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Fecal-FT-NIRS as a Noninvasive Tool for Assessing Diet Quality of Mediterranean Deer

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ABSTRACT

In order to assess the diet quality of two Mediterranean deer species we developed and validated a Fourier transform near-infrared diffuse reflectance spectroscopy methodology on feces (Fecal-FT-NIRS) for the determination of acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, C:N ratio, and enzymatic digestibility of organic matter (EDOM). We used rumen contents and fecal samples from 149 red deer (*Cervus elaphus hispanicus*) and 111 fallow deer (*Dama dama*) from southeast Spain ($n = 520$ observations). Spectra from the feces were related with rumen conventional chemical analysis through chemometric regression with partial least-squares (PLS). Specific predictive equations from red and fallow deer data separately were generated, as well as merged equations after pooling all deer samples. All the predictive equations had a high linearity with high correlation coefficients ($r = 0.8 – 0.99$). The selected equations had a reliable accuracy considering the root-mean-square errors of prediction (RMSEP), calibration (RMSEC), and cross-validation (RMSECV) in relation to the range of values for which the NIRS calibration was set for each parameter. Broad-based equations from combined samples were demonstrated as being useful for all nutritional parameters determination in red and fallow deer simultaneously. Equations obtained for the red deer data were also successfully applied to fallow deer and vice versa for NDF, ADF, C:N, and lignin determination, while for EDOM assessment the specific equations for each species were more accurately applied. Once validated, the Fecal-FT-NIRS technique can be considered as a suitable noninvasive tool for monitoring deer diet quality variations in Mediterranean environment. This method has the possibility to overcome interspecific barriers of direct fecal analysis by using rumen digesta as its reference method.

Introduction

The N and C content of vegetation is one of the plant characteristics of vital importance for herbivores, involving all the metabolic processes, as well as the cellular structure. Because N is an element of the amino acids that make up proteins, it is the most common constituent used to evaluate the nutritional quality of the diet of wild (Leslie and Starkey, 1987; Irwin et al., 1993; Hodgman et al., 1996; Blanchard et al., 2003) and domestic ruminants (Arman et al., 1975; Moore et al., 2004). This is positively correlated with the digestibility of the diet as well (Irwin et al., 1993). Increased C:N ratio due to reductions in the concentrations of N and increases in structural carbohydrates lead to foliar quality decrease (Couture et al., 2012).

Other constituents such as fiber, the main component of the plant cell wall, have been widely studied since their analysis was developed in the 1960s by Van Soest and coworkers (Van Soest, 1963; Van Soest and Wine, 1967) as indicators of forage quality. Lignin content also represents the structural investment of plants, which influences their physical properties via their involvement in architectural support and defense (Freschet et al., 2010). Because digestion in ruminants is mostly inversely correlated with the rate of lignification (Hatfield and Fukushima, 2005), studying this issue is important because it gives useful information about the quality of diet consumed. Similarly, the use of cellulases appears to be a good alternative for evaluating forage quality and a method that could offer a high correlation with in vivo results in digestibility studies (Jones and Theodorou, 2000).

However, all these analyses are unsuitable for addressing many of the nutritional ecological questions involving large amounts of samples because they are too laborious and expensive. Dietary evaluation analysis has traditionally involved laboratory-based wet chemistry techniques, which especially for lignin are difficult and require skilled personnel in order to achieve accurate and reproducible results. The cost of chemicals and time are high when using these standard approaches, and many of the analyses require large quantities of

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Table 1
ADF, NDF, lignin, ratio C:N, and EDOM (% w/w) of red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) rumen samples measured by wet chemical analysis used as references for PLS calibration and for external validation of equations.

		ADF		NDF		Lignin		C:N		EDOM	
	N	mean ± SD	min-max	mean ± SD	min-max	mean ± SD	min-max	mean ± SD	min-max	mean ± SD	min-max
					Used in chemometric calibration and internal cross-validations						
Cervus	127	49.7 ± 5.2	35.2-61.5	66.8 ± 6.4	46.3-85.6	31.9 ± 5.5	22.6-78.8	10.6 ± 2.1	7.4-18.1	31.9 ± 7.0	21.3-55.9
Dama	90	44.4 ± 5.5	26.8-58.9	64.4 ± 8.3	39.8-93.5	27.9 ± 4.3	18.0-38.0	10.7 ± 2.9	6.5-19.4	35.7 ± 8.3	21.3-68.2
					Used in additional external validation						
Cervus	22	47.1 ± 4.6	39.9-56.8	63.2 ± 7.8	45.1-74.9	29.7 ± 4.2	23.5-43.7	9.8 ± 1.5	7.4-13.5	34.6 ± 7.5	23.7-51.3
Dama	21	44.5 ± 5.7	32.1-54.0	61.8 ± 7.2	40.7-72.7	26.2 ± 2.3	22.1-30.4	10.1 ± 2.7	6.9-17.9	34.9 ± 5.4	23.3-44.7
All deer	260	47.1 ± 5.9	26.8-61.5	65.1 ± 7.4	39.8-93.5	29.8 ± 5.3	18.0-78.8	9.8 ± 2.5	6.5-19.4	33.6 ± 7.6	21.3-68.3

hazardous chemicals such as concentrated sulfuric acid (Van Soest et al., 1991).

