The prediction of *in vitro* digestibility of forages by near infrared reflectance spectroscopy

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Introduction

Digestibility of feeds is an essential parameter determining their nutritive value and thus its accurate estimation is required by most of feeding systems.

Currently, several *in vitro* techniques are commonly used for routine analyses in commercial laboratories as they are less expensive and time-consuming than the *in vivo* method and produce repeatable data highly correlated to those obtained with a reference *in vivo* procedure (Goering and Van Soest,[1] Jones and Hayward,[2] Tilley and Terry[3]). Nevertheless, *in vitro* techniques may be also expensive and time-consuming methods. Therefore, an alternative is desirable when a large-scale testing of feedstuffs is required.

Near infrared reflectance spectroscopy (NIRS) has been successful in predicting, rapidly and accurately, chemical composition and digestibility in a diverse group of feeds, including forages quality (Norris *et al.*[4]). Nevertheless, to our best knowledge, there is little information comparing the application of NIRS to predict *in vitro* digestibility of different feed constituents or the same constituent but measured according different techniques.

The aim of this study was to compare the calibrations obtained either with the Tilley & Terry or with the Goering & Van Soest procedures, which are two of the most used *in vitro* techniques in the feed evaluation for ruminants.

Material and methods

Feed samples

One hundred and seven herbage samples, harvested from meadows located in farms across the mountain of León (Northwest of Spain), were used in this study. Samples were oven dried at 60ºC (for two days) and then ground, to pass 1 mm screen, for laboratory analysis.

The samples were selected to provide a wide range in botanical and chemical composition and thus in digestibility.

Chemical composition

Dry matter (DM), ash and crude protein (N×6,25) were determined by the proximate procedures outlined by the AOAC[5]. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the procedures proposed by Van Soest *et al.*[6] and Goering and Van Soest,[1] respectively. Also, acid detergent lignin (ADL) was determined according to Goering and Van Soest[1] procedure.

In vitro digestibility

In vitro technique of Tilley and Terry[3] was used to determine *in vitro* dry matter (DMD_{TT}) and organic matter digestibility (ODM_{TT}). *In vitro* dry matter (DMD_{GV}) and neutral detergent fibre digestibility ($NDFD_{GV}$) were determined according to the Goering and Van Soest[1] procedure. All methods were performed with the modifications proposed by the ANKOM-DAISY procedure (Bochi Brum *et al.*[7]). Measurements were made in duplicate and standards were included in each method. Merino ewes fed alfalfa hay were used to obtain the ruminal fluid needed for the incubations.

Near infrared technology

Samples were scanned at 2 nm intervals over the NIR spectral range (1100 to 2500 nm) using a spectrophotometer model InfraAlyzer 500 (Bran+Luebbe GmbH, Norderstedt, Germany). Samples were scanned by quadruplicate using two different cells and recording the spectral data as log (1/*R*). The mean spectrum was used for each sample.

Sixty-two samples were selected on the basis of their chemical composition for calibration purpose. The remaining 45 samples were used as validation set.

All calibrations were obtained using partial least square regression (PLSR). Different mathematical treatments based on first and second derivatives were used. All the calibration equations were tested and the optimum model for each parameter was selected on the basis of minimising the standard error of prediction (*SEP*).

Results and discussion

The mean value, range and standard deviation of DMD_{TT}, OMD_{TT}, DMD_{GV} and $NDFD_{GV}$ are summarised in Table 1. As can be observed, the means and the standard deviations were similar for each parameter between both sets and, in any case, the differences were less than 10% and 25% respectively, so it could be considered that samples used for validation were comparable to those used for calibration purposes (Windham *et al.*[8]).

Calibration and validation results for the different *in vitro* parameters are shown in Table 2. The best mathematical treatment for the prediction of DMD_{TT} and DMD_{GV} was a second derivative with a gap of 4 and a segment of 4 data points, and a first derivative with a gap of 4 and a segment of 4 data points, respectively. The coefficient of determination (R^2), the coefficient of determination adjusted for the degrees of freedom (R^2 adj) and the standard error of calibration (*SEC*) values were better for the model estimating DMD_{TT} than that corresponding to DMD_{GV}. However, the robustness of the last one seemed to be larger, as suggested the lowest *SEP* and the highest RPD. It could be related to the different number of terms selected in each model. In fact, increasing the number of terms in the model to predict OMD_{TT} decreased *SEC* but increased *SEP* (*SEC* values were 2.38 and 1.68, and *SEP* values were 2.20 and 2.31 for equations including 3 and 7 factors, respectively).

Table 1. Range, mean and standard deviation of the calibration and validation sets.

