# A fecal near-infrared reflectance spectroscopy-aided methodology to determine goat dietary composition in a Mediterranean shrubland<sup>1</sup>

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**ABSTRACT:** An ecologically sound approach to the problem of brush encroachment onto Israeli rangeland might be their utilization by goats, but better knowledge of the feeding selectivity and ability of goats to thrive in encroached areas is required to devise viable production systems. Direct observation of bites could provide precise and accurate estimates of diet selection, but construction of a sufficiently large database would require too much time. The present study describes the first attempt to construct fecal near-infrared reflectance spectroscopy (NIRS) calibrations of the botanical and nutritional composition of the diet, and of the total intake of free-ranging goats, based on reference values determined with bite-count procedures. Calibration of fecal NIRS was based on 43 observations encompassing 3 goat breeds and 4 periods (spring, summer, and fall of 2004, and spring of 2005). Each observation comprised 242 min of continuous recording of the species and bite-type category selected by a single animal, on each of 2 consecutive days. The mass and chemical guality of each species and bite-type category-a total of more than 200,000 bites—were determined by using the simulated bite technique. Associated feces were scanned in the 1,100- to 2,500-nm range with a reflectance monochromator. Fecal NIRS calibrations had reasonable precision for dietary percentages of the 3 main botanical components: herbaceous vegetation (as one category;  $R^2 = 0.85$ ), *Phillyrea latifolia* ( $R^2 = 0.89$ ), and tannin-rich *Pistacia lentiscus* ( $\mathbb{R}^2 = 0.77$ ), with SE of cross-validation (SECV) of 7.8, 6.3, and 5.6% of DM, respectively. The R<sup>2</sup> values for dietary percentages of CP, NDF, IVDMD, and polyethylene glycol-binding tannins were 0.93, 0.88, 0.91, and 0.74, respectively, with SECV values of 0.9, 2.1, 4.3, and 0.9% of DM, respectively. The R<sup>2</sup> values for intakes of herbaceous vegetation, P. latifolia, and P. lentiscus were 0.80, 0.75, and 0.65, with SECV values of 71, 64, and 46 g of DM/d, respectively. The R<sup>2</sup> values for the daily nutrient intakes were below 0.60. Fecal NIRS data can be used to expand the databases of botanical and nutritional dietary composition when observed and resident animals graze simultaneously, but intakes should be calculated from fecal NIRS-predicted dietary DM composition and an independent evaluation of DMI.

Key words: browse, diet composition, feces, goat, near-infrared reflectance spectroscopy

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#### **INTRODUCTION**

Adverse socioeconomic conditions (El Aich et al., 1995) have caused extensively raised goat populations

in Mediterranean shrublands to dwindle, which has led to a large increase in the cover of woody brush species, an increased fire hazard, and reduced biodiversity (Perevolotsky and Seligman, 1998). Utilization by goats might be an ecologically sound approach to this problem, but better knowledge of their feeding selectivity and their ability to thrive in encroached areas is required to devise viable production systems.

Direct observation could provide precise and accurate estimates of diet selection (Agreil and Meuret, 2004), but the method is too time-consuming for construction of a sufficiently large database to clarify the effects of season, breed, and location on the propensity of goats

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to consume browse species. Fecal near-infrared reflectance spectroscopy (NIRS) can determine both the chemical (Leite and Stuth, 1995) and the botanical (Landau et al., 2004a) composition of goat diets, although the methodology must be applied with care. Walker et al. (2002) showed that applying calibration equations developed in one feeding trial to fecal samples gathered in another (i.e., external validation) yielded predictions of low accuracy. Indeed, Coleman et al. (1995) stated that NIRS equations cannot be extrapolated beyond the conditions represented in calibration samples, and Landau et al. (2005) demonstrated that similar structures of calibration and validation populations are a prerequisite for successful external validation of fecal NIRS equations. The present study describes the first attempt to construct fecal NIRS calibrations of the botanical and nutritional composition of the diet, and of the total intake of free-ranging goats, based on reference values determined with a bite-count methodology.

## MATERIALS AND METHODS

#### Grazing Site

The study was conducted at the south of the Mount Carmel ridge, Israel (32°25' N, 34°52' E), which is characterized by an average yearly rainfall of 600 mm and a 180-d rainy season from October to April. The ecosystem is a disturbed Mediterranean woodland (garrigue), characterized by steep, rocky slopes with sparse patches of shallow soil. The vegetation is dominated by low trees (mainly *Phillyrea latifolia* L.) and tall shrubs [mastic tree, *Pistacia lentiscus* L. and *Calicotome villosa* (Poir.) Link] that form 2- to 3-m high coppices around islets that are sometimes covered with climbing Rubia tenuifolia D'Urv., Clematis cirrhosa L., and Smilax aspera L. Isolated common (Quercus calliprinos, also named Quercus coccifera Webb) and Tabor (Quercus ithaburensis Decne) oak trees, as well as carob (Ceratonia siliqua L.) and buckthorn (Rhamnus lycioides L.) trees can also be found. Occasional bushes of Ephedra foemina Forskk., Asparagus stipularis Forskk., and Sarcopoterium spinosum L. Spach grow between the coppices. From January to mid-May, green annual herbaceous vegetation covers the soil patches.

Five fenced 0.1-ha plots and 4 unfenced plots that differed in aspect, slope, and botanical cover were used. Over the course of 4 seasons—spring, summer, and fall 2004, and spring 2005—foraging was rotated among the plots according to vegetation availability.

# Animals and Management

The goats were kept according to ICACG (Israel Council on Animal Care Guidelines, 1994).

