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Fecal NIRS prediction of dietary protein percentage and in vitro dry matter digestibility in diets ingested by goats in Mediterranean scrubland☆

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Abstract

A method to elucidate the nutritional value of goats' diets in ligneous environments is needed. Near infrared spectroscopy (NIRS) can be applied to fecal material to establish statistical relationships between the reflectance of fecal samples in the near infrared region and the diets consumed. We tested fecal NIRS for the prediction of dietary crude protein content (CP) and in vitro dry matter digestibility (DMD) in Damascus goats browsing Mediterranean scrubland dominated by *Pistacia lentiscus* and *Phyllirea latifolia*. Two datasets consisting of pairs of feces and dietary data were used; for the first ($n = 151$), goats were hand-fed different ratios of legume hay and concentrate ($n = 60$), or combinations of three browse species and concentrate ($n = 91$), while for the second ($n = 153$), data were collected during 10 observation days with grazing goats. On these days, pairs of dietary information and feces were derived from one observed goat but feces were also sampled from 12 to 15 resident goats grazing in the same paddock. Each dataset served for the validation of NIRS equations established with the other. For CP, in dataset 1, the values of R^2 for calibration were 0.98 (all data) and 0.90 (only browse) and the standard error of cross validation (SECV, internal validation) was 0.50%. When samples from dataset 2 were used for external validation, R^2 varied between 0.55 (all goats) and 0.85 (observed goats) and the standard error of prediction (SEP, external validation) was 2.2 and 1.6% in the same order. Using dataset 1, we determined that the condition for 95% confidence that two goats ate the same diet was a Mahalanobis distance (H) of less than 0.5 between their fecal spectra. Using dataset 2, the values of R^2 for calibration varied between 0.90 (all goats) and 0.92 (only observed goats and residents with $H < 0.5$ from their fecal spectra) and SECV was approximately 0.50%. When samples from dataset 1 were used for external validation, R^2 was 0.47 (calibrations with all goats) and 0.50 (only observed goats and resident goats with $H < 0.5$ from the observed goats), and SEP varied between 0.8 and 1.2% in the same order. Lower R^2 and accuracy were found for in vitro DMD than those for CP. A dataset containing feces and dietary information from observed goats

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seems to be accurate and cost-effective to implement fecal NIRS determination of dietary CP and DM digestibility in grazing goats.

Keywords: Goat; Range management; Near infrared; Mediterranean pasture; Grazing

1. Introduction

Goats are used for brush control and ecological management of Mediterranean scrubland (Perevolotsky and Seligman, 1998). Farmers are willing to cooperate with communities in this important role on the condition that profitability is not impaired, i.e., the diets of goats are compatible with their production goals. They need a method to evaluate the daily intake of nutrients and in particular of protein, the first limiting factor on the range, in order to supplement the animals, if needed. In heterogeneous environments, this information can be acquired by time-consuming observations and hand-clipped reconstituted diets (Kababya et al., 1998), but such technology is not relevant for farm conditions.

The importance of fecal chemical composition in understanding nitrogen and energy status in goats was demonstrated by Nunez-Hernandez et al. (1992). The "fecal NIRS" technology, pioneered in goats by Leite and Stuth (1995) is aimed at predicting the dietary percentages of nutrients (Leite and Stuth, 1995; Landau et al., 2004) or botanical composition (Walker et al., 2002; Landau et al., 2004). The method relies on the multivariate calibration of the relationship between dietary composition and the spectra of feces scanned with a near infrared spectrometer (NIRS), followed by a validation process.

A typical database for fecal NIRS consists of pairs of fecal spectra and nutrient analyses (reference values, in NIRS jargon) of the diets consumed by the animals, therefore, the value of NIRS calibrations depends strongly on the quality of dietary information keyed into the database. In stall-fed goats, samples of individual goat diets and residues can be easily obtained, making for easy determination of the nutrients ingested (Landau et al., 2004). In contrast, the assessment of diets consumed by goats in heterogeneous environments is a complex task: throughout a year, the average number of species ingested by goats, on a daily basis, in Mediterranean woodland and scrubland, was 9 (Kababya et al., 1998). Some authors have coped with that complexity by using oesophageally fistulated animals. The dietary composition established from oesophageally cannulated animals and fecal samples collected from non-fistulated resident animals grazing in the same paddocks have served to establish fecal NIRS calibrations of dietary CP and in vitro digestibility in goats and beef cattle (Leite and Stuth, 1995 and Lyons and Stuth, 1992, respectively). However, diets selected by cannulated animal may be different from those of non-cannulated resident animals (Coates et al., 1987; Jones and Lascano, 1992), and animal welfare considerations limit the use of cannulated animals. Direct observation (Kababya et al., 1998; Agreil and Meuret, 2004) could be used as an alternative to cannulated animals as means to obtain the dietary information needed in fecal NIRS calibrations involving grazing small ruminants. In order to determine if an observed sheep is representative of the resident counterparts, Agreil and Meuret (2004) have used the Mahalanobis spectral distance (H) measured between the NIRS fecal spectra of observed and resident sheep based on the assumption that in a randomly distributed population, 99% of the population has H numbers smaller than 3. There is no information regarding the optimal ratio of observed to resident animals for fecal NIRS determination of goats' diets. Stall-feeding goats with reconstituted diets consisting of browse species is also attractive: Coates (1998) reported that better calibration statistics for dietary content of CP and digestibility were obtained in stall-fed than oesophageally cannulated grazing animals, probably because dietary reference values are more precise under confined condition. Given the complexity of goat feeding behaviour, it is clear that multi-species diets must be used for calibration because, according to Coleman et al. (1995), NIRS equations cannot be extrapolated beyond the conditions represented in calibration samples.