In contrast, the near-infrared spectrometry (NIRS) methodology offers many advantages over standard methods: It employs simple sample preparation methods (drying and grinding) and, once validated, avoids the problems of organic and other chemical waste disposal, using nontoxic or corrosive reagents (Mark et al., 2002). Except for the high initial cost of the acquisition of equipment, it is low cost, chemical free, and rapid uses nondestructive analyses (Landau et al., 2004).

The feasibility of predicting diet quality by analyzing near infrared spectra of the feces, termed "Fecal-NIRS," has been established for a long time (Lyons and Stuth, 1992). Directly using fecal NIR spectra related to attributes of the diet is considered a novel and exciting application to NIRS spectroscopy (Dixon and Coates, 2009). Because chemical composition can be predicted from fecal spectra as accurately as from direct analysis of feeds (Landau et al., 2004), near infrared reflectance has been widely applied in the agricultural industry to evaluate the nutritional quality of domestic herbivore feeds (Leite and Stuth, 1995; Walker et al., 2002; Boval et al., 2004; Landau et al., 2005, 2006, 2008; Fanchone et al., 2009), as well as to monitor the nutritional status and ecology of grazing herbivores (Dixon and Coates, 2009), including wild deer (Dryden, 2003).

However, to our knowledge no previous information is available on using infrared spectroscopy techniques on fecal samples to predict diet quality for Spanish wild deer. As for its limitations, NIRS can offer high prediction percentages only when the samples belong to the same population type as that used to set up the equation.

Therefore the main aim of this study was to develop an NIRS methodology in order to monitor diet quality of two free-ranging Spanish deer populations. Predictive equations suitable for ADF, NDF, lignin, C:N ratio, and EDMO determinations to assess the diet quality of red (*Cervus elaphus hispanicus*) and fallow (*Dama dama*) deer were performed by using Fecal-FT-NIRS coupled with a PLS as a chemometric regression method. In order to find a global equation useful for determining diet quality in both species, we tested our equation by pooling red and fallow deer samples in addition to testing the samples separately.

Table 2
PLS calibration statistics of ADF predictive equations for red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and both species conjunctly. Second derivative and 10 PLS factors were used.

Equation	RER[1]	RMSEC[2]	RMSEP[3]	RMSECV[4]	Corr. Coeff.	Spectral region
1.Cervus	**5.25**	**0.60**	**5.01**	**5.06**	**0.99**	**1657.8-2223.7**
2.Cervus	5.04	0.56	5.22	4.58	0.99	1377.-2398.1
3.Dama	**8.67**	**0.63**	**3.7**	**6.27**	**0.99**	**1377-2398.1**
4.Dama	8.25	0.51	3.89	6.91	0.99	1657.8-2223.7
5.Merged	**4.58**	**1.12**	**7.56**	**7.19**	**0.98**	**1100-2500**
6.Merged	4.20	1.22	8.25	7.70	0.98	1000-2500

(Equations: 1. Cervus, 3. Dama, 5. Merged = Equation from chemometric calibration of red deer, fallow deer, all deer samples, respectively). The gray lines represent the best of the 6 equations represented in the table.

[1]Residual error value.
[2]Root-mean-square error of calibration.
[3]Root-mean-square of standard error of prediction.
[4]Root-mean-square error of cross-validation.

Methods

Deer Sampled and Area Studied

The material used in this study consisted of rumen content and feces ($n = 260$ x 2), which were collected from 149 deer (*Cervus elaphus hispanicus*) and 111 fallow deer (*Dama dama*) hunted during 2008 and 2009 in Lugar Nuevo (LN), an enclosed official estate located in the Sierra de Andújar Natural Park, in the southeast of Spain (38°9′N 4°3′W). Animals were eating while hunted and samples of 250 g of rumen contents and 5–10 g feces were collected directly from the rectum immediately thereafter. Recently collected rumen contents and feces were kept under refrigeration in field coolers for less than 1 hour and then kept at -20°C until further laboratory analysis. Only deer with nonmacerated rumen contents were sampled, as advised by Djordjevic et al. (2006), in order to avoid as much as possible partially digested materials.

Ruminal Content Chemical Analyses

Van Soest and coworkers assayed a system for characterizing the fibrous portion of forages (Van Soest, 1963; Van Soest and Wine, 1967). This system divided the cell walls into fractions, which were insoluble in ADF or NDF solutions. So, following the standard method of Van Soest and Wine (1967), first the dry matter content of the 260 rumen samples was determined by drying in an oven to constant weight using the standard methods (AOAC, 1995), and then NDF and ADF were estimated.

The enzymatic digestibility of organic matter (EDOM) was calculated in two steps: 1) extracting the feed with a neutral detergent solution and 2) incubating with a cellulose enzyme solution (McLeod and Minson, 1982). The pepsin-cellulase method described by Jones and Hayward (1975) was used, considering certain modifications in the cellulase concentration (1 g/L solution), incubation time (24 or 48 h), and type of final wash (hot distilled water or acetone). The enzymatic digestion was carried out by adding 25 ml of enzyme solution to each sample, incubation time 24 h at 40°C. After incubation, samples were vacuum filtered and the residue was washed with hot distilled water and then oven dried at 105°C until reaching constant weight. Thereafter, the crucibles were incinerated at 550°C for 1.5 h in an electric muffle furnace, cooled in desiccators to room temperature and reweighed to obtain the indigestible organic matter.