In the spring and fall of 2004, the flock consisted of adult Damascus goats (n = 12). In summer 2004, these were culled and replaced with Damascus (n = 9), Boer

(n = 9), and Mamber (n = 9) yearlings, managed as 3 separate groups. The groups were led out to forage in the mornings and were housed at night in a dirt-floored and roofed building. During fall 2004 and spring 2005, foraging was rotated among seven 0.1-ha fenced plots and an unfenced area according to vegetation availability. The animals were shepherded only in the unfenced area. Adult and yearling does received a daily ration of 90 and 138 g of DM, respectively, of a commercial concentrate (Ambar Feed Mills Ltd., Hadera, Israel) containing 18% CP (DM basis).

# Collection of Dietary Data for Calibration

The dietary data required for the calibration of the fecal-NIRS procedure were collected in 2 stages. The first stage comprised direct and continuous observation of individual animals to determine the number of bites removed, by plant species and bite-type category. The second stage comprised collection of representative samples of each species and bite-type category for the determination of their mass and quality.

Observations on Goats and Observation Data Processing. Observations (n = 45) encompassed diets selected by adult Damascus does and yearling Boer, Mamber, and Damascus goats. The respective numbers of observations were 10, 11, 12, and 12. Goats were observed in 5 plots in 4 seasons: spring, summer, and fall 2004, and spring 2005. Each observation comprised 2 consecutive days of observation on the same animal. The distribution of observations among goat breeds and seasons is presented in Table 1.

Observations of foraging behavior were initiated after a 5-d period of acclimation to a new plot and always encompassed the entire day's foraging for the observed animal. Observations began between 0630 and 1040 h (average 0800 h) and terminated between 1030 and 1440 h. The duration of an observation day ranged from 213 to 300 min (average 242 min), with 85% of the observations lasting between 235 and 245 min. A complete observation (i.e., pair of observation days) was double this length. The observers were T. Glasser (n = 23), H. Muklada (n = 18), and a postgraduate student (n = 4).

An effort was made to observe as many animals as possible, but only 30 goats eventually served as the focal animals. Individual animals were not used for observations if the continuous presence of an observer at a distance of approximately 1 m interfered visibly with their normal foraging behavior. Observations were recorded with a voice-activated digital MP3 recorder. When a focal goat began to eat, the recorder was operated, time was automatically recorded, and the observer recorded a sequence of codes that combined species and bite-type category (small, medium, or large; leaf, stem, or fruit). A few of the bite-type categories defined consumption units that were not bites in the usual sense of the term. For example, *E. foemina* was consumed by severing a relatively long section of branch and then

Year	Season	Breed	Age	n	$BW \pm SEM, kg$
2004	Spring	Damascus	Adults	3	$53.5 \pm 1.3$
	Summer	Damascus	Adults	7	$51.2~\pm~1.0$
	Fall	Boer	Yearling	4	$20.9~\pm~1.1$
		Mamber	Yearling	4	$18.1~\pm~0.7$
		Damascus	Yearling	5	$31.8 \pm 0.5$
2005	Spring	Boer	Yearling	$7^{1}$	$31.6~\pm~1.7$
		Mamber	Yearling	$8^{1}$	$26.7~\pm~0.5$
		Damascus	Yearling	$7^{1}$	$36.4~\pm~0.7$

Table 1. Mean BW of the goats examined in the 45 observations, according to year, season, and breed

<sup>1</sup>Not all different individuals.

bringing it into the mouth by chewing; therefore, in summer 2004, each consumption unit of this species was recorded as the number of centimeters consumed. Table 2 shows the number of bite-type categories defined for each species in each year-season combination.

To trim periods of silence from recorded electronic voice files (.mp3) during the 4-h observations, timesignal (every 30 s) files (.wav) were created (Cool Edit Pro version 2.0, Adobe Systems, Inc., San Jose, CA) and combined with the voice files. Silent periods were trimmed by using Sonic Foundry (version 6.0, Sonic Foundry Inc., Madison, WI). This procedure resulted in significantly shorter files (1 to 1.5 h). The bite count and time data from the trimmed files were then manually keyed into an Excel spreadsheet. A total of 195,660 true bites and 27,921 consumption units (species-category combinations that were not true bites) were recorded.

Simulated Bites Collection. To estimate the goats' intake and the quality of the diets they selected, a technique of manually simulated grazing was used. Bitelike samples and samples representing consumption units that were not true bites were clipped so that the sample collection combined species and bite-type cate-

**Table 2.** The number of bite-type categories defined for each species in each year-season combination

Species	Spring 2004	Summer 2004	Fall 2004	Spring 2005	Total
True bites					
Allium sp.	_	_	_	1	1
Asparagus aphyllus	3	3	3	3	12
Asphodelus ramosus	2	1	3	5	11
Calicotome villosa	8	_	2	5	15
Ceratonia siliqua	_	3	2	_	5
Clematis cirrhosa	3	_	_	5	8
Cyclamen persicum	_	_	_	1	1
Eryngium creticum	_	_	_	1	1
Euphorbia sp.	_	_	_	1	1
Olea europaea	3	_	_	_	3
Phillyrea latifolia	4	3	4	4	15
Pistacia lentiscus	3	3	5	3	14
Pistacia palaestina	_	3	_	_	3
Prasium majus	_	_	_	3	3
Quercus calliprinos	_	3	_	_	3
Quercus ithaburensis	_	2	_	_	2
Rhamnus lycioides	3	3	3	4	13
Rubia tenuifolia	3	3	3	6	15
Sarcopoterium spinosum	3	_	1	5	9
Scabiosa prolifera	_	_	_	1	1
Sinapis arvensis	_	_	_	1	1
Tamus communis	_	_	_	3	3
Unidentified	1	1	_	_	2
Other consumption units					
Ephedra foemina	_	1	6	4	11
Herbaceous, dry	2	4	1	_	7
Herbaceous, green	3	_	_	1	4
Smilax aspera	3	3	5	5	16
Grand total	44	36	38	62	180