Finally, adequate validation procedures are critical to ensure the robustness of calibrations under a

complexity of diets selected by goats. Two estimates of accuracy are used to evaluate NIRS calibrations: standard error of prediction (SEP) represents the variability in the difference between predicted values and reference values when the equation is applied to an external (i.e., not used in any step of the calibration) validation data set, SECV represents the variability in the difference between predicted values and reference values when the equation is applied sequentially to subsets of data from the calibration data set. The SECV procedure may give over-optimistic results, in particular if data are replicated, but is justified in situations with randomly selected calibration samples from a natural population (Naes et al., 2002b). Fecal NIRS equations of dietary CP and DOM percentages developed in goats ranging in the Savannah region of Texas were found by external validation to be robust under conditions prevailing in southern Texas (Leite and Stuth, 1995). However, no statistical criteria for the possible extension of their validity to other environments have ever been published to our knowledge.

In the framework of a program aimed in establishing methodologies to monitor Mediterranean ecosystems, the aims of this study were to investigate: (i) if the dietary percentages of CP and in vitro DMD in goats feeding in Mediterranean scrubland can be predicted by fecal NIRS calibrations developed in confined goats; (ii) statistical considerations based on spectral distances for the elaboration of fecal NIRS calibrations by using observed and resident ranging goats.

2. Materials and methods

For this study, we used two datasets consisting, in total, of 304 pairs of feces and dietary data derived from 15 Damascus and 12 Saanen goats. Dataset 1 ($n = 151$) was based on an experiment with goats fed under confinement. A preliminary analysis of this dataset is presented by Landau et al. (2004). In dataset 2 ($n = 153$), data were obtained from grazing goats and from Saanen goats fed hay and concentrate from a previous experiment (Landau et al., 2002).

2.1. Animals and diets for dataset 1

Twelve Damascus yearling goats (mean weight of 38.5 ± 0.7 kg) were used for this study. The goat facility consisted of roofed individual dust-floor pens (1.7 m × 1.7 m) and of a roofed collective corral where animals were placed in between tests. The individual pens were sufficiently large not to alter the daily pattern of intake or activity of the goats. Pens were closed together in order to reduce the cage effect on behaviour. Each pen had a 15-l water bucket and a trough divided into two separate compartments for concentrate and hay. A shelf was placed under each trough in order to facilitate residue collection. Diets comprised of concentrate in combination with legume hay ($n = 60$) or four species of browse ($n = 91$): *Pistacia lentiscus* L., *Phyllirea latifolia* L., *Calicotome villosa* (Poiret) Link, and *Pinus brutia* Ten (Table 1). Browse branches were cut daily. Diets were weighed and distributed once every morning. Rough composition is presented in Table 1 and diets are fully detailed in Landau et al. (2004). The study consisted of twelve 10-day tests. On the morning of day 6 of each test, pens were thoroughly cleaned of any residues before the distribution of rations. On days 7–10, residues were

Table 1
Composition of diets during the tests carried out in confinement (dataset 1)

Dietary components	*n*	Mean DMI (g/day)	Percentage of component in diet (% of DM)
Legume hay	60	564	57.2–79.5
Concentrate		252	20.5–42.8
P. lentiscus	18	738	55.2–72.7
Concentrate		411	27.3–44.8
P. latifolia	22	878	56.2–78.8
Concentrate		411	21.2–43.8
P. lentiscus	12	246	13.3–25.2
P. latifolia		646	46.5–57.8
Concentrate		348	28.3–28.7
P. brutia	12	895	63.6–78.2
Concentrate		364	21.8–36.4
P. brutia	12	317	16.8–43.8
P. lentiscus		466	32.9–60.1
Concentrate		277	17.4–34.8
P. brutia	12	301	9.1–37.5
P. lentiscus		233	12.1–27.1
P. latifolia		429	23.3–45.2
Concentrate		278	25.7–39.0
P. latifolia	3	623	42.1–47.7
C. villosa		492	30.7–37.8
Concentrate		295	19.8–20.2

collected every morning before feeding, and weighed on a scale with ±0.5 g accuracy. On days 9–10, feces were grab-collected at three different times in the morning, mid-day and evening. Mean intake of days 6–9 was taken as the daily intake of each goat. This procedure resulted in 151 pairs of faeces and diet (Table 1) of which 60 pairs resulted from hay-and-concentrate diets, and 91 from diets comprising of one to three browse species in addition to concentrate.

