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NIRS-aided evaluation of faecal output in goats browsing on Mediterranean woodland

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SUMMARY – Long chain n-alkanes, such as C36, are reliable markers of faecal output (FO), but their chemical extraction is time-consuming. We hypothesized that polyethylene glycol (PEG), analysed by near infrared spectrometry (NIRS), can replace C36 for the estimation of FO in browsing goats. This was verified in eight Sarda goats browsing lentisk-based woodland, and dosed every morning with C36 (65 mg/goat/day) and PEG 4000 (50 g/goat/day). Faeces were sampled twice daily for 2 days, after 9 days of adaptation. Calibrations relying on the whole NIR region (1100 to 2500 nm), or relying only on segments not involved in PEG-tannin bonds (>2280nm), were calculated (R^2 = 0.999, SECV = 0.32% and R^2 = 0.998, SECV = 0.45%, respectively). Group estimates of FO, using C36 or PEG, did not differ when the restricted NIR equation was utilized. However, assuming that the FO value provided by C36 analysis is correct, FO tended to be overestimated (P<0.14) for PEG, when the whole NIR region equation was utilized.

Keywords: Nutrition, digestibility, pasture, PEG, NIRS.

RESUME – *"Evaluation avec NIRS de l'excrétion fécale chez des chèvres pâturant dans les forêts méditerranéennes". Les alcanes à chaîne longue, comme le C36, sont des marqueurs fiables de l'excrétion fécale, mais leur extraction chimique exige beaucoup de temps. Nous avons fait l'hypothèse que le polyéthylène glycol (PEG), analysé par spectrométrie dans le proche infra-rouge (NIRS) peut remplacer le C36 pour l'estimation de l'excrétion fécale de chèvres se nourrissant de ligneux. Cela a été vérifié en utilisant 8 chèvres Sardes pâturant dans un bosquet de lentisque, dosées chaque matin avec l'alcane C36 (65 mg/goat/day) et le PEG 4000 (50 g/goat/day). Les fèces ont été collectées deux fois par jour, après 9 jours d'adaptation. Des calibrations comprenant tout le domaine du proche infrarouge (1100-2500 nm) ou excluant les segments de liaison PEG-tannins (>2280 nm) ont été calculées (R^2 = 0,999, SECV = 0,32% and R^2 = 0,998, SECV = 0,45%, respectivement). L'excrétion des fèces a été semblable pour la méthode C36 ou celle du PEG quand l'équation restrictive a été utilisée. Toutefois, en assumant que la valeur du FO fournie par C36 est correcte l'excrétion fécale tendait à être surestimée (P < 0,14) par le PEG, quand l'équation basée sur tout le domaine proche infra-rouge a été utilisée.*

Mots-clés : *Nutrition, digestibilité, pâturage, PEG, NIRS.*

Introduction

Efforts are underway to use goats to reduce the abundance of brush in Mediterranean ecosystems where brush encroachment increases the danger of fire, limits recreational value and decreases biodiversity. In goat production systems relying on brush clearing, a contradiction exists between optimal nutrition for productive purposes (and economical profitability for the farmer), and maximal intake of browse required by the community. A compromise management, based on the monitoring of feed intake, must ensure that the energy balance is sufficient to sustain productivity and body condition at key periods.

It is a challenging task to monitor feed intake in ranging goats, due to the highly heterogeneous environment that they exploit and their selective behaviour (Kababya *et al*., 1998). Even when feed ingredients are determined, *in vitro* digestibility measurements are not informative of the *in vivo* value (Perevolotsky *et al*., 1993). The determination of faecal output (FO) may be a way to circumvent this difficulty. This is because 81% and 74% of the variation in the intake of organic matter and of digestible organic matter, respectively, can be predicted by FO in goats feeding on browse (Núñez-

Hernández *et al*., 1992). Because it is unpractical to carry out total collection of faeces in ranging goats, the evaluation of FO requires the use of indigestible markers. Only external markers are relevant to determine FO in ranging goats, because a pre-requisite for using internal markers is knowledge of the diet.