gories, according to the recorded foraging behavior. The intake of herbaceous vegetation was evaluated by cutting "estimated mouthful" samples and intake of E. foemina by clipping phyllode segments of various lengths. This resulted in a total of 17,555 bite-like samples, of which 4,188, 3,095, 5,072, and 5,200 samples were collected in spring, summer, and fall 2004, and spring 2005, respectively. The DM contents were assessed immediately after collection by drying the bitelike samples at 60°C for 48 h in a forced-air oven. Greater temperatures could not be used because of the volatile components, especially phenolics, in browse foliage. Bite weights were then calculated by combining species and bite-type categories. Total species daily intakes were calculated as the weighted product of the number of bites and category bite-weights and summed into total intake for each 2-d observation.

Laboratory Analysis of Simulated Bites and Calculation of Nutrient Intakes. To obtain amounts large enough for laboratory analysis, bite-like samples were merged into 180 species and bite categories, to yield 41, 40, 40, and 59 samples for spring, summer, and fall 2004, and spring 2005, respectively. The samples were then ground to pass a 1-mm sieve. The IVDMD was evaluated according to the methods of Tilley and Terry (1963). The CP was assayed by using an automated Kjeldahl method (976.05; AOAC, 1990), and NDF and ADF were assayed according to the methods described by Goering and Van Soest (1970). The content of polyethylene glycol-binding tannins (**PEG-b-T**) was determined by NIRS without extraction, according to the methods of Landau et al. (2004b).

The intakes of CP, NDF, ADF, in vitro-digestible DM, and PEG-b-T were calculated from the sum of bites per species and category, multiplied by estimated bite weight, expressed on a DM basis, and the chemical composition and dietary nutrient percentages were calculated as nutrient intakes divided by DMI, in which the latter comprised pasture plus concentrate.

# Collection of Feces for Calibration

The goats stayed in the same plot for at least 3 d after an observation day. On the second and third days, feces were grab-collected from the anus of the observed goats in the morning, at midday, and in the evening, and a composite sample for all the times and both days for each animal was dried at  $60^{\circ}$ C in a ventilated oven for 48 h and ground to pass a 1-mm sieve. At 0600 h on the days of fecal sampling, the animals (without feed or water restriction) were weighed with a model Merav 2002 electronic balance, with an accuracy of ±10 g (Shekel Balances, Rosh Ha-Ayin, Israel).

# NIRS Procedures

*Preparation of Fecal Samples.* Fecal samples were redried at 60°C for 1 h, allowed to equilibrate in a desiccator at ambient temperature for 1 h, packed into sample cells with a near-infrared-transparent quartz cover glass, and scanned at wavelengths from 1,104 to 2,492 nm in 2-nm increments with a Foss NIRSystems (Hoganas, Sweden) model 5000 NIR reflectance monochromator spectrometer to collect near-infrared spectra as log(1/R), where R is reflectance.

NIRS Calibration Equation Development. Before the calibration equations were developed, raw spectral data were transformed with the standard normal variance and detrend procedures to remove the nonlinearity caused by light scattering (Barnes et al., 1989). Mathematical treatments were used to enhance spectral differences of "1, 4, 4, 1" or "2, 6, 6, 1," in which the numbers represent the derivative, the gap width over which the derivative is calculated, the number of points in a moving average (i.e., first smoothing procedure), and the nanometers over which the second smoothing is applied, respectively (Infrasoft International, 1999). Population outliers were searched for by using the Mahalanobis distance between each of the fecal samples and a mean spectrum of the calibration population (Shenk and Westerhaus, 1991). A modified partial least squares regression (Martens and Naes, 1987) was used to develop the calibration equations, in which stored NIRS spectra from fecal samples were the independent variables and nutritional attributes were the dependent reference data.

The calibration precision was evaluated according to the multiple  $\mathbb{R}^2$  (i.e., the proportion of variability in the reference data accounted for by the regression equation). The SE of calibration defined the variability in the differences between predicted and reference values. The calibration accuracy was evaluated by cross-validation and expressed as the SE of cross-validation (**SECV**). The SECV is the average root mean square difference between the predicted and reference values when the equation is calculated and applied sequentially to subsets (of which there were 4 in the present study) of data from the calibration data set. The SECV procedure may give overly optimistic results, especially if the data are replicated, but is justified in situations in which the calibration samples are randomly selected from a natural population (Naes et al., 2002). When regressions of observed vs. predicted values were examined, the closeness of slopes to unity and of intercepts (bias) to zero served as criteria for the usefulness of the calibrations.