2.2. Pastures and animals (dataset 2)

The study was conducted at the south of the Carmel ridge, Israel (32°25′N, 34°52′E). The region is characterized by an average yearly rainfall of 600 mm, and a rainy season of 180 days from October to April. The ecosystem can be described as disturbed Mediterranean woodland (garrigue) featuring steep, rocky slopes with scarce patches of shallow soil. The vegetation was dominated by low trees (mainly *P. latifolia*) and shrub-like low trees (*P. lentiscus* and *C. villosa*) forming a 2–3 m high coppice round islets sometimes covered with climbing *Rubia tenuifolia* Dum.-Urville, *Clematis cirrhosa* L. and *Smilax aspera* L. Some isolated green (*Quercus calliprinos* Webb) and Tabor (*Quercus ithaburensis* Decaisne) oak trees, as well as carob (*Ceratonia siliqua* L.), and buckthorn (*Rhamnus alaterna* L.) trees could also be found. Some bushes of *Ephedra foemina* Forskk., *Asparagus stipularis* Forskk., *Sarcopoterium spinosum* L. Spach were occasionally located between the coppices. From January to mid-May, green annual herbaceous vegetation covered the soil patches. Observations were conducted in three paddocks of 0.1 ha each. In one of the paddocks grazed in summer, shrubs had been removed to ground level in spring 2003, in order to diversify the diets. A group of 12–15 goats grazed daily from 08:00 to 13:00 h under the supervision of an observer.

2.3. Observations and feces sampling (dataset 2)

Observations of goat foraging behaviour were conducted on 10 different periods of 1 day from February to August, 2004. Candidates for observation were not used if the continuous presence of the observer at a distance of approximately 1 m interfered visibly with normal foraging behaviour. After a 5-day period of acclimation to a new spaddock and presence of the observer, the observations were initiated as described by Kababya et al. (1998) with the difference that only a single, different goat was observed continuously throughout each of the 10 observations days. Observations were recorded using a voice-activated tape-recorder. In brief, when a focal goat started to eat, the observer recorded the time, the word “eat”, the species grazed or browsed, the number of bites removed and their size and anatomical category (small, medium or large, leaf, stem or fruit). In order to estimate the quality of the diet selected by the goats, a hand-simulated grazing technique was employed in which bite-like samples were clipped from the bushes previously grazed by the observed animal. Goats stayed on the same paddock for at least 3 days after an observation day. On the second and third days, feces were grab-collected from the observed goat as well as her resident counterparts (n = 11–14) at three different times in the morning, mid-day and evening and a composite sample within animal across times and days was used for NIRS scanning.

2.4. Chemical analyses

All samples (feed and feces from database 1, bite-like samples and feces from database 2) were air dried at 60 °C for 48 h in a ventilated oven and ground to pass a 1 mm sieve. Samples were re-dried at 60 °C for 1 h and left to equilibrate in a desiccator at ambient temperature for 1 h before scanning. Crude protein (CP) was analyzed in diets according to AOAC (1984). In vitro digestibility of dry matter (IVDMD) was according to Tilley and Terry (1963). These attributes in percentages, botanical composition, and intake values were used to derive the percentages of nutrients later to be used as reference values in the NIRS calibrations.

2.5. NIRS and statistical procedures

Fecal samples were packed into sample cells with a near-infrared transparent quartz cover glass and scanned between 1104 and 2492 nm in 2 nm increments using a Foss NIRSystems 5000 NIR reflectance monochromator spectrometer (Foss Tecator, Hoganas, Sweden) in order to collect NIR spectra as log(1/R) where R = reflectance. Before development of calibration equations, raw spectral data were transformed with the standard normal variance (SNV) and detrend procedures to remove non-linearity that results from

light scattering (Barnes et al., 1989). Mathematical treatments were used to enhance spectral differences where "1, 4, 4, 1" or "2, 6, 6, 2", where the numbers represent the derivative, gap width over which the derivative is calculated, the number of points in a moving average, i.e., first smoothing procedure, and the number of nm over which the second smoothing is applied, respectively (ISI, 1999). Calibration equations were developed on the treated spectral data, using the modified partial least-squares routine of the WinISI II software (ISI, 1999). Before final calibration, equations were calculated, outlier passes were made to remove observations with $T > 2.5$ (ISI, 1999). The quality of prediction by equations was evaluated by the coefficient of determination (R^2), i.e., the proportion of variability in the reference data accounted for by the regression equation and the standard error of calibration (SEC) that represents the variability in the difference between predicted values and reference values. The accuracy of calibrations was evaluated by the aid of cross-validation (with SECV as estimate of quality).

Principal component analysis was conducted to verify that goats fed similar diets excrete feces of similar spectral characteristics. For dataset 1, the standardized Mahalanobis distance (H) for the nine best scores between the fecal spectra of goats fed a test diet and those of goats fed reference diets identical or different from the test diets was calculated. The distance was calculated to the population centroid by using an average computed spectrum. In addition, nearest neighbor distances were calculated between each sample of the tested diet and each of three randomly selected spectra from goats fed the same or another diet given as reference diet. In dataset 2, for each observation day separately, the Mahalanobis (H) distances between the fecal spectra of the observed and each of the resident goats, and between the spectrum of each goat and the average fecal spectrum of all the goats, were calculated (see formulas in Naes et al., 2002a).

Prediction equations for dataset 2 were first established using only spectra from observed animals. In the second step, equations were successively recomputed by adding animals with H distances less than 0.5, 1.0, 2.0 and 3.0. Finally, a calibration was computed, including all animals.

For external validation, goats from database 1 that consumed diets containing both *P. lentiscus* and *P. latifolia* served to validate calibrations derived from dataset 2. Calibrations derived from dataset 1 were validated in two steps according to the recommendation of Coleman et al. (1995) that NIRS equations should not be extrapolated beyond the conditions represented in calibration samples. Calibrations derived from goats fed browse in dataset 1 were validated using fecal spectra and diets from observed or all grazing goats in dataset 2. Calibrations derived from all goats, i.e., including those fed hay and concentrate, were validated with all dataset 2, i.e., including spectra from Saanen goats fed hay and concentrate. R^2 and the standard error of prediction served to assess the quality of validation and T-tests were used to check the slopes of curves against unity.