Parameter	Calibration set (n=62)			Validation set (n=45)		
	Range	Mean	SD	Range	Mean	*SD*
DMD_{TT}	63.1–88.0	79.2	7.62	63.7–87.6	79.4	7.29
OMD_{TT}	62.2–88.9	79.6	6.87	63.3–88.1	79.2	7.61
DMD_{GV}	63.7–88.1	78.7	6.24	65.0–88.7	78.6	7.53
$NDFD_{GV}$	39.8–70.3	59.6	6.90	40.1–73.6	58.8	7.70

DMD_{TT}: *in vitro* dry matter digestibility measured according to Tilley & Terry method; **OMD_{TT}**: *in vitro* organic matter digestibility measured according to Tilley & Terry method; **DMD_{GV}**: *in vitro* dry matter digestibility measured according to Goering & Van Soest procedure; **$NDFD_{GV}$**: *in vitro* neutral detergent fibre digestibility measured according to Goering & Van Soest procedure

As expected, DMD_{TT} and OMD_{TT} were highly correlated ($r = 0.99$; $p<0.05$). Therefore, it was not surprising that the best mathematical treatment to predict OMD_{TT} was the same as for DMD_{TT}. Moreover, accuracy of the prediction of both DMD_{TT} and OMD_{TT} was also comparable.

On the other hand, the calibration and validation statistics for OMD_{TT} were in agreement with those of Van Waes *et al*.[9] (R^2=0.85; *SEP*=2.05) for grass and maize samples.

Table 2. Calibration and validation statistics for DMD_{TT}, OMD_{TT}, DMD_{GV}, $NDFD_{GV}$

Parameter	p	R^2	R^2 adj	*SEC*	*SEC*V	*SEP*	*RPD*	*CV*
DMD_{TT}	7	0.965	0.961	1.31	1.79	2.44	2.98	3.08
OMD_{TT}	7	0.969	0.965	1.28	1.71	2.35	2.92	2.97
DMD_{GV}	3	0.862	0.855	2.38	2.56	2.20	3.42	2.80
$NDFD_{GV}$	11	0.905	0.884	2.40	3.90	3.50	2.20	5.95

p: number of terms in the equation; ***R*²**: coefficient of determination; ***R*² adj**: coefficient of determination adjusted for the degrees of freedom; ***SEC***: standard error of calibration; **SECV**: standard error of cross validation; ***SEP***: standard error of prediction; **RPD**: ratio performance deviation; ***CV***: coefficient of variation of the validation set

Table 3. Coefficients of correlation between chemical composition and *in vitro* digestibility

Parameter	CP	NDF	ADF	ADL
DMD_{TT}	0.86	-0.86	-0.87	0.31
OMD_{TT}	0.88	-0.86	-0.86	0.33
DMD_{GV}	0.84	-0.89	-0.88	0.31
$NDFD_{GV}$	0.77	-0.68	-0.72	0.12

DMD_{TT}: *in vitro* dry matter digestibility measured according to Tilley & Terry method; **OMD_{TT}**: *in vitro* organic matter digestibility measured according to Tilley & Terry method; **DMD_{GV}**: *in vitro* dry matter digestibility measured according to Goering & Van Soest procedure; **$NDFD_{GV}$**: *in vitro* neutral detergent fibre digestibility measured according to Goering & Van Soest procedure; **CP**: crude protein; **NDF**: neutral detergent fibre; **ADF**: acid detergent fibre; **ADL**: acid detergent lignin

The best predictive equation for $NDFD_{GV}$ was obtained using a second derivative with a gap of 10 and a segment of five data points, respectively. Nevertheless, either calibration or validation statistics were poorer than for the other *in vitro* parameters, despite that a higher number of terms were selected in the equation of prediction (11). It may be attributed to the fact that NIR spectra are related to both the physical structure and the chemical composition of the samples, and $NDFD_{GV}$

showed lower coefficients of correlation with the chemical composition than DMD_{TT}, OMD_{TT} or DMD_{GV}.

Nevertheless, differences in the precision of the reference methods could also explain these results. In this sense, consecutive analytical procedures are required for determining NDF content of the feed and residues of incubation and, probably for that, the coefficient of variation for the determination of $NDFD_{GV}$ (4.46%) was higher than for DMD_{TT} (1.77%), OMD_{TT} (2.17%) or DMD_{GV} (2.01%).

Conclusion

The present study has demonstrated that, in herbage samples of different botanical composition, NIRS can be used to predict accurately dry matter and organic matter digestibility measured with different analytical procedures. Nevertheless, prediction of NDF digestibility was less successful, partly due to the slightly lower precision of the reference method.

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