For lignin determination we used the Tappi T222 om-88 norm (1998). In this method, lignin is defined as the insoluble component

Table 3
PLS calibration statistics of NDF predictive equations for red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and both species conjunctly. Second derivative and 10 PLS factors were used.

Equation	RER[1]	RMSEC[2]	RMSEP[3]	RMSECV[4]	Corr. Coeff.	Spectral region
7.Cervus	**4.46**	**1.74**	**8.81**	**8.73**	**0.96**	**2083.3-2500**
8.Cervus	4.92	2.47	7.98	10.52	0.92	2066.1-2377
9.Dama	**4.2**	**1.75**	**12.8**	**9.70**	**0.98**	**2083.3-2500**
10.Dama	5.67	2.28	9.48	8.99	0.96	2066.1-2377
11.Merged	**5.95**	**3.63**	**9.03**	**8.97**	**0.86**	**2083.3-2500**
12.Merged	6.24	4.3	8.6	9.22	0.80	2066.1-2377

(Equations: 1. Cervus, 3. Dama, 5. Merged = Equation from chemometric calibration of red deer, fallow deer, all deer samples, respectively). The gray lines represent the best of the 6 equations represented in the table.

[1]Residual error value.
[2]Root-mean-square error of calibration.
[3]Root-mean-square of standard error of prediction.
[4]Root-mean-square error of cross-validation.

in a 72% sulfuric acid solution. The lignin content measured with this method had good correlation with the lignin content determined by direct methods such as analytical pyrolysis in woody elements (Alves et al., 2006), and this is the most widely accepted analytical method for determining the lignin content in woody constituents (Carrier et al., 2011). We chose this sulfuric acid method instead of the acid detergent fiber lignin method (ADL), most used in herbaceous and grazing ruminants, because of the high content of woody elements in the diet of deer, which are browsers during long constraint periods eating forbs and woody elements (Bugalho and Milne, 2003; Azorit et al., 2012).

Elemental Analyzer (Thermo Finnigan Flash EA1112) by Micro-Dumas Combustion was used for total N and C analysis, and then the C:N ratio was obtained. Samples from rumen for these determinations were previously freeze-drying lyophilized and ground to uniformity.

Fecal Spectral Data Collection

Fecal samples were freeze-dried lyophilized, then dried to constant mass in an oven at 60°C. Each sample was ground with a grinder (Bosch, MKM 6003), controlling the particle size (12-18 μm), because this has a great effect on the spectra NIR (Casler and Shenk, 1985; Windham, 1987), although the fineness of the particles is less important than the size distribution of uniform particles in a sample (Shenk and Westerhaus, 1993a, 1993b; Dryden, 2003). We acquired spectra using a near-infrared reflectance spectrophotometer FT-NIR Antaris (MDS) equipped with an integrated sphere efficiency above 95% and an InGaAs detector for working in the region of 833–2630 nm. Samples were scanned over the wavelength range 1000–2500 nm. After each sample measurement, the glass of the sampler where the sample was placed was cleaned with distilled water and dried carefully afterwards. The scan number was optimized to 160 scans, by adequate signal-to-noise ratio, uniting in a single spectrum.

Chemometric Analysis and Calibration Model Development

Spectral data and chemometric analysis for PLS calibration were processed using TQ Analyst 6.1.1 and software from the Thermo Nicolet Corp. PLS regression is based on spectral decomposition in which the original variables are replaced by the so-called latent variables, which are linear combinations of the original ones. For this calculation PLS uses the spectral and concentration information resulting from ruminal content chemical analyses and maximizes the covariance between them, thus achieving latent variables that are directly related to the constituents of interest. In the initial