3. Results

3.1. Do goats feeding on similar diets have similar fecal spectra?

Table 2 presents Mahalanobis distances computed with data from goats in dataset 1 that received *P. lentiscus* and *P. latifolia* as parts of their diets. H is computed to have unit variance, hence $H = 1$ when the test and reference diets were the same. All animals fed a diet with *P. lentiscus* as the sole source of browse had $H > 3$ when compared to a group average spectrum or to a randomly selected spectrum, when the browse source of the reference diet was *P. latifolia*, and vice versa. Thus, these browse sources produce very different fecal spectra which NIRS is capable of distinguishing. Furthermore, all goats fed a test diet always excreted feces that did not differ from the average spectrum of their group, i.e., $H < 3$. However, within diets, when the reference spectrum was selected randomly, the average H ranged between 1.9 and 2.5, and some of the goats (17%) had spectra which were quite different ($H > 3$) from three reference animals that consumed the same diet. In other words, when comparing to a single animal, diets which are truly the same could appear to be different if the criterion for difference was $H > 3$.

In a theoretical paddock offering two browse species, observed and resident goats can consume a diet that consists of either one of the two species or a combination of both. Would fecal spectra classify diets correctly? If goats consumed two browse species, most of them (11/12) were correctly classified as outliers by

Table 2
The Mahanalobis (H) distance between the fecal spectra of goats (left column) fed concentrate and *P. Platifolia* ($n = 17$), *P. lentiscus* ($n = 18$) or *P. latifolia* + *P. lentiscus* ($n = 12$) and the average fecal spectrum, or three random fecal spectra of goats fed reference diets

Tested diet	Reference diet	Diets serving as reference			
		Average fecal spectrum		Random fecal spectra	
		Mean H	$H > 3$	Mean H	$H > 3$
P. latifolia	*P. latifolia*	1.0	0/17	1.9–2.5	1–3/17
P. lentiscus	*P. lentiscus*	1.0	0/18	1.9–2.0	2–4/18
P. latifolia	*P. lentiscus*	4.4	17/17	4.5–5.2	17/17
P. lentiscus	*P. latifolia*	4.5	18/18	4.6	18/18
P. latifolia + *P. lentiscus*	*P. latifolia*	4.3	11/12	4.0–4.4	11–12/12
P. latifolia + *P. lentiscus*	*P. lentiscus*	4.1	12/12	3.9–4.1	11–12/12
P. latifolia + *P. lentiscus*	*P. latifolia* + *P. lentiscus*	1.0	0/12	1.9–2.5	1–5/12

using the H distance relative to reference goats that consumed only one browse species. However, up to 5/12 goats were false outliers on the basis of $H > 3$, relative to counterparts that consumed the same mixed diet. It is clear, therefore, that an H threshold of 3 is not adequate to classify diets when fecal spectra of a group ("residents") are compared with the spectrum of an individual because the H value representing the spectral distance between a reference goat and counterpart goats eating the same diet can frequently be more than 3.

Notwithstanding, in all analyses, considering 47 fecal samples that resulted from *P. lentiscus* and *P. latifolia* diets in dataset 1, irrespective of the reference spectrum, all fecal samples with $H < 0.5$ belonged to goats that consumed a diet identical to that of the reference (Fig. 1). In analyses with up to three browse species (not shown), only 6% of fecal samples with $H < 0.5$ from a reference goat were misclassified. To summarize, $H < 0.5$ can be considered a threshold value at 95% confidence to ascertain that two goats eat the same diet.

3.2. Diets consumed by goats in dataset 2

Diets consumed by observed goats are presented in Table 3. As expected, the relative proportions of *P. latifolia* and *P. lentiscus* in goats' diets were higher when observations were carried out in the paddocks where no brush clearing had been carried out.

The spectral distance between average group spectra and the spectrum of observed animals were lower than 3 (not shown) at the exception of one observation day. The spectral distance from resident to observed goats was greater than 3 for 0 to 6/15 resident goats, depending on observation day (Table 4). When fecal spectra of observed and resident goats were compared, When all observation days are merged, the spectral distance between observed and resident goats is less than 0.5 for 22% of the fecal samples, and less than 3 for 80% of the samples (Fig. 2).

3.3. Calibration and validation performances

Calibrations developed with confined goats were very satisfactory with R^2 greater than 0.94 (Table 5) for the whole of dataset 1. R^2 was lower for goats fed browse, but values were still greater than 0.85. SECV values were similar when all goats or only those fed browse in dataset 1 were used.

