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Polyethylene Glycol, Determined by Near-Infrared Reflectance Spectroscopy, as a Marker of Fecal Output in Goats

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We report the application of NIR spectroscopy to determine the fecal concentration of poly(ethylene glycol) (PEG, MW 6000) used as an external marker of fecal output in goats. Calibration was carried out, using the modified partial least-squares method (MPLS), combining all wavelengths in the 1100−2500 nm range, with high linearity ($R^2 = 0.99$). In goats fed at maintenance level, the recovery of PEG in feces was complete, and the estimation of fecal output was accurate, when a moderate dose of PEG was given (20 g/d). A higher dose of PEG (40 g/d) was associated with underestimation of fecal output, probably because PEG interacted with water metabolism. Using PEG and its NIRS-aided analysis to determine fecal output is accurate, simple, and cheap. However, the feasibility of this new method must be verified in goats feeding on tannin-containing diets, and in goats at high feeding level.

KEYWORDS: NIR; goats; ruminants; nutrition; digestion; poly(ethylene glycol)

INTRODUCTION

The first step in the determination of nutrient utilization in animals, i.e., the assessment of fecal output, can be obviously achieved by total collection of feces. Total collection requires housing of animals in individual crates, or harnessing them with a feces-collection device. Individual housing or harnessing may stress the animals, thereby impairing normal feeding behavior and thus biasing the measured fecal output. In addition, these methods are irrelevant to farm situations. In cattle, the low dry matter (DM) content of feces makes total collection impractical.

Therefore, techniques based on indigestible markers have been developed to estimate fecal output. An ideal marker of fecal output should be unabsorbed along the gastro-intestinal tract (GIT) and should not interfere with digestive and absorptive processes in the different parts of the GIT (*1*). Poly(ethylene glycol) (PEG) is an indigestible polymer when its degree of polymerization is high enough (MW of 3000 and higher), and it has been used as an external marker to measure fecal output in cattle (*2*). In early studies, PEG was recovered gravimetrically (*3*) or measured by using turbidimetry (*4*). An improved turbidimetry method, based on the formation of an emulsion from PEG and trichloracetic acid in the presence of barium ions (*5*), was implemented, but turbidity was shown to be time-dependent (*6*), and PEG recovery was dose-dependent (*2*). Tritiated PEG was also prepared for digestion studies (*7*) because reproducible results could not be obtained by using the previous methods, but it appeared that part of PEG was not totally recovered in the aqueous phase (*8*). More recently, high-performance liquid chromatography has been used to quantify PEG in urine (*9*), but we are not aware of its use for feces. Notwithstanding analytical drawbacks, all the methods cited above require tedious pre-extraction of PEG from the biological matrix, and include the use of hazardous chemicals.

Near-infrared reflectance spectroscopy (NIRS) has been recently used for the quantitative determination of PEG in the pharmaceutical industry (*10*). No preparation, apart from oven-drying and subsequent grinding, is needed for NIRS analysis of organic components in feces (*11*). Therefore, using PEG and its NIRS-aided analysis would potentially be a simple, environment-friendly, and cheap new methodology for the evaluation of fecal output in animals.

The use of PEG as a marker of fecal output has been neglected because the turbidimetric method of PEG determination is laborious, and also because the rate of PEG recovery seemed to be related with the PEG dose administered to the animal (*2*).

The aim of the present study was to elaborate a NIRS-aided procedure for the analysis of PEG in feces, and to re-visit the value of PEG as an external marker of fecal output in goats, and, in particular, the effects of dose on PEG recovery.

MATERIALS AND METHODS

Animals. Four multiparous, nonpregnant, and dry Israeli Saanen goats, weighing 72.0 kg (SD ± 6.2 kg), were housed in individual

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metabolic cages equipped with automatic feeders (Ankom Technology, Fairport, NY) that provided 1/12 of the diet every 2 h. Water was freely available.

Feed. Goats were offered a diet consisting of 60% of vetch hay of good quality (15.4% CP, on DM basis) and 40% of a commercial concentrate (18.4% CP, Matmor Ltd., Ashod, Israel). The concentrate consisted of 30% barley grain, 17% corn grain, 10% oats, 10% cottonseed meal, 10% wheat bran, 5.5% rapeseed meal, 5% sunflower meal, 3% soybean meal and 3% corn gluten meal, 3.5% soybean oil, and 3% of a vitamin and mineral premix. Goats were acclimatized to cages and feeds for 10 days to achieve steady-state conditions, and this was followed by two experimental periods of 8 days each. Diets were offered at 90% ad libitum to prevent refusals during the collection period. Daily intakes of hay and concentrate were 715 (SE 23) and 311 (SE 11.0) g on a DM basis, respectively.