# RESULTS

#### Reference Value Database

Table 3 shows the partition according to species and season of the 195,660 individual true bites and 27,921 consumption units (species-category combinations that were not true bites) recorded. Figure 1 shows the variation in the bite weights obtained for each species that was attributed to a year-season combination and bitetype category in the 180 merged samples subjected to

Species	Spring 2004	Summer 2004	Fall 2004	Spring 2005	Total
True bites					
Phillyrea latifolia	2,775	6,745	20,249	18,525	48,294
Rhamnus lycioides	765	2,863	4,123	29,528	37,279
Smilax aspera	302	2,698	11,818	11,611	26,429
Sarcopoterium spinosum	538	_	1,694	21,468	23,700
Pistacia lentiscus	952	2,085	6,045	8,263	17,345
Rubia tenuifolia	248	2,705	2,291	10,881	16,125
Asparagus aphyllus	400	2,472	6,451	746	10,069
Calicotome villosa	1,722		287	5,013	7,022
Euphorbia sp.		_		2,665	2,665
Clematis cirrhosa	323	_		1,898	2,221
Asphodelus ramosus	91	70	231	1,305	1,697
Ceratonia siliqua	_	689	38	,	727
Prasium majus	_	_		720	720
Quercus calliprinos	_	340		_	340
Allium sp.	_	_		291	291
Pistacia palaestina	_	153		_	153
Tamus communis	_	_		140	140
Cyclamen persicum	_	_		124	124
Sinapis arvensis	_	_		68	68
Scabiosa prolifera	_	_	_	62	62
Eryngium creticum	_	_		61	61
Quercus ithaburensis	_	53			53
Unidentified	21	20			41
Olea europaea	34	_		_	34
Other consumption units					
Ephedra foemina	_	17,221	341	918	18,480
Herbaceous, dry	310	5,417	1,460	_	7,187
Herbaceous, green	84	·		2,746	2,830
Smilax aspera	_	_	165	·	165

**Table 3.** The total number of bites recorded for each species in each year-season combination<sup>1</sup>

<sup>1</sup>Values are totals for all bite-type categories within a species.

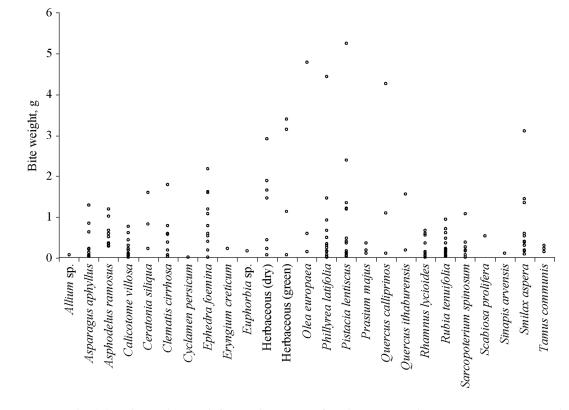
laboratory analysis. Fifty-three percent of true bites and consumption units weighed (DM basis) less than 0.25 g, 16% weighed 0.25 to 0.5 g, 15% weighed 0.5 to 1.0 g, and 16% weighed more than 1.0 g. The largest bite weights were noted for P. lentiscus (5.2 g, spring 2004), Olea europaea (4.8 g, spring 2004), P. latifolia (4.4 g, fall 2004) and Q. calliprinos (4.3 g, summer 2004). After total species daily intakes were calculated (Appendix Table A1, g/d), it appeared that 2 observations from fall 2004 (observations 9 and 10) recorded extremely low intakes (349 and 506 g/d, compared with an average of  $1.086 \pm 45$  g/d for the whole data set), which strongly suggested impaired health. Their associated fecal NIRS reflectances in several wavelengths, that is, around 1,900 nm (C = O stretch in  $COH_2$ ), 1,920 nm (C = O stretch in CONH), and 1,940 nm (water), were atypical and the spectra featured the greatest Mahalanobis values in the data set. Because the objective was to devise a dietary predictive methodology for healthy animals, these 2 observations were discarded from the data set used for fecal NIRS calibrations.

The intakes of CP, NDF, ADF, in vitro-digestible DM, and PEG-b-T were calculated from the sum of bites per species multiplied by mass per bite and the chemical compositions of the bites. The wide variety of nutrient contents in bite-like samples is depicted in Figure 2. The content of CP varied between 3.5 and 23.7% of DM, and that of PEG-b-T varied between 0 and 27% of DM. Intakes as grams per day and as percentages of DMI are given in Appendix Table A2. For readers interested in seasonal patterns of foraging selectivity, the order of observations parallels the order of seasons in Table 1. Minimal and maximal ranges for dietary percentages on a DM basis were 5.6 to 13.0% CP, 38.5 to 56.7% NDF, 23.6 to 36.4% ADF, 32.3 to 67.5% IVDMD, and 3.6 to 11.6% PEG-b-T.

# Fecal NIRS Calibrations

Examination of the Mahalanobis spectral distances from the mean fecal spectra showed that 65% of the standardized H values were below 1, 31% were between 1 and 2, and the remainder were between 2 and 3. In other words, no spectral outliers (H >3 from individual spectra to the population centroid; Shenk and Westerhaus, 1991) were found in the fecal spectra used for calibration.

Calibrations for dietary percentages were run directly, independently of those of absolute rates of intake (g/d). In other words, dietary percentages were not calculated by dividing a fecal-NIRS-obtained estimate of nutrient intake by a fecal-NIRS-obtained estimate of



**Figure 1.** Bite weight (g) values obtained for each species for the various bite-type categories and year-season combinations (n = 180).

DMI. Because *P. latifolia* was the main tree, *P. lentiscus* was the main encroaching brush species, and herbaceous vegetation was important for nutritional reasons, calibrations are given only for these botanical entities, in addition to dietary attributes.