As in other ruminants, chrome-oxide (Kababya *et al*., 1998) can be used to assess FO. Faecal material needs to be ashed or wet-digested before chrome is oxidized to chromate, acidified, diluted, and assayed by atomic absorption spectrometry (see review by Yakoulaki *et al*., 1993). An alternative to chrome is n-alkanes. The recovery of the C36 n-alkane given to goats feeding on browse was 94.7% (Decandia *et al.,* 2000). In order to be extracted from faeces, alkanes need to be saponified, extracted in ethanol, before GLC analysis (Decandia *et al.,* 2000; Brosh *et al.,* 2003). In addition, n-alkanes are expensive, which prevents their widespread use for monitoring purposes.

Long-chained polyethylene glycol (PEG, MW>4000) can be used as marker of faecal output in goats, using a near infrared spectroscopy (NIRS) procedure that does not require chemical extraction of PEG from the faeces, but only oven-drying and grinding. When given in excessive doses, PEG interferes with water metabolism and its excretion in faeces is not steady, but otherwise recovery is 100% (Landau *et al*., 2002). However, a limiting factor of using PEG as a marker of FO could be that PEG forms complexes with tannins. These complexes are formed by hydrogen bonding between the (ether) oxygen of the PEG glycol chain and the phenolic hydroxyl in tannins (Jones, 1965). The 2274 nm wavelength is related with stretching and bending of C=0 and O-H bonds, and partly with CH_2 deformation. The best correlation between an individual wavelength and PEG is found at 2280-2288 nm (Murray *et al*., 1995; Landau *et al.,* 2002). From 2278 nm to the end of the NIR region, all wavelengths feature CH stretch and CH_2 deformations that are not involved in the PEG-tannin bond. Restricting the calibration to these wavelengths represents, therefore, an appealing approach. However, light scattering problems are known to affect distal part of the NIR region, and restricting the number of wavelengths available for fitting data decreases the accuracy of NIR calibration equations. We showed before, using quebracho tannins as model of condensed tannins, in indoors, well-controlled studies with goats, that it is possible to use PEG, assayed by NIRS, as marker of FO when diets contain tannins, at the condition that the calibration models involve only wavelengths featuring chemical moieties that are not involved in PEG-tannin bonds (Landau *et al*., 2003).

The aim of the present study was to assess in goats, managed under farm condition and feeding on tannin-rich woodland, if PEG can replace the indigestible n-alkane C36 as marker of faecal output. This was done by using calibrations models over the whole NIR region, or restricted to NIR regions where PEG and tannin do not interact.

Materials and methods

Animals and diets

The study was conducted at the Bonassai experimental farm (41° N latitude) in Sardinia (Italy) during the summer of 2002. Eight mature Sarda goats weighing 48.9±5.6 kg, at the end of lactation (190±10 days in milk), were used for the experiment. They were grazing for 6 h daily on 2.5 hectares lentisk-based woodland (Decandia *et al*., 2000), and supplemented with ryegrass hay (200 g/goat/day) and a commercial concentrate (16% CP, 200 g/goat/day). The experiment consisted of two periods of 9 days each, in which each goat received or did not receive PEG, i.e. four goats received PEG in period 1 and four goats received PEG in period 2. The n-alkane C36 (65 mg/goat/day) was administered in a paper bung every morning before turned to pasture in both periods. The period without PEG was needed to collect faeces used to elaborate NIRS calibrations for the prediction of PEG in faeces. Grab samples of faeces were collected twice daily during the last 2 days of each period, at 08:30 and 15:30. Intake at pasture was assessed by direct observation of bites, and re-constitution of diet eaten by hand plucking of vegetation (Kababya *et al*., 1998), as is described by Decandia *et al*. (2003).