Validations with external samples yielded lower R^2 values than calibrations (Table 5 versus Table 6). The validations, using data from observed and resident goats of dataset 2, of calibration equations obtained from browse-fed goats in dataset 1 featured low bias and acceptable SEPs, but extremely low R^2 values for CP and in vitro DMD (Table 6) with negative slopes for in vitro DMD. This was putatively attributed to the low range of CP and in vitro DMD in the validation set and justified using calibrations from all dataset 1 and the inclusion of fecal spectra from Saanen goats to broaden the ranges of CP and DMD in the validation set. When this was done, the quality of validations, as expressed by higher R^2 and slopes closer to 1, was improved. R^2 decreased when resident goats were included in the validation population. Overall, for CP and in vitro DMD, the R^2 values of validations were reasonably high (0.85

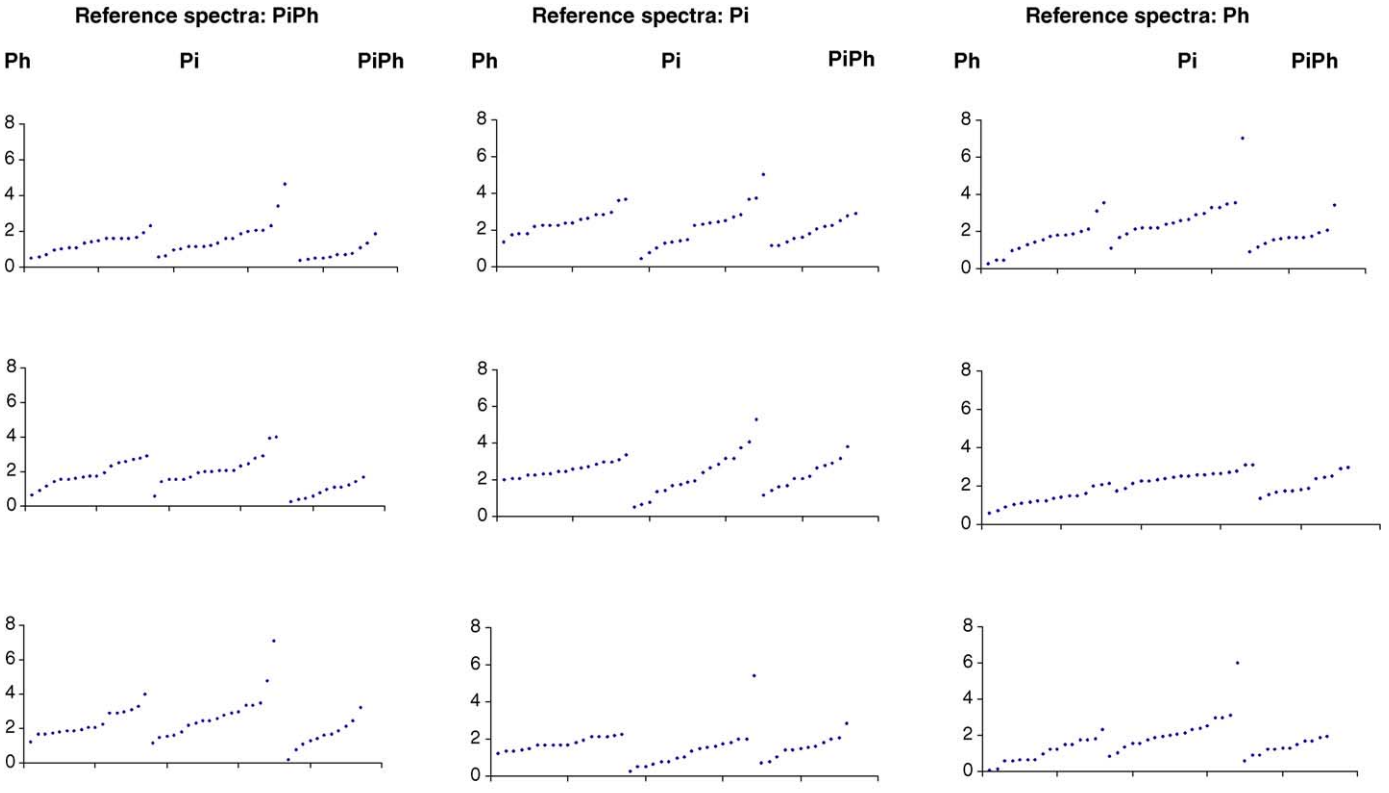

Fig. 1. Mahalanobis distance between 47 tested and three randomly picked fecal NIRS spectra. Goats consumed *P. latifolia* (Ph), *P. lentiscus* (Pi) or a combination of both browse species (PiPh).

Table 3
The intake at pasture (g of DM) and the composition of diets (% of DM) consumed by observed goats in paddocks where bushes had been removed (R) or not removed (NR) to ground level (dataset 2)

Month	Febraury	March	April	August	August	August	August	August	August	August
Paddock treatment	NR	NR	NR	R	R	R	R	R	NR	NR
Observation day	1	2	3	4	5	6	7	8	9	10
Intake	836	504	545	540	716	610	661	553	345	267
Diet composition										
Concentrate	11.6	19.2	17.7	17.1	12.9	15.1	14.0	16.7	26.8	34.5
Herbaceous	2.3	2.3	24.8	43.9	28.8	33.2	31.5	44.9	25.9	8.2
P. latifolia	26.1	22.9	26.8	1.3	2.8	14.0	6.9	15.1	23.5	42.3
P. lentiscus	35.4	40.4	15.8	0.5	1.6	3.9	3.0	4.8	21.9	7.9
Q. calliprinos	0.0	0.0	0.0	7.5	8.5	0.1	8.4	0.3	0.0	0.0
Q. ithaburensis	0.0	0.0	0.0	0.0	6.2	0.3	0.0	0.1	0.0	0.0
C. siliqua	0.0	0.0	0.0	0.9	5.4	8.8	4.1	0.0	0.0	1.7
R. alaternus	1.4	1.4	1.6	0.2	2.0	2.4	0.5	1.0	0.3	2.6
R. tenuifolia	1.8	0.5	0.6	5.2	10.8	0.1	0.7	2.3	0.0	0.0
S. aspera	4.8	0.5	2.7	12.5	7.3	1.0	2.9	6.0	1.4	1.8
E. foemina	0.0	0.0	0.0	7.1	7.7	0.1	1.6	0.0	0.0	0.1
A. stipularis	3.3	2.4	1.4	3.8	6.0	20.9	26.4	8.8	0.3	0.9
A. ramosus	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. cirrhosa	9.5	3.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C.vilosa	3.4	4.1	4.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O. europea	0.0	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. spinosum	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chemical composition										
CP	10.4	10.6	10.9	8.0	8.1	7.1	7.8	8.1	9.3	10.4
In vitro DMD	49.0	49.4	60.6	50.2	47.1	43.8	45.7	46.0	50.5	48.0