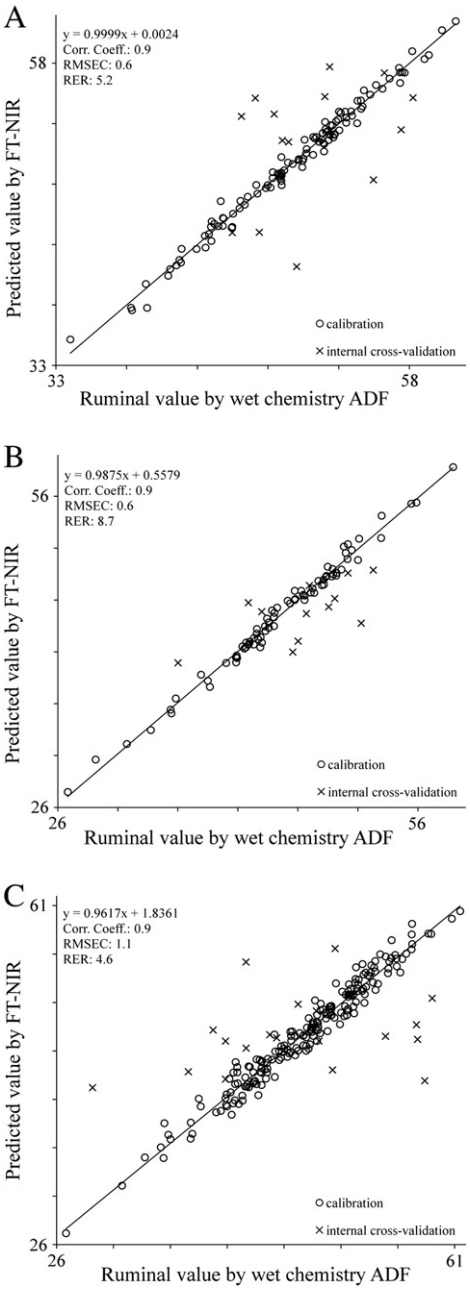

Fig. 1. Linear regression relationship between Fecal-FT-NIR predicted values and ADF values measured using standard wet chemistry techniques. **A,** ADF calibration model from red deer set (equation 1. Cervus). **B,** ADF calibration model from fallow deer set (equation 3. Dama). **C,** ADF calibration model from all deer sets (equation 5. Merged).

database only two values were eliminated before performing calibration because their being out of range may have been due to mistakes detected during the analysis with standard chemical methods. Later, no outliers were detected within a minimum standardized H distance of 6.0 from their nearest neighbors (Shenk and Westerhaus, 1993a, 1993b). Different equations are obtained by combining distinct spectral regions with different mathematical pretreatments of the spectrum, first and second derivatives. The best model was found when the correlation coefficient *(r)* was the higher value and the RMSEC was the lower. The predictive ability of the calibration model, as well as its accuracy, were assessed by calculating the root-mean-square error of cross-validation (RMSECV) of the

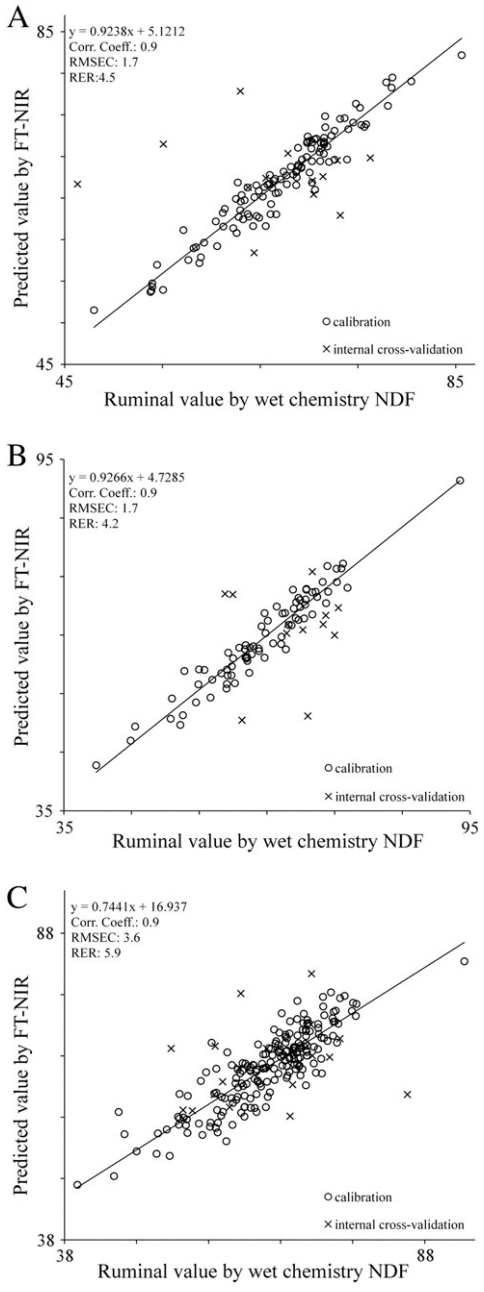

Fig. 2. Linear regression relationship between Fecal-FT-NIR predicted values and NDF values measured using standard wet chemistry techniques. **A,** NDF calibration model from red deer set (equation 7. Cervus). **B,** NDF calibration model from fallow deer set (equation 9. Dama). **C,** NDF calibration model from all deer sets (equation 11. Merged).

standard error of prediction (RMSEP). We assessed the accuracy of NFD, ADF, lignin, C:N ratio, and EDOM prediction through equations obtained with the data of red and fallow deer separately, as well as after pooling all deer samples together.

Predictive Equations: Selection and Validation

The quality of calibration was assessed from the correlation coefficient and the root-mean-square error of calibration, while the predictive ability of the calibration model and its accuracy were assessed by calculating the RMSECV and standard RMSEP. The residual error value (RER) was also used as a validation statistic. These indicators are dimensionless statistics, meaning they can be compared on the same basis between different models (AACC, 1999; Williams, 2001; Workman, 2001; Feam, 2002). The RMSEP measures the accuracy of prediction (the difference between the true and estimated values). We used RER as range/RMSEP, which together with the RMSECV provides the average uncertainty that can be expected for prediction of true samples. With values of RER ≥ 4 the calibration is acceptable for sample screening, with 8–12 the calibration is acceptable for quality control, and if RER ≥ 12 the calibration is good for quantification (Millmier et al., 2000).

Table 4
PLS calibration statistics of lignin predictive equations for red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and both species conjunctly. Second derivative and 10 PLS factors were used.