PEG analyses

Faeces were collected from all goats during the no-PEG period. Faeces were dried in a forced-air oven at 60ºC for 48 h, ground through a 1-mm sieve, and pooled. In order to build-up calibration

models, faeces were mixed, in duplicates, with PEG (MW 4000), to final PEG concentrations ranging from 0, 1.25, 2.5, 3.75, 7.5, 10, 15, and 20%. Bi-distilled water (30 ml) was added to mixtures to facilitate homogenization. Samples (n=32) were then re-dried and ground. Before scanning, samples were dried in an oven at 50º C for 2 hours to stabilize moisture and placed in a desiccator for 1 hour to cool to ambient temperature. Faecal samples were scanned, using a Foss NIRSystems 5000 monochromator (Foss Tecator, Hoganas, Sweden). Reflectance spectra were recorded from 1100 to 2500 nm in 2 nm steps as Log (1/R), where R represented reflected energy.

The modified partial least-squares (MPLS) regression method was used to establish a relationship between the first derivate of log (1/R) and PEG concentrations in faeces-PEG mixtures. This was done by using the WinISI II software (ISI, 1999). Corrections of NIR spectra for particle size were carried out by using the SNV (standard normal variate) and detrend procedure (Barnes *et al*., 1989). The predictive ability of calibration models was tested by using cross-validation, i.e. dividing the whole set of samples into 6 sub-sets, and calibrating PEG in 5 sub-sets while validating equations on the remaining sixth; or by using randomly half of the samples for calibration and the remainder for validation. Three different calibration models were developed. Calibration 1 (CAL1) relied on all wavelengths from 1100 to 2500 nm, calibration 2 (CAL2) relied on the NIR segment 2280-2288 nm, and calibration 3 (CAL3) on all wavelengths greater than 2280 nm. The rationale of this choice was: (i) to assess if the analytical PEG recovery differs, when calculated according to a calibration that includes NIR segment putatively involved in the PEG-tannin bonding, by comparing CAL1 to CAL2 and CAL3; and (ii) to elaborate a compromise between a too short segment of calibration and the risk of light-scattering, by comparing CAL2 and CAL3.

The linearity and the accuracy of calibrations were assessed by the coefficient of determination (R^2) between predicted and actual values, and by the standard error of cross validations (SECV). Once calibrations were calculated, collected faeces samples were scanned, and the PEG content of faeces was predicted according to ISI (1999) procedures. Faecal output was calculated as the ratio between the daily dose of PEG dose and faecal PEG concentration derived from NIRS measurements. The amount of PEG recovered was calculated as the product of actual faeces excretion by faecal PEG concentration.

Alkane analyses

Faeces samples were bulked in each period for each goat and oven-dried at 60°C to measure n-alkane concentrations according to Molle *et al.* (1998). The samples were saponified by direct treatment with ethanolic KOH (10%) at 90°C overnight and alkane extraction with hexane was performed at controlled temperature (60°C). The extracted samples were assayed by on-column injection on a 30 m x 0.530 mm (I.D.) megabore column in a Varian model 3400 CX gas chromatograph. The faecal output was then measured using faeces C36 concentration corrected by its recovery rate (Dove *et al*., 1989).

Statistical Analyses

Values for FO, calculated from n-alkane analysis, were compared to figures calculated from PEG concentration by paired T-tests.

Results and discussion

Values for R^2 and SECV for CAL1, CAL2 and CAL3 were (0.999; 0.32%); (0.972, 1.29%) and (0.998; 0.45%), respectively. These values for CAL1 are similar to those published before (Landau *et al.,* 2002). The decrease in accuracy, when using a restricted number of wavelengths, i.e. increase in SECV from 0.32% to 1.29% was of the same order as described before (from 0.13% to 0.8%; Landau *et al*., 2003). It seems that CAL3 represents a desirable compromise, in which NIR segments involved in PEG-tannin bonding are eliminated, without affecting too much the accuracy of prediction (i.e. SECV increases from 0.32% to 0.45%).