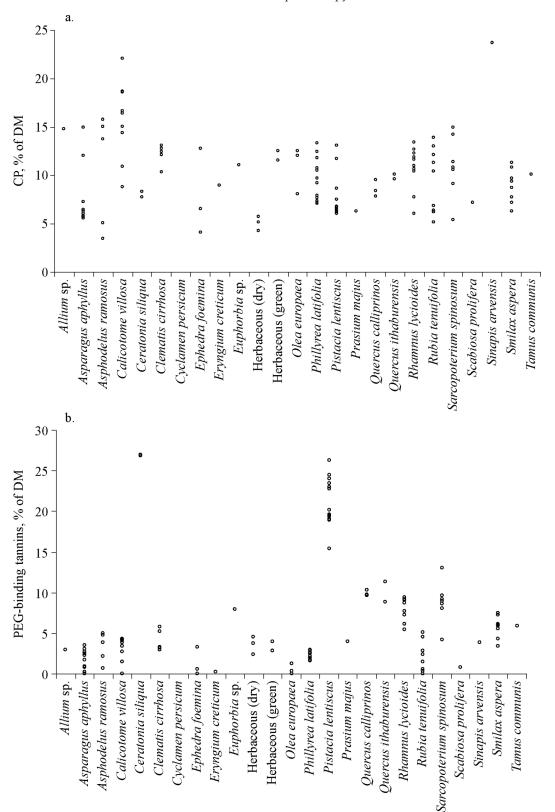
The performance of the fecal NIRS calibrations is summarized in Table 4. Overall, the  $R^2$  value for the calibration of total daily nutrient intakes (not shown) were low: 0.18 for DM, 0.59 for CP, 0.13 for NDF, 0.52 for in vitro-digestible DM, 0.50 for PEG-b-T, and 0.20 for ADF; as for botanical intakes, the  $R^2$  value for the rates of intake of both herbaceous vegetation and *P. latifolia* was greater than the  $R^2$  for *P. lentiscus* (0.80 and 0.65, respectively). The rates of intake of herbaceous vegetation and *P. latifolia* (i.e., 157 and 122 g/d) were predicted with respective SECV values of 71 and 64 g/d. The slopes of actual vs. predicted values of herbaceous vegetation and *P. latifolia* intakes were close to 0.80; they differed from unity (P < 0.01) and had nonzero (27 g/d; P < 0.05) intercepts.

Compared with the intake predictions, better calibration and cross-validation statistics were obtained for fecal NIRS-predicted dietary percentages. The R<sup>2</sup> values for *P. latifolia*, herbaceous vegetation, and *P. lentiscus* were 0.89, 0.85, and 0.77, respectively, with respective SECV values of 6.3, 7.8, and 5.6% of DM, and averages of 17.6, 22.3, and 8.7% of ingested DM. The slope of the relationship between the bite-count-estimated and fecal NIRS-predicted values for *P. latifolia* was 0.90 [i.e., different from (P < 0.05) but still reasonably close to unity] and the intercept did not differ from zero (P = 0.10).

Within the calibrations for nutritional attributes, the lowest  $R^2$  value (0.74) was found for PEG-b-T, with all others being close to 0.90. The accuracy of the PEG-b-T calibration (SECV of 0.88 for an attribute average of 4.83% of ingested DM) was also the lowest. The SECV values of the dietary percentages of CP, NDF, ADF, and in vitro-digestible DM were low, relative to average values for these attributes, but only the CP and in vitro-digestible DM calibrations could be considered as to-tally unbiased, with slopes not significantly different from unity, and intercepts not significantly different from zero.

# DISCUSSION

Fecal NIRS calibrations for dietary chemical composition of free-ranging goats have been reported before, but this is the first report of fecal NIRS with reconstituted diets based on bite counts and on the simulated bite method for reference values. Compared with the use of fistulated animals (Leite and Stuth, 1995), the bite count methodology has 3 advantages: 1) information is obtained for the entire grazing days; 2) the same animal is used for diet estimation and fecal sampling; and 3) diets selected by fistulated animals may be different from those of unfistulated residents (Coates et al., 1987).



**Figure 2.** (a) Crude protein and (b) polyethylene glycol (PEG)-binding tannin contents (% of DM) obtained for each species for the various bite-type categories and year-season combinations (n = 180).

**Table 4.** Standard errors of calibration (SEC) and of cross-validation (SECV) of fecal NIRS calibrations (n = 43) of dietary composition (% of ingested DM, including concentrate)

	-		-				-	
Constituent	$Math^1$	Mean	SD	SEC	$\mathbb{R}^2$	SECV	Slope	Intercept
Botanical								
Herbaceous	2, 6, 6, 1	22.3	12.5	4.9	0.85	7.8	0.87	2.8
Phillyrea latifolia	2, 6, 6, 1	17.6	13.1	4.3	0.89	6.3	0.90	2.0
Pistacia lentiscus	2, 6, 6, 1	8.7	9.8	4.6	0.77	5.6	0.78	2.1
Nutritional								
CP	2, 6, 6, 1	11.0	2.29	0.62	0.93	0.87	0.95	0.70
NDF	2, 6, 6, 1	41.9	3.74	1.30	0.88	2.14	0.75	10.0
ADF	2, 6, 6, 1	25.3	2.88	0.98	0.89	1.69	0.90	2.4
IVDMD	2, 6, 6, 1	56.4	10.6	3.16	0.91	4.27	0.92	4.4
Polyethylene glycol-binding tannins	2,6,6,1	4.8	1.44	0.73	0.74	0.88	0.75	1.2

<sup>1</sup>Numbers represent the derivative, the 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 nanometers over which the second smoothing is applied.

However, the esophageal extrusa samples collected by Leite and Stuth (1995) consisted of the diet actually consumed by animals, whereas in this study the diets were simulated with bite counting, with inherent risk of errors, particularly in the estimation of bite weights.