Equation	RER[1]	RMSEC[2]	RMSEP[3]	RMSECV[4]	Corr. Coeff.	Spectral region
13.Cervus	5.54	0.36	4.54	5.32	0.99	2223.7-1374.7
14.Cervus	**5.86**	**0.62**	**4.29**	**5.13**	**0.99**	**2449.2-1286.5**
15.Dama	6.93	0.20	2.88	4.25	0.99	2223.7-1374.7
16.Dama	**6.89**	**0.31**	**2.90**	**3.85**	**0.99**	**2449.2-1286.5**
17.Merged	6.49	1.55	4.58	6.99	0.96	1666.6-1333.3
18.Merged	**5.85**	**1.6**	**5.08**	**5.74**	**0.96**	**2449.2-1286.5**

(Equations: 1. Cervus, 3. Dama, 5. Merged = Equation from chemometric calibration of red deer, fallow deer, all deer samples, respectively). The gray lines represent the best of the 6 equations represented in the table.
[1]Residual error value.
[2]Root-mean-square error of calibration.
[3]Root-mean-square of standard error of prediction.
[4]Root-mean-square error of cross-validation.

Results

Wet Chemical Analysis and Fecal-FT-NIRS Spectra

Through chemical analysis we determined the ADF, NDF, lignin, C:N ratio, and EDOM contents of red and fallow deer ruminal samples and used it for both chemometric calibrations ($n = 217$) and external validation ($n = 43$) (Table 1). The overall mean ADF content was 47.1 ± 5.9, while the mean NDF content was 65.1 ± 7.4. The overall mean lignin content was 29.8 ± 5.3, the C:N ratio was 9.8 ± 2.5, and mean digestibility was 33.6 ± 7.6 considering the average for both deer species. These sets of samples, randomly selected from a natural population throughout the whole year, had a wide range of values appropriate for NIRS calibrations. We detected significant seasonal differences ($P < 0.001$) for all diet constituents. Validation sets had similar means and SD to the full data set, and the variability in the concentration of parameters in the samples was also considered suitable for developing an NIR calibration.

Acid Detergent Fiber Content

PLS calibration statistics and different prediction equations for ADF results for the model of the total variability were performed. The most significant results with PLS models using different spectral regions are summarized in Table 2. The second derivative and 10 PLS factors were used in all cases, and latent variables were selected on the basis of the RMSECV, which should be minimized. The lowest calibrations error (RMSEC) was used as an indicator of calibration quality, while the accuracy of prediction of the models was assessed on RMSEP, which should be as low as possible and similar to RMSECV, as well as the RER, which was considered as the better test for the quality of the model (Millmier et al., 2000). Through linear regression we related the values predicted by Fecal-FT-NIR and the reference values of the selected calibration equations derived from deer, fallow deer, and

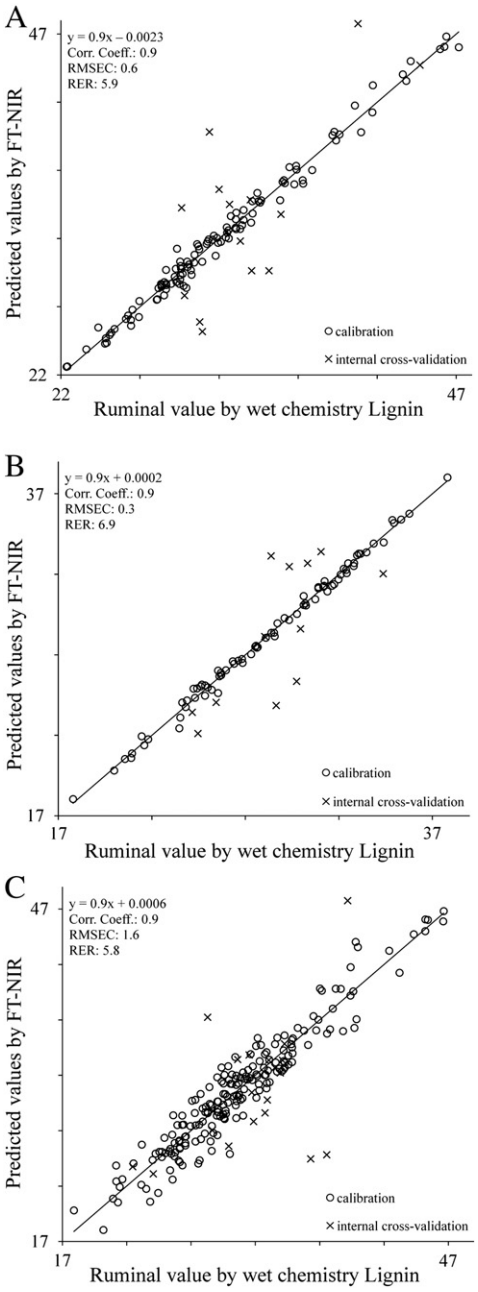

Fig. 3. Linear regression relationship between Fecal-FT-NIR predicted values and lignin values measured using standard wet chemistry techniques. **A,** Lignin calibration model from red deer set (equation 14. Cervus). **B,** Lignin calibration model from fallow deer set (equation 16. Dama). **C,** Lignin calibration model from all deer sets (equation 18. Merged).

pooled samples (Fig. 1). The selected equations had a high linearity with correlation coefficients *(r)* around 0.99, and a reliable accuracy considering the RMSEP, RMSEC, and RMSECV in relation to the range of values for which the NIRS calibration was set for each parameter. The RER index ranged from 4.20–8.67 (Table 2, Fig. 1).