The calculated PEG faecal concentrations, on DM basis, ranged between 5.6 and 8.8% (pooling

all data from the three calibrations) at the exception of one goat in one period, for which the PEG estimate was less than 4%, i.e. abnormally low. These data were discarded. The variability of FO was similar, when assessed by using C36 n-alkane or PEG (Table 1). Individual estimates for FO yielded by C36 and PEG (Fig. 1) did not correlate well (best correlation with CAL2, R = 0.49), but the average estimates of FO (Table 1) did not differ when calculated with C36 n-alkane (726 g/goat/day) or PEG using CAL3 (729 g/goat/day; paired t-test, P = 0.94) or CAL2 (766 g/goat/day, paired t-test, P = 0.43). The actual value for FO in the present study is unknown, but for the sake of simplicity, we assume that the value for FO provided by C36 analysis is correct, because its accuracy has been shown before in goats feeding on browse (Decandia *et al.*, 2000). Under this assumption, FO tended to be overestimated (805 g/goat/day, P<0.14) by PEG, when the whole NIR range was utilized (CAL1), as found before (Landau *et al.*, 2003), while correct estimations were obtained when a calibration included only wavelengths greater than 2280 nm (CAL3).

Table 1. Faecal output (DM, g/d, mean and standard error) and dry matter digestibility (DMD), calculated by using, as external marker, C36 n-alkane, or PEG. PEG was calibrated by using the whole NIR range (CAL1), the 2280-2288 nm NIR segment (CAL2), or the whole NIR region above 2280 nm (CAL3). The correlation evaluates the relation between the two markers of FO. The t-test significance refers to the differences between estimates of FO

	C36	PEG-CAL1	PEG-CAL2	PEG-CAL3
FO	729 ± 120	805 ± 83	766 ± 111	726 ± 105
t-test (C36, PEG)		P<0.14	P<0.43	P<0.94
R (C36, PEG)		0.35	0.49	0.33
DMD	0.52	0.47	0.50	0.52

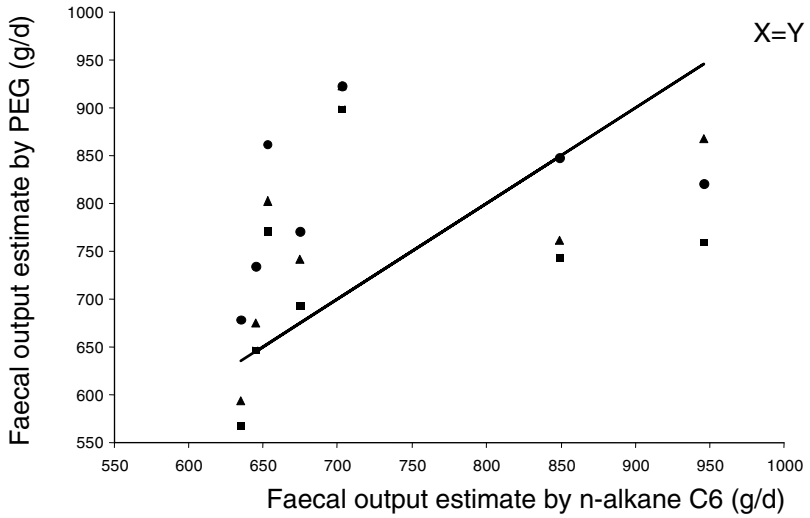

Fig. 1. The relation between estimates of faecal output obtained by using n-alkane C36 or PEG as external indigestible markers. PEG-derived estimates of FO were obtained with NIRS calibrations in the whole NIR region (CAL1, ●), the 2280-2288 nm segment (CAL2, ▲), or the 2280-2500 nm segment (CAL3, ■).

According to Decandia *et al.* (2003), the average group digestibility of DM in the present study was 52%, when calculated by using C36 n-alkane. PEG-aided evaluations of digestibility were 47, 50 and 52% when CAL1, CAL2 and CAL3 were used (Table 1). This comforts the conclusion that PEG, assayed by NIRS, and using the CAL3 calibration is a potential alternative to n-alkane assayed by gas chromatography for the determination of digestibility.

Our results, obtained under farm conditions, suggest that PEG can replace C36 n-alkane as external marker to estimate FO in goats feeding on tanniferous browse. A prominent advantage is that the NIRS-aided analysis of PEG requires only drying and grinding of the samples, whereas the n-alkane analysis by gas chromatography requires chemical extraction.

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