Digestibility Trial. On days 1−8 (period 1) and 9−16 (period 2) of the experiment (following the 10-day adaptation period) goats were orally dosed at 08:00 a.m with 20 or 40 g/d of PEG (MW 6000, Renex, ICI CC&P, Chocques, France) dissolved in 70 mL of water, using disposable plastic syringes. Total collection of feces and urine was carried out for the last 3 days of each experimental period. Because female goats were used, urine was collected via Foley catheters inserted to the urethra and fixed with leucoplast tapes along the hind leg, to prevent mixing of urine with feces. During the last 24 h of each experimental period, feces was collected and weighed every 4 h.

Chemical Analyses. To calibrate PEG in feces, fecal samples were collected during the last 3 days of adaptation from goats, dried in a forced-air oven at 60 °C for 48 h, ground through a 2-mm sieve using a Wiley mill (Arthur Thomas, Philadelphia, PA), and pooled. Feces were then mixed, in duplicates, with PEG (MW 6000, Renex, ICI CC&P, Chocques, France), at increments of 0.5%, to final PEG concentrations ranging from 0 to 10%. Bi-distilled water (30 mL) was added to mixtures to facilitate homogenization. Samples ($n = 42$) were then re-dried and ground. Before scanning, samples were dried in an oven at 50 °C for 2 h to stabilize moisture and placed in a desiccator for 1 h to cool to ambient temperature. Spectra were scanned, using a Foss NIRSystems 5000 reflectance apparatus (Foss Tecator, Hoganas, Sweden). Reflectance was recorded in 2-nm steps as Log (1/*R*), where *R* represented reflected energy.

Spectral Data and Statistical Analyses. The modified partial least-squares (MPLS) method, combining all wavelengths in the 1108−2492 nm range in increments of 4 nm, was first used to establish a relationship between the first derivate of log(1/*R*) and the PEG concentrations of feces−PEG mixtures, using the WinISI II software (*12*). Corrections of NIR spectra for particle size were carried out by using the standard normal variance (SNV) and detrend procedure (*13*). The robustness of calibration was tested by using cross-validation, i.e., dividing the whole set of samples into 6 subsets, and calibrating PEG in 5 subsets while validating equations on the remaining sixth; or by using randomly half of the samples for calibration and the remainder for validation. The spectral data were analyzed to detect outliers. This was done by using the "SCORE" procedure of ISI based on principal component analysis (*12*), and using algorithms that rank samples according to their distance from the average spectrum of the file (*14*), with cutoff values of 1.5 and 2.5 for "*T*" and "*H*" (*15*, *16*), respectively.

Correlations between the first and the second derivative of Log(1/*R*) at increments of 2 nm, and fecal PEG concentrations, were computed using ISI procedures (*12*) to identify potential single wavelengths for PEG calibrations.

Once calibrations were calculated, collected feces samples were scanned, and the PEG content of feces was established according to ISI (*12*) procedures. Fecal output was calculated as the ratio between the daily dose of PEG dose and fecal PEG concentration derived from NIRS measurements. The amount of PEG recovered was calculated as the product of actual feces excretion by fecal PEG concentration.

Actual values for fecal output were compared to figures calculated from PEG concentration by paired *T*-test. Similar comparisons were carried out for actual PEG intake and PEG recovered. The effects of PEG dose and hour of collection on fecal DM and PEG concentrations were evaluated, using a repeated measurements procedure of GLM, with goat (dose) as the term of error (*17*).

Table 1. Accuracy of Prediction by NIR Spectroscopy of Fecal PEG Concentration, Using MPLS Calibration and the Calibration/Validation or Cross-Validation Methods: SE Units Are %, on DM Basis

calibration		validation	
SE calibration	R^2	SE validation	R^2
0.26	0.99	0.46	0.98
cross validation			
SE calibration	SE cross validation	R^2	
0.19	0.32	0.99	

RESULTS AND DISCUSSION

The first purpose of this study was to assess the feasibility of NIRS analysis of PEG in goats' feces. After removal of two outlier points − that possibly resulted from errors in weighing the components of two individual PEG−feces mixtures − the calibration of PEG concentration in feces, using the MPLS method and the first derivative of Log(1/*R*), was of very high predictive value ($R^2 = 0.99$, **Table 1**). The slope of the correlation between predicted and actual values for PEG concentration was 0.99 and did not differ from 1.0. Accuracy, as measured by SE of validation, was 0.46 and 0.32%, using classical validation and the cross-validation procedure, respectively. Calibrations based on the second derivative of log(1/*R*) were of lesser predictive value.