Neutral Detergent Fiber Content

The PLS calibration statistics and performance of six predictive equations for NDF determination are shown in Table 3. The selected equations for NDF determination had high linearity with correlation coefficients *(r)* ranging between 0.80 and 0.98 and a reliable accuracy with RMSEP, RMSEC, and RMSECV near the standard deviations of the reference data of values for which the NIRS calibration was set for each parameter, as well as their range. The RER index ranged from 4.2–6.24 (Table 3, Fig. 2). Fig. 2 plots the regression of the better calibration.

Table 5
PLS calibration statistics of C:N predictive equations for red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and both species conjunctly. Second derivative and 10 PLS factors were used.

Equation	RER[1]	RMSEC[2]	RMSEP[3]	RMSECV[4]	Corr.Coeff	Spectral region
19.Cervus	3.69	0.50	2.88	2.18	0.97	1686.9-2376.9
20.Cervus	**3.17**	**0.52**	**3.35**	**2.68**	**0.96**	**1600-1800.2**
21.Dama	4.87	0.97	2.65	3.56	0.94	2500-2200.22
22.Dama	**4.39**	**0.52**	**2.94**	**3.36**	**0.98**	**1686.9-2376.9**
23.Merged	4.89	1.59	2.64	3.05	0.80	2190.1-2455.8
24.Merged	**4.01**	**0.55**	**3.22**	**3.00**	**0.97**	**1000-2500**

(Equations: 1. Cervus, 3. Dama, 5. Merged = Equation from chemometric calibration of red deer, fallow deer, all deer samples, respectively). The gray lines represent the best of the 6 equations represented in the table.
[1]Residual error value.
[2]Root-mean-square error of calibration.
[3]Root-mean-square of standard error of prediction.
[4]Root-mean-square error of cross-validation.

Lignin Calibration Model and Predictive Equations

Several equations for the prediction of chemical components were obtained separately with the data of red and fallow deer and also after pooling all deer samples to generate a broad-based equation. The second derivative and 10 PLS factors were used in all cases. Following the statistical indicators we selected six predictive equations for determining lignin. All models for lignin determination gave correlation coefficient values > 0.96 and acceptable RMSEC. The best equations were 14.Cervus, 16.Dama, and 18.Merged with RMSEP and RMSECV values ranging from 2.88–5.08 and 3.85–6.99, respectively (Table 4, Fig. 3).

C:N Ratio Calibration Model

Table 5 shows the PLS calibration statistics and performance of the best index prediction equations for C:N. Fig. 4 shows the best calibration regressions. The quality of the calibration models and the high correlation of the NIR spectra of the samples were confirmed by a high correlation coefficient in fallow deer from 0.96 to 0.98 (Table 5, Fig. 4). In this case the equations had reliable accuracy with RMSEP, RMSEC, and RMSECV near the standard deviations of the reference data values for which NIRS calibration was set. The RER index ranged from 4.89–3.17 (Table 5, Fig. 4).

Enzymatic Digestibility of Organic Matter

The PLS calibration statistics and better performance of EDOM equations are shown in Table 6. The quality of the calibration models and the high correlation of the NIR spectra of the samples' digestibility were verified by a high correlation coefficient for red deer and fallow deer of 0.96–0.99 (Table 6, Fig. 5). In this case the red deer equations had a higher standard RMSECV and RMSEP in relation to the range of reference values, so a lower RER than in the case of the fallow deer and merged equations was obtained (Table 6).

Discussion

Samples of rumen measured by wet chemistry analysis for ADF and NDF in both species were similar to those resulting from analysis in

