems to be a feasible and reliable means for predicting some DM (a_{DM}, $a + b_{DM}$, $EDDM_{0.02}$ and $EDDM_{0.06}$) and CP (b_{CP} and $a + b_{CP}$) ruminal degradability parameters more accurately than chemical composition data, probably because NIR spectra provide more comprehensive information on the chemical composition of forages and can take into account other kinds of information related, for example, to the particle size of the sample or the molecular structure of the proteins. Nevertheless, the CP degradability parameters were less successfully predicted than the parameters for the ruminal DM degradation. This could be due, perhaps, to errors of the reference method, such as the microbial contamination of the residues of incubation. To improve the efficiency of NIRS technology, it is important to understand the relative contributions of the different sources of error, particularly those associated with the reference method, in order to minimise the impact of those factors which contribute most to the overall error. Furthermore, although the number of samples used in the present study was enough to test the ability of NIRS to predict these kinds of parameters, maybe a broader population could have improved the robustness of these equations.

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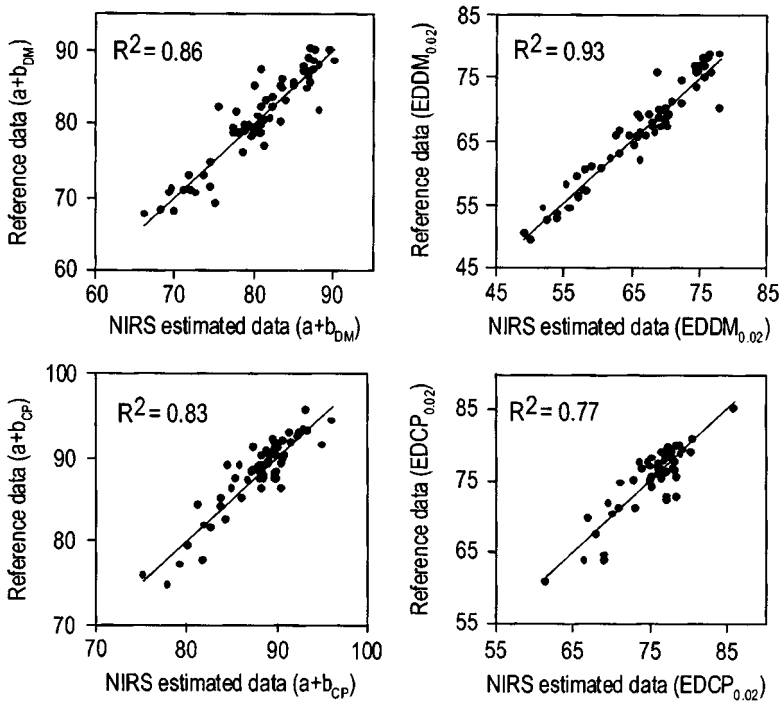

Figure 1. Relationship between *in situ* reference data corresponding to 62 herbage samples and those predicted by NIRS technology.

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