Good correlations (**Figure 1**) were found between the first derivative of Log(1/*R*), measured at particular NIR segments, and PEG concentrations in the PEG−feces mixtures (**Figure 1**). This was the case for the 1160−1162 nm (C═O stretch; $R = -0.96$), 1166−1176 nm (2nd overtone of CH stretch, CH═CH bond; $R = -0.96$), 1190−1200 nm (2nd overtone of CH, CH_3; $R = -0.95$), 1694−1700 nm (CH stretch, $R = -0.96$, as found also by others (*10*)), 1816−1820 (OH stretch, $R = 0.97$), 2186 nm (NH bond, $R = -0.96$), 2200 nm (CH stretch, C═O, $R = -0.97$), 2278−2286 nm (CH stretch and CH deformation, $R = -0.96$), and 2330 nm (CH stretch, CH2 deformation, $R = -0.96$) NIR segments. However, not all correlations resulted from the identification of chemical bonds belonging to the PEG molecule. A prominent example is the good correlation between Log(1/*R*) and the concentration of PEG, measured at 2186 nm. The PEG molecule does not contain N atoms. Therefore, the good correlation obtained at this segment is probably the mere effect of dilution of N-containing bonds in the feces matrix, which could be obtained by mixing feces with any N-free material. In contrast, the correlation obtained at the 2276−2286 nm is one of chemical significance. When single wavelength calibration was carried out, using only the 2276−2286 nm NIR segment (CH-stretch and CH2 deformation), the determination of PEG concentration in feces was accurate, yet at lesser accuracy ($R^2 = 0.96$; SE of calibration = 0.6%) than by using the MPLS procedure (**Table 1**). The spectra of feces samples containing 0, 2, 5, 7.5, and 10% PEG are shown in **Figure 2**, featuring their easy identification at this particular NIR segment. This finding could be of practical interest for the use of PEG as a marker of fecal output when diets contain tannins, as hydrogen bonding between oxygen of the PEG chain and phenolic hydroxyl groups (*18*) does not involve chemical moieties that are comprised in this NIR segment. The spectra of feces from one goat, sampled at different hours of the day, and dosed with 0, 20, or 40 g/d PEG are depicted in **Figure 3**, clearly showing three well-defined populations according to the PEG dose, all along the NIR range, and, more evidently, in the 2276−2286 nm NIR segment.