In the present study, 10 species or groupings accounted for slightly more than 90% of the estimated total intake. They were, in descending order of total consumption, P. latifolia, green herbaceous vegetation, P. lentiscus, S. aspera, R. lycioides, R. tenuifolia, S. spinosum, dry herbaceous vegetation, and E. foemina. Because bites can be counted accurately by an experienced observer, accurate estimation of the bite weight for these species is critical for successful estimation of intake. Published data are limited regarding the bite weight of goats foraging in Mediterranean shrubland, and bite weights, which represent an intermediate stage in intake calculations, are rarely published. Bite weights reported in the present study are similar to those reported by Aharon et al. (2006) and Z. Henkin (ARO, Newe Yaar, Israel, personal communication) for R. lycioides, S. aspera, S. spinosum, and herbaceous vegetation, and those reported by Decandia et al. (2000) and M. Decandia (IZCS, Bonassai, Italy, personal communication) for P. lentiscus and P. latifolia. Intake values for the above species in the present study were comparable to those reported by Kababya et al. (1998) and Decandia et al. (2000).

The successful use of fecal NIRS to predict the percentage of leafy spurge (*Euphorbia esula*) in confinement experiments has been demonstrated (Walker et al., 1998). Fecal NIRS also enabled prediction of the botanical composition of individual browse species in mixtures of 4 species (Landau et al., 2004a), but calibrations based on confinement experiments were not sufficiently robust when applied to free-ranging shrubland conditions (T. Glasser, unpublished), probably because of the complexity of the goats' diets (see Appendix Table A1).

To our knowledge, the data in Table 4 represent the first use of fecal NIRS to determine the botanical composition of diets consumed by free-ranging goats. This was enabled by using individual observation data to provide reference values for calibration. Nevertheless, the R<sup>2</sup> values for the calibrations of *P. latifolia* and *P. lentiscus* obtained in the present study were lower (0.89 and 0.77, respectively; Table 4) than those obtained in wellcontrolled confinement experiments with the same plant species (0.94 and 0.95, respectively; Landau et al., 2004a). In the confinement experiments, the SECV values for percentages of *P. latifolia* and *P. lentiscus* were 6.3 and 7.0% of DM, respectively. The SECV/mean ratio was 15 to 20%, compared with 35 to 65% in the present study. It is probable that some of the difference arose from noise in the fecal spectra generated by the variety of species ingested, and that some of it arose from errors in the estimation of bite number and weight. Walker et al. (2002) characterized fecal NIRS calibrated in controlled conditions but used under field conditions as "interval scale of measurement," with sufficient accuracy, for example, to compare treatment effects on diet preference, but not to estimate the actual composition of the diet.

In contrast, we contend that the present calibrations for nutritional attributes, at least for CP and IVDMD as percentages of DM ingested (Table 4), have more general predictive potential according to the criteria of Williams (2001). The  $\mathbb{R}^2$  values of our present calibrations for dietary CP and IVDMD (0.93 and 0.91, respectively; Table 4) are similar to those obtained by Leite and Stuth (1995) using grazing, esophageally fistulated goats (0.94 and 0.92, respectively), but lower than those reported by Landau et al. (2004a) for goats that were hand-fed with browse diets (0.98 for both attributes). The SECV values, relative to the means of the respective nutritional attributes, were low, indicative of satisfactory accuracy.

As reported previously for goats that were hand-fed in confinement with combinations of Mediterranean browse (Landau et al., 2004a), fecal NIRS calibrations of dietary percentages are more precise and accurate than those of absolute rates of intake (Table 4). This was expected because NIRS is primarily a methodology aimed at determining chemical composition (i.e., percentages). A similar result was reported by Boval et al. (2004) for cattle. Therefore, one would expect to obtain a more accurate estimate of absolute nutrient intake rate by multiplying the dietary percentages obtained from fecal NIRS measurements by an independently estimated total DMI. Calculation of DMI requires knowledge of fecal output and of the digestibility of a representative diet. Fecal output can be accurately determined by means of indigestible markers such as chromium sesquioxide (Kababya et al., 1998), a longchain n-alkane (Decandia et al., 2000), or polyethylene glycol (Landau et al., 2003). Digestibility can also be estimated fairly by fecal NIRS (this study). Further research is needed to verify that fecal NIRS can be used to obtain accurate estimates of nutrient intakes.

What could be done to improve fecal NIRS calibrations (i.e., get greater  $R^2$  and lower SECV values for equations)? Obtaining dietary reference values by observing animals at pasture is time-consuming. This restricts the size of data sets that can be used for fecal NIRS calibrations. We have previously shown in a confined experiment (Landau et al., 2005) that in a given population of goats, the Mahalanobis distance between the fecal spectra of goats ingesting the same diet is always less than 0.5. Therefore, selected feces from resident animals that are not observed but graze temporarily and spatially with focal animals could be used to increase the size of data sets, with the Mahalanobis distance used as a criterion. However, this approach has to be taken cautiously because there is a risk of increased fecal spectral redundancy (Shenk and Westerhaus, 1991) and the quality of reference values would by definition be lower in resident than in focal animals.

Last, in the future, fecal NIRS calibrations based on bite counts will need to be continuously updated to encompass the spectral variety associated with new grazing conditions, as recommended by Coleman et al. (1995) for all NIRS procedures.