and 0.73, respectively), the slopes did not differ from 1 (1.05 and 1.25, with $t = 0.64$ and 0.15, respectively). The predicted mean value did not differ from the actual mean value, but the values of SEP were three times greater than those of SECV.

The number of observations in dataset 2 (Table 7) was too small to allow calibration of fecal NIRS equations without including residents. Resonably high R^2 and low SECV were obtained in the prediction of CP when residents with fecal spectra distance $H < 0.5$ from the observed goat were included in the calibration. The R^2 and SECV values in the CP calibration, 0.90 and 0.5, respectively, were little affected by further inclusion of fecal samples from resident goats with higher H values. The calibrations of in vitro DMD showed relatively poor R^2 values, even though SECV values were acceptable for all of them. For external

Table 4
The Mahanalobis (H) distance between the fecal spectra of observed goats and resident goats (database 2) during 10 observation days

	Average H	Number of residents with $H > 3$
Day 1	2.08	3/12
Day 2	1.36	1/12
Day 3	2.02	3/12
Day 4	2.44	6/12
Day 5	2.20	6/12
Day 6	1.37	1/15
Day 7	1.58	4/15
Day 8	1.10	0/15
Day 9	1.18	1/15
Day 10	1.80	3/15

Table 5
Calibration performance of diet composition in dataset 1

	N	Mean	S.D.	R^2	SEC	SECV
All dataset 1						
CP	142	12.3	2.9	0.98	0.42	0.50
In vitro DMD	144	59.7	10.4	0.97	1.71	2.14
Only diets with browse						
CP	80	10.0	0.96	0.90	0.29	0.43
In vitro DMD	81	52.1	5.40	0.85	2.13	2.28

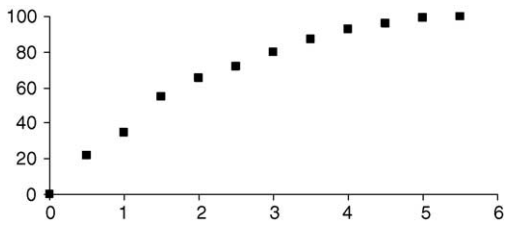

Fig. 2. Cumulative partition of H distances from resident to observed goats' fecal spectra, calculated separately for each observation day ($n = 153$). x-axis: Mahalanobis distance, y-axis: cumulative percentage.

validations (Table 8), R^2 values were low and slopes deviated significantly from 1, but the predicted and actual means for dietary CP were similar, and SEP was reasonably low (0.76–1.2, depending on the number of resident goats included in calibration). This indicates that the two sources of error precision (low R^2) and accuracy (slope not equal to 1) were off-setting. The SEP for in vitro DMD was extremely high, showing impaired robustness of the calibration to external validation.

Table 6
External validation with dataset 2 of fecal NIRS equations from dataset 1

	SEP	Means		Slope	R^2
		Predicted	Actual		
Calibration from browse only					
Validation samples					
Observed					
CP	1.1	9.1	9.1	0.71	0.21
In vitro DMD	5.7	49.0	48.5	−0.73	0.15
Observed + all residents					
CP	1.4	9.0	9.2	0.39	0.06
In vitro DMD	5.2	48.8	48.7	−0.36	0.04
Calibration from all dataset 1					
Validation samples					
Observed + Saanen					
CP	1.6	12.6	13.4	1.05	0.85
In vitro DMD	7.5	62.6	60.0	1.25	0.73
Observed + resident ($H < 1$) + Saanen					
CP	1.7	10.3	11.4	0.98	0.69
In vitro DMD	6.4	53.2	53.4	1.1	0.54
Observed + all residents + Saanen					
CP	2.2	9.5	11.2	0.98	0.55
In vitro DMD	6.5	50.8	53.2	0.95	0.40

Table 7
The calibration performance of fecal NIRS in dataset 2, using dietary reference values from observed goats only or from observed and resident goats, selected according to the spectral distance (Mahalanobis standardized H) between their feces and those of observed goats

	n	R^2	SEC	SECV
Only observed goats				
CP (% of DM)	10	0.21	1.21	1.53
In vitro DMD (%)	10	0.22	4.06	4.95
Observed + residents ($H < 0.5$)				
CP (% of DM)	35	0.92	0.35	0.48
In vitro DMD (%)	35	0.74	1.62	2.39
Observed + residents ($H < 1$)				
CP (% of DM)	51	0.88	0.43	0.57
In vitro DMD (%)	50	0.70	1.85	2.12
Observed + residents ($H < 2$)				
CP (% of DM)	91	0.90	0.40	0.53
In vitro DMD (%)	91	0.82	1.74	2.20
Observed + residents ($H < 3$)				
CP (% of DM)	111	0.90	0.40	0.52
In vitro DMD (%)	110	0.77	1.98	2.31
Observed + all residents				
CP (% of DM)	139	0.90	0.39	0.46
In vitro DMD (%)	141	0.84	1.69	2.10