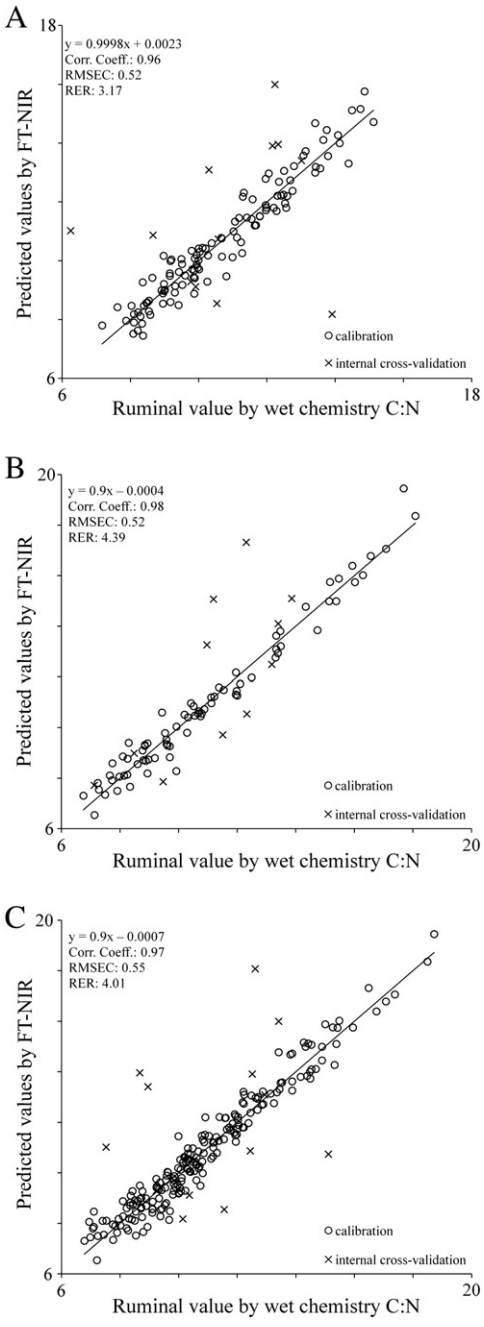

Fig. 4. Linear regression relationship between Fecal-FT-NIR predicted values and measured values using standard wet chemistry techniques. **A,** C:N calibration model from red deer set (equation 20. Cervus). **B,** C:N calibration model from fallow deer set (equation 22. Dama). **C,** C:N calibration model from all deer sets (equation 24. Merged).

goats (Cerón et al., 1996; Illius et al., 1999) and other deer species (Hodgman et al., 1996). The values of lignin in goats were lower than those found in our study, due to a difference between the feeding of farm goats and feeding of deer in our study, which were in an environment having to adapt to different food availability with seasonal variations. Although seasonal variation was not measured, overall ADF, NDF, and lignin concentrations in rumen digesta were lower from fallow deer and lower EDOM from red deer. This may be due to the fact that red deer browse more than fallow deer (Azorit et al., 2012).

Monitoring the nutritional quality, well-being, and diet selection of free-ranging deer has been an essential part of big game management for a long time (Hodgman et al., 1996). Studies on nutritional

Table 6
PLS calibration statistics of EDOM predictive equations for red deer (*Cervus elaphus*), fallow deer (*Dama dama*), and both species conjunctly. Second derivative and 10 PLS factors were used.

Equation	RER[1]	RMSEC[2]	RMSEP[3]	RMSECV[4]	Corr. Coeff.	Spectral region
25.Cervus	**3.55**	**1.45**	**13**	**20.33**	**0.99**	**1000- 2500**
26.Cervus	1.86	5.25	24.8	22.59	0.96	2022.6-2416.6
27.Dama	**6.36**	**0.69**	**7.39**	**10.89**	**0.99**	**1000- 2500**
28.Dama	4.86	1.94	9.67	10.26	0.97	2022.6-2416.6
29.Merged	**5.82**	**1.22**	**8.01**	**8.06**	**0.98**	**1000- 2500**
30.Merged	4.55	1.55	10.3	9.22	0.98	1000- 2500

(Equations: 1. Cervus, 3. Dama, 5. Merged = Equation from chemometric calibration of red deer, fallow deer, all deer samples, respectively). The gray lines represent the best of the 6 equations represented in the table.

[1]Residual error value.
[2]Root-mean-square error of calibration.
[3]Root-mean-square of standard error of prediction.
[4]Root-mean-square error of cross-validation.

quality attributes such as diet fiber variation, as well as digestive efficiency, are also needed in order to properly understand adaptation mechanisms in ecological research (Robbins et al., 1995; Perez-Barbería et al., 2004; Codron et al., 2007). But many of the nutritional ecological questions involve large amounts of samples difficult to obtain in wild deer in the natural environment. Fecal-NIRS methodology using fecal spectra, without requiring fecal wet chemical analysis, is an interesting tool for noninvasive monitoring of deer diet quality in the natural environment, allowing us to obtain the suitable amount of data required in ecological research. Furthermore, by using rumen digesta samples consisting of the combination of plants eaten by deer, this study became an integrative assay, allowing us to monitor directly the diet selected by deer.

We developed and validated a Fecal-FT-NIRS coupled with PLS for diet deer quality assessment of free-ranging Spanish deer. The prediction of dietary ADF, NDF, lignin, C:N ratio, and EDOM in red and fallow deer can be accomplished via fecal NIRS. Equation performance statistics (*r*, RMSEP, RMSEC, RMSECV, RER) are similar to previous reports for both wild and domestic ruminants.

ADF, NDF, lignin, and C:N ratio content are chemical properties of the diet and relatively easily measured. Digestibility, on the contrary, is more difficult to determine, owing to the effects of the animal in question; it is not just a chemical component of the feed. Thus it follows that the prediction of diet digestibility with fecal NIRS would be less successful than that of ADF, NDF, lignin, and C:N ratio, the inherent error associated with quantifying diet digestibility being greater than that associated with diet fiber, lignin, and nitrogen. The success of an NIRS prediction equation for any diet constituent will ultimately depend on the reliability of the chemical reference method. This will of course include all aspects of the process including sampling, not just the chemical technique in and of itself.

Sampling rumen and feces obtained directly from the animal instead of collecting them from the ground improved the identification of samples and their conservation and avoided the contamination of the samples, as well as the degradation of the main constituents.