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Allium sp. $-^1$ <th></th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>9</th> <th>7</th> <th>8</th> <th>6</th> <th>10</th> <th>11</th> <th>12</th> <th>13</th> <th>14</th> <th>15</th> <th>16</th> <th>17</th> <th>18</th> <th>19</th> <th>20</th> <th>21</th> <th>22</th> <th>23</th>		1	2	3	4	5	9	7	8	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23
	Allium sp.	-	l	I	I	I	I	I	I				I	I	I	l		I	I	I	I	I	I	I
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Asparagus aphyllus	55	24	15	41	93	255	348	97	2	5 C	1	25		2	20		ũ	0	1	1	5 C	77	104
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Asphodelus ramosus	6	11	11	I	I	I		20			38	9			16	0				က	12		0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Calicotome villosa	53	40	60								0	5	0	0	10	0	0	0	0	2	1	1	7
	Ceratonia siliqua	I			10	79	107	54			6		က			I					4	4	I	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Clematis cirrhosa	159	30	11												Ι					I		I	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Cyclamen persicum													I		Ι					I			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Ephedra foemina				77	110	1	21	0		1				5 L			66		45	14	41	24	0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Eryngium creticum															I								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Euphorbia sp.																							
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Herbaceous											299	360	476	276	161	133	171	33	249	53	17	161	35
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Herbaceous, dry			211	471	454	406	416	497	179	44					I					I			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Herbaceous, green	26	16													I					I			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Olea europaea		24																					
	Phillyrea latifolia	436	237	288	14	40	170	91	167	162	224	152	414	629	607	510	291	501	707	755	272	310	385	396
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Pistacia lentiscus	591	407	172	5	23	48	40	53	151	42	56	15	285	166	7	332	144	179	546	239	111	154	171
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Prasium majus															I					I			
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Quercus calliprinos				81	167	0	111	က							I								
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Quercus ithaburensis					55	4		1															
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Rhamnus lycioides	17	14	18	2	33	30	7	11	0	14	89	11	25	15	17	0	26	27	က	25	22	15	52
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Rubia tenuifolia	30	ũ	9	57	57	1	10	26	0	0	59	80	7	6	6	1	14	1	00	12	00	1	5
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Sarcopoterium spinosum		0	29								131	87		1	16		00	35	4	94	56	10	15
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Scabiosa prolifera															I					I			
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	Sinapis arvensis																							I
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Smilax aspera	80	Ð	30	134	110	12	38	66	10	6	93	96	30	40	177	9	673	145	55	187	209	102	91
$1,458 \hspace{0.1in} 814 \hspace{0.1in} 852 \hspace{0.1in} 890 \hspace{0.1in} 1,222 \hspace{0.1in} 1,035 \hspace{0.1in} 1,137 \hspace{0.1in} 941 \hspace{0.1in} 506 \hspace{0.1in} 349 \hspace{0.1in} 917 \hspace{0.1in} 1,029 \hspace{0.1in} 1,453 \hspace{0.1in} 1,124 \hspace{0.1in} 943 \hspace{0.1in} 766 \hspace{0.1in} 1,128 \hspace{0.1in} 1,667 \hspace{0.1in} 907 \hspace{0.1in} 795 \hspace{0.1in} 50 \hspace{0.1in} 50$	Tamus communis																							
	DMI	1,458	814	852	890	1,222	1,035	1,137	941	506	349	917	1,029	1,453	1,124	943	766	1,608	1,128	1,667	007	795	930	878

APPENDIX

$Observation^{1}$	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Allium sp.	I	0	0	0	6	0	1	4	5	0	0	0	1	0	1	1	0	1		I	I
Asparagus aphyllus	0	31	21	က	5 L	4	2	31	9	23	4	1	0		0	0	13	9	1	0	
Asphodelus ramosus	32	13	36	26	35	29	29	16	9	15	29	33	29	19	4	5	7	2	15	18	62
Calicotome villosa	33	19	48	130	15	4	48	13	4	36	11	38	80	36	17	6	13	4	27	15	
Ceratonia siliqua	I		I		I														I		I
Clematis cirrhosa	17	48	48	73	00	က	18	6	5	18	23	20	14	34	10	24	68	43	10	4	11
Cyclamen persicum	0	0		0	0	1	0		0	0	0	0									
Ephedra foemina		6	9	1	123	645	13	29	6	28	15		85	6	9	31	63	16	15	11	10
Eryngium creticum																	00	1	0	က	
<i>Euphorbia</i> sp.		34	12		4	ũ	5	1	1	96	63	68	30	47	9	10		0	38	20	
Herbaceous																					
Herbaceous, dry			I		I				I	l			l			I	l				I
Herbaceous, green	320	245	179	301	728	521	515	732	854	455	600	311	540	320	317	273	226	295	352	198	170
Olea europaea						I									I	I			I		
Phillyrea latifolia	13	57	31	43	149	236	266	410	295	87	69	178	84	163	105	73	156	128	84	111	216
Pistacia lentiscus	25	69	39	38	156	69	47	36	50	27	84	206	133	125	46	46	27	41	51	43	15
Prasium majus		9	1	9	7	2	က	4	0	11	13	18	11	က	1	1	0	1	က	1	5
$Quercus\ calliprinos$					Ι					I			I						I		I
Quercus ithaburensis																					
Rhamnus lycioides	107	188	93	159	262	206	115	42	19	56	37	38	79	116	216	193	51	67	154	72	116
Rubia tenuifolia	215	236	427	137	131	71	56	29	22	6	29	216	75	73	46	153	23	35	32	18	29
Sarcopoterium spinosum	130	92	73	167	17	ũ	19	27	27	211	161	71	16	24	42	59	57	28	124	110	351
Scabiosa prolifera										16	5								7	4	
Sinapis arvensis				1	1	က	0		1	1	0	0									
Smilax aspera	က	2	1	65	50	86	123	45	34	6	9	58	265	323	130	164	329	350	9	က	7
Tamus communis			က	5 C	က	0	4	9	0			2 2			0	I	2	I	0		I
DMI	895	1.051	1.020	1.157	1.703	1.893	1.264	1.432	1.338	1.100	1.150	1.262	1.371	1.293	946	1.041	1.038	1.017	917	631	993