Table 8
Validation, using fecal samples of goats fed diets containing *P. lentiscus* and *P. latifolia* in dataset 1 ($n = 36$), of calibrations sourcing from dataset 2

Calibration used	SEP	Means		Slope	R^2
		Predicted	Actual		
Observed + residents ($H < 0.5$)					
CP	1.2	9.5	9.2	0.36	0.50
In vitro DMD	3.4	50.8	49.8	0	0.10
Observed + all residents					
CP	0.83	9.6	9.2	0.62	0.47
In vitro DMD	8.6	42.7	49.8	0.40	0.25

4. Discussion

Because the aim of the fecal NIRS methodology in the present study was to predict the chemical composition of diets, its accuracy can be considered optimal if it compares positively to that of direct NIRS analyses of the diet components. The SECV values of direct predictions are approximately 0.9% for Mediterranean browse (Meuret et al., 1993) and 1% for a variety

of pasture grasses (Garcia-Ciudad et al., 1993). The SECV for NIRS-aided determination of in vitro DMD of natural pasture varies between 2.4 and 3.5% (Landau et al., 2005). The values of SECV in Table 5 derived from confined goats, and in Table 7, including observed and resident grazing goats, compare favorably with these figures.

Fecal NIRS calibrations are intended to be robust in a variety of conditions. The SECV procedure is justified only when calibration samples are randomly selected from a natural population, which was not the case in the calibrations presented in these tables. The risk that they provide over-optimistic estimates of accuracy, evoked by Naes et al. (2002b), is evidenced in the present study by the fact that SECV values (internal validation) are much smaller than SEP (external validation).

Looking at the calibration of dataset 1 established from browse-fed goats, it appears that its validation with grazing goats yields precise group results (no bias), low SEP (1.1–1.4%) but low R^2 and slopes that differ from 1. This is probably because the ranges of nutrient contents in the calibration set were low, with a coefficient of variation (CV, S.D./mean) of 10%. This statistical outcome of limited data in the extremes was also noted for cattle diets by Lyons and Stuth (1992). In the present study, when CV was doubled by including all goats in dataset 1, external validations were improved, and SEP, relative to the means, was unaffected (SEP 1.1% for an average of 9.1% CP is statistically similar to SEP 1.6% for an average of 13% CP). Comparing values in Tables 5 and 6, it appears that for CP and in vitro DMD, SEP was at least threefold higher than SECV. For these two parameters, we still consider that the calibrations based on dataset 1 are relatively robust to external validation because slopes do not deviate from 1, and mean predicted values are reasonably close to actual values, i.e., bias is small (less than 1% of dietary CP).

Lyons and Stuth (1992) reported R^2 values ranging between 0.45 and 0.93, and prediction errors ranging between 0.90 and 1.2%, in external validations of dietary CP with cattle. With goats, Leite and Stuth (1995) reported higher SEV (calculated with internal samples) than in calibrations derived from datasets 1 and 2: 1.28 versus 0.50% (Tables 5 and 7), respectively. Even though dietary CP was in a range between 4 and 26% in their study, compared with 9–16% in ours, prediction errors for internal validations are still lower in both datasets of our study than in theirs, relative to the means of dietary CP.

Table 7 presents the first fecal NIRS calibrations of dietary quality based on direct behavioural observations on grazing ruminants to our knowledge. Based on studies in cattle (Coates, 1998; Boval et al., 2004), it was expected that goats fed in confinement would provide reliable data for fecal NIRS calibrations of dietary CP. However, it is promising that data from observed grazing goats are not inferior to those of confined goats, and that all calibrations of dietary CP in our study are not inferior to those developed with oesophageally cannulated goats. However, the higher SEPs in our study, compared with Leite and Stuth (1995), suggest that their fecal NIRS calibrations were better fitted to predict dietary CP in external samples.

On the other hand, it seemed doubtful that confined animals could serve for calibrations aimed at predicting dietary attributes in grazing animals because the variety of feeds selected by goats (6–11 botanical components, with ever-changing chemical composition, Table 3) results in a huge variety of fecal spectra that cannot be simulated in browse-fed confined goats. On the other hand, it was uncertain that observations on grazing goats could provide dietary information good enough to be used for fecal NIRS predictions of dietary components. However, relative to the ranges of dietary CP represented in calibrations, SEPs obtained with confined or grazing goats were similar.