The accuracy of the predictive equations for fecal NIRS compared with wet chemistry values was good, owing to the fact that all models gave correlation coefficient values > 0.80, as obtained in goats (Glasser et al., 2008), which are considered to be excellent (Williams, 2001; Workman, 2001). In addition, the low RMSECV values, indicative of satisfactory accuracy, as well as the acceptable RMSEC and RER in relation to the range of reference values, also indicate the usefulness of Fecal-FT-NIRS for estimating red and fallow deer diet quality parameters. Lignin is a noncarbohydrate cell wall component, the structure of which is highly complex and variable according to diet. We obtained useful equations for lignin determination with low errors of calibrations ranging between 0.2 and 1.6,

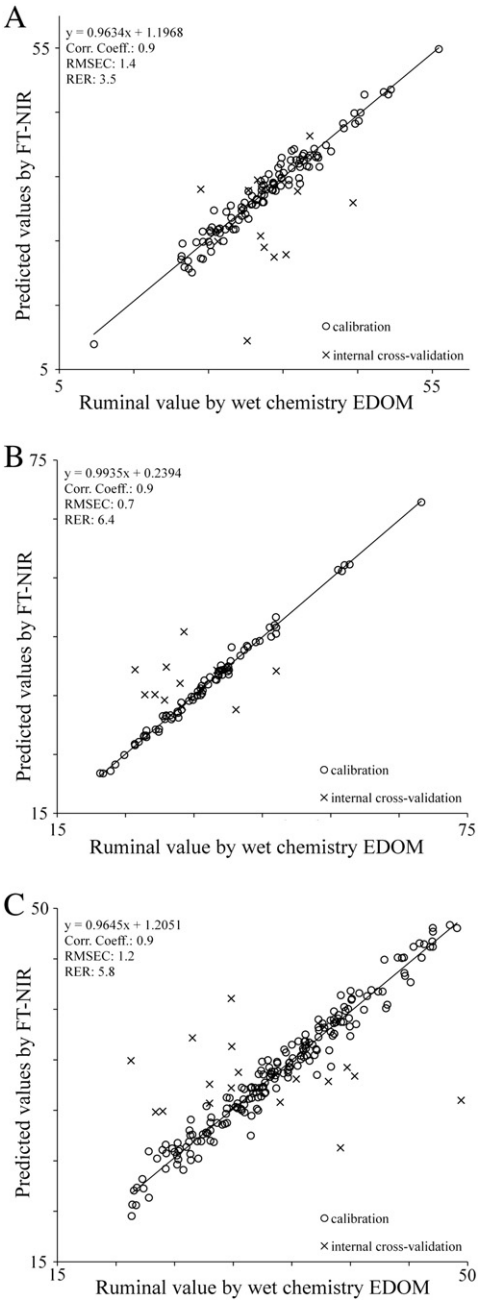

Fig. 5. Linear regression relationship between Fecal-FT-NIR predicted values and EDOM values measured using standard wet chemistry techniques. **A,** EDOM calibration model from red deer set (equation 25. Cervus). **B,** EDOM calibration model from fallow deer set (equation 27. Dama). **C,** EDOM calibration model from all deer sets (equation 29. Merged).

lower than those reported in deer (Keating, 2005), and correlation coefficients from 0.96 to 0.99, which are considered to be excellent (Williams, 2001; Workman, 2001). These are greater than those obtained in pasture (Pullanagari et al., 2011) and herbage ingested by sheep (Fanchone et al., 2007) but similar to acacia (Yao et al., 2010) and other woody lignin determinations (Hodge and Woodbridge, 2004). RMSEP and RMSECV values were slightly higher than similar studies but all within acceptable limits (Dryden, 2003).

The degree of accuracy and precision obtained with fecal NIRS for the measurement of dietary ADF, NDF, lignin, C:N ratio, and EDOM in red and fallow deer indicates that this technique could be employed to obtain near real-time assessments of both animal nutrient status and forage resource quality.

Although incompatibility for prediction models between species has been found in previous studies (Aufrère et al., 1996), our broad-based equations from combined red and fallow deer samples were useful for ADF, NDF, lignin, C:N ratio, and EDOM determination in red and fallow deer simultaneously, producing a single model to obtain the values for both species. Moreover, equations obtained for the red deer data were also successfully applied to fallow deer and vice versa, as was corroborated by an external validation, correctly determining new samples not included in the calibration set. Regarding the statistical quality indicators, the equations can be classified as good for sample screening and quality control (Millmier et al., 2000) and good enough for accurate determinations in order to monitor variations in free-ranging Mediterranean deer diet.

Implications

The calibration of fecal samples with a spectrophotometer in infrared reflectance (NIRS) requires time, expertise, and a large number of samples with known reference values with precision and reliability for each parameter analyzed. However, except for the initial cost of the NIRS equipment acquisition, analysis of samples is not expensive. Once validated by statistical indicators and even by an external verification of the calibration equations ability in order to predict new samples from the same deer populations, Fecal-FT-NIRS appears to be an integrative, noninvasive, simultaneous tool for monitoring diet quality variations of two deer species. Allowing rapid analysis of large amounts of fecal samples, this technique should be considered of interest to be used in ecological research monitoring of Mediterranean deer. This method had the possibility to overcome interspecies barriers of direct fecal analysis by using rumen content as its reference method.

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