**Table A2.** Intake at pasture (g/2 d) and proportion of DM of CP, NDF, ADF, IVDMD, and polyethylene glycol-binding tannins (Tan) that served to calculate reference values for fecal near-infrared reflectance spectroscopy (NIRS) calibrations

			Inta	ke, g/2 d				Prop	ortion of	f DM, %	
Observation	CP	NDF	ADF	IVDMD	Tan	DMI	CP	NDF	ADF	IVDMD	Tan
1	133	672	472	643	136	1,458	9.1	46.1	32.4	44.1	9.3
2	71	369	264	326	88	814	8.7	45.4	32.4	40.1	10.8
3	73	446	285	376	53	852	8.6	52.3	33.4	44.2	6.2
4	55	473	305	384	42	890	6.2	53.1	34.3	43.2	4.7
5	82	624	413	508	82	1,222	6.7	51.1	33.8	41.6	6.7
6	58	481	320	335	65	1,035	5.6	46.4	30.9	32.3	6.3
7	71	619	408	449	67	1,137	6.3	54.5	35.9	39.5	5.9
8	59	534	338	400	46	941	6.3	56.7	35.9	42.5	4.9
$9^{1}$	35	254	169	184	48	506	6.9	50.2	33.4	36.4	9.5
$10^{1}$	24	180	120	125	20	349	6.9	51.5	34.5	35.9	5.8
11	52	492	334	328	51	917	5.6	53.7	36.4	35.8	5.5
12	62	528	351	385	41	1,029	6.1	51.3	34.1	37.4	4.0
13	91	680	449	509	104	1,453	6.3	46.8	30.9	35.1	7.1
14	74	504	335	412	67	1,124	6.6	44.8	29.8	36.7	5.9
15	63	417	281	385	35	943	6.7	44.3	29.8	40.9	3.8
16	51	302	208	247	89	766	6.6	39.5	27.1	32.3	11.6
17	104	728	501	628	97	1,608	6.5	45.2	31.2	39.1	6.1
18	80	434	298	430	70	1,128	7.1	38.5	26.4	38.1	6.2
19	111	663	455	577	157	1,667	6.7	39.8	27.3	34.6	9.4
20	60	385	271	320	84	907	6.6	42.4	29.8	35.3	9.2
21	54	325	229	316	53	795	6.7	40.9	28.8	39.7	6.7
22	61	412	281	349	62	930	6.5	44.3	30.2	37.5	6.6
23	60	369	257	321	63	878	6.8	42.0	29.3	36.5	7.2
24	117	382	232	605	42	895	13.0	42.6	26.0	67.5	4.7
25	131	435	285	664	59	1,051	12.4	41.4	27.1	63.2	5.6
26	133	428	288	676	39	1,020	13.0	42.0	28.2	66.3	3.9
27	$100 \\ 145$	500	316	732	63	1,020 1,157	12.6	43.2	27.4	63.3	5.5
28	207	739	465	1,102	92	1,703	12.0 12.2	43.4	27.3	64.7	5.4
29	235	853	586	1,102 1,262	83	1,893	12.2 12.4	45.1	31.0	66.7	4.4
30	155	546	340	813	56	1,055 1,264	12.4 12.3	43.2	26.9	64.3	4.5
31	$155 \\ 172$	637	386	940	$50 \\ 52$	1,204 1,432	12.0	44.5	26.9	65.6	3.6
32	160	609	356	902	52 50	1,432 1,338	12.0	44.5 45.5	26.5 26.6	67.4	3.7
33	134	503	301	302 706	60	1,350 1,100	11.9 12.2	45.5	20.0 27.4	64.2	5.5
34	139	$501 \\ 521$	308	753	65	1,100 1,150	12.2 12.1	45.3	27.4 26.7	65.4	5.6
35	155 152	515	$303 \\ 341$	758	05 79	1,150 1,262	12.1 12.1	40.8	20.7 27.0	60.0	6.2
36	161	515 594	$341 \\ 381$	883	79 74	1,202 1,371	12.1 11.8	40.8 43.3	27.0 27.8	64.4	5.4
37	153	$532 \\ 532$	351	$\frac{883}{782}$	74	1,371 1,293	11.8	43.3 41.2	27.8 27.1	60.4	5.9
						,					
38 39	$115 \\ 128$	$\frac{385}{424}$	$\begin{array}{c} 241 \\ 274 \end{array}$	$\begin{array}{c} 601 \\ 669 \end{array}$	55 56	$946 \\ 1,041$	$12.2 \\ 12.3$	$\begin{array}{c} 40.7\\ 40.7\end{array}$	$25.4 \\ 26.3$	$63.5 \\ 64.2$	$5.8 \\ 5.4$
						,		40.7 42.2		64.2 62.7	
40	123	438	290 272	651	51 59	1,038	11.9		27.9		4.9
41	120	420	272	648	52	1,017	11.8	41.3	26.7	63.7	5.1
42	111	392	236	578	53	917 621	12.1	42.7	25.7	63.0	5.8
43	77	262	159	388	37	631	12.2	41.5	25.3	61.4	5.9
44	121	392	235	603	60	993	12.2	39.5	23.6	60.8	6.1
45	122	400	254	616	53	998	12.3	40.1	25.5	61.7	5.4

 $^{1}$ Data from observations 9 and 10 were not used for the fecal NIRS calibrations.