In the present study, as in the study by Leite and Stuth (1995), part of the pairs of feces diets belonged to resident animals, but these authors did not raise the question of their accuracy. The purpose of the statistical work with spectral distances was to ascertain that fecal spectra from resident animals may be safely used in the calibration, assuming that their diet was identical to that of the observed goat. Shenk and Westerhaus (1991) used a standardized H distance of 3 from the mean to exclude spectral outliers from a plant population and a threshold of 0.6 from sample to sample to eliminate spectrally redundant samples from a calibration set. Indeed, in browse-fed goats from dataset 1, we found that H distances between fecal spectra less than 0.5 give 95% confidence that the goats ate the same diet. The risk of spectral redundancy is more limited in feces than that in plant samples because numerous factors in addition to the plants eaten – in particular individual goat digestibility – add variety to fecal

spectra. Thus, the diet composition of residents with $H < 0.5$ can be safely deemed similar to that of observed goats on a daily basis. When this was done, the R^2 and SECV for dietary CP were as good in dataset 2 (pasture, Table 7) as in dataset 1 (confinement, Table 5), showing the potential of goat observations to implement fecal NIRS calibrations of dietary attributes in grazing animals. However, including only residents with $H < 0.5$ from an observed goat represents a waste of chemical information contained in 80% of fecal samples (Fig. 2). Lyons and Stuth (1992) and Coates (1998) for cattle, and Leite and Stuth (1995) for goats, used fecal samples from all residents to calibrate fecal NIRS equations of dietary CP and in vitro digestibility. Can animals with greater H distance from the observed goats be legitimately included in the calibration set?

At distances above 0.5 from an individual spectrum, H did not discriminate samples for diet composition in dataset 1 (Fig. 1). Globally, when computed from average spectra for a given diet, the number of samples with $H > 3$ is almost nil (Table 2), but when computed from randomly picked samples, the number of samples in the tested population with $H > 3$ can be as high as 20%. In other words, observed animals cannot be statistically considered equivalent to the mean of the group, and the assumption of Agreil and Meuret (2004) that $H < 3$ ensures similarity of diets is not supported by our results in goats. When using for fecal NIRS calibrations of botanical composition fecal spectra distant more than 0.5 in H from those of observed animals, there is a risk of keying in false reference values. Does that mean that spectra with $H > 0.5$ must be discarded? The answer depends on what attributes are to be predicted. The variety of feeds available for goats in dataset 2 was considerable. Percentages of CP varied between 7 (browse) and 22% (grasses) in February, and between 5 (dry grasses) and 16% (climbing plants) in summer, but dietary CP was kept in a relatively narrow range of 7.1–10.9% throughout all observation days. Kababya et al. (1998) demonstrated before that selectivity in goats ranging in scrubland results in steady CP concentration throughout the year. We hypothesize that differences in species composition were not necessarily accompanied by considerable differences in the percentages of dietary CP; therefore, the CP data keyed for calibrations may have been only slightly biased. Indeed, including gradually fecal samples with higher H values did not affect R^2 and SECV values for CP. When resident goats were associated to observed animals for external validations (Table 6), R^2 was decreased but prediction errors were slightly improved. Calibrations of dietary CP that included all resident goats or only those with $H < 0.5$ showed similar performance when submitted to external validation with external samples (goats fed combinations of *P. lentiscus* and *P. latifolia*). Our data shows that, at least for the determination of dietary CP, most resident animals can also be used for calibrations without deleterious effects.

Throughout our study, the R^2 values were higher, and errors of validation or prediction lower for CP than for in vitro DMD, as found by Coates (1998) for cattle. Boval et al. (2004) and Coates (1998) also came to the same conclusion by comparing the respective calibration performances of NIRS for dietary CP and in vivo OMD in confined steers. In cattle fed grass, the relationship between in vitro and in vivo DMD is not always straightforward (Coates, 1998), but in goats, this relationship is even more complex. This is because the NIRS spectrometer scans feces and "sees" chemical bonds resulting from in vivo indigestibility, whereas reference values on which equations rely are derived from an in vitro procedure. Leite and Stuth (1995) have lengthened the incubation time of the in vitro procedure and re-termed it "in vivo OMD". This correction may be helpful for Texas Savannahs but not for Mediterranean browse because the microbial fermentation in the in vitro procedure is impaired by the tannins contained in the browse. This results in underestimation of the energy value of browse for goats (Perevolotsky et al., 1993). A possible solution for this discrepancy could be to neutralize tannins by using PEG in the in vitro procedure (Jones and Palmer, 2000), or by using a Dacron bag in situ procedure (Kababya et al., 1998) and then verifying that the modified digestibility values are closer to those of in vivo, and use these values for fecal NIRS calibrations. We hypothesize that once a better appraisal of digestibility is available, fecal NIRS will be improved.

When comparing two strategies of fecal NIRS implementation, i.e., experiments in confinement versus goat observations at pasture, one has to take into account not only accuracy, but also economical cost. The best procedure would be to use only observed animals for calibration and for external validations, but the amount of labour necessary to implement fecal NIRS calibrations would be unrealistic. For a similar level

of accuracy, the strategy based on observed/resident goats was less costly labour and required fewer facilities than the strategy relying on feeding experiments in confinement.

5. Conclusion

Fecal NIRS has the potential to predict the dietary CP percentage in goats fed browse in confinement or freely grazing in browse-rich Mediterranean landscapes. If the aim is to predict CP percentage in grazing goats in a cost-effective way, the data necessary to implement robust fecal NIRS equations are more likely to be collected by goat observations than by attempts to imitate their diets under confined conditions. The spectral distance between feces excreted by resident and observed goats may be useful to decide if resident animals have consumed diets that are similar to those of observed animals. In this study, the maximal spectral distance to ensure that two goats had similar diet composition was very low ($H < 0.5$).

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