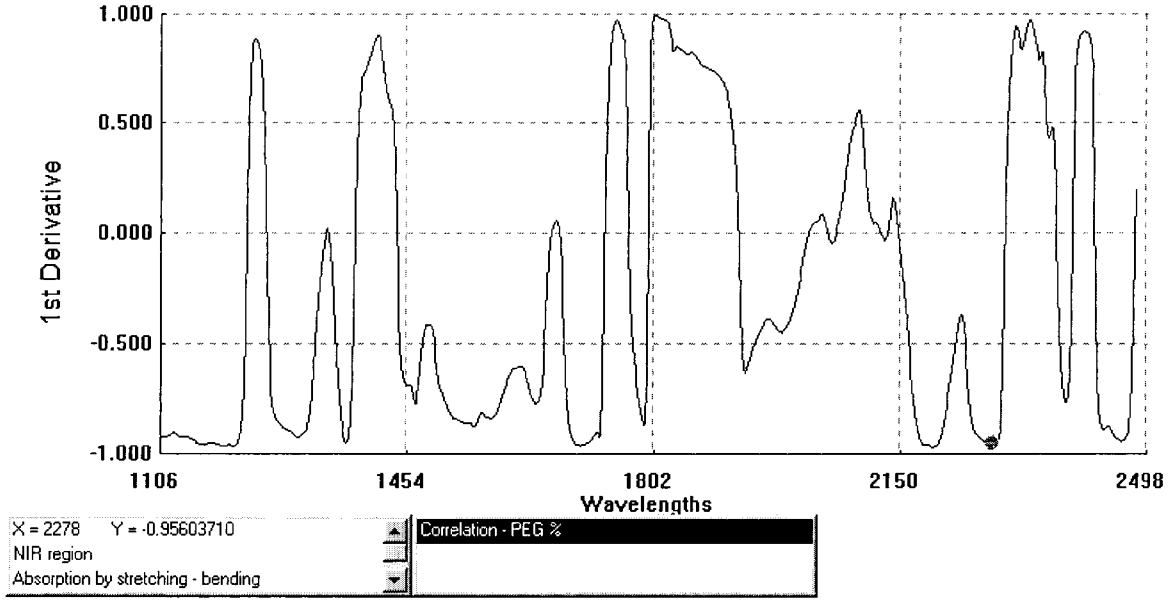

Figure 1. Correlation coefficients (*Y*-axis) between the 1st derivative of Log(1/*R*) at particular wavelengths in the NIR range and PEG concentration in feces–PEG mixtures ($n = 42$)

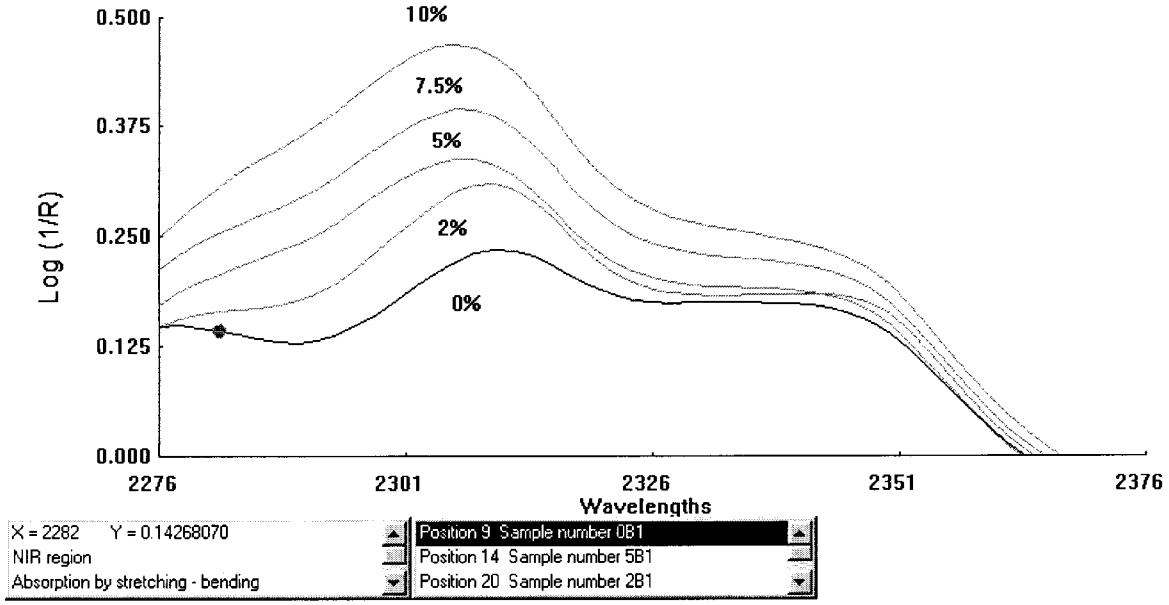

Figure 2. NIR spectra in the 2270–2350 nm wavelength segment of feces–PEG mixtures used for calibration of PEF in feces, after standard normal variance and de-trend procedure.

Once the accuracy of NIRS analysis of PEG in feces was demonstrated, we used our data to re-visit the issue of PEG as a marker of fecal output. In order for PEG to be adopted as a marker of fecal output, this molecule must be shown not to interfere with steady state condition along the GIT (*1*). When all data were pooled, fecal DM content was affected by time of sampling ($P < 0.001$; **Figure 4**), which seems contradictory to strict steady state condition. The higher PEG dose significantly decreased the content of DM in feces, compared with the lower dose (57.8 and 47.8%, SE 1.3%, $P < 0.005$), but the effect of sampling time on fecal DM content was more significant in goats given 40 g/d of PEG than in goats given 20 g/d of PEG ($P < 0.03$ and $P < 0.06$, respectively). This is consistent with the recent finding (*20*) that PEG reduces the mean retention time of the liquid phase in the GIT in goats, and that the osmotic effect of PEG inhibits water and electrolyte absorption by the jejunum and the ileum in humans (*21*). Therefore, in order for PEG not to interfere with the digestive process (*1*), low doses of the polymer must be administered.

Overall, time of sampling affected the fecal concentration of PEG ($P < 0.04$; **Figure 4**). However, this effect was significant ($P < 0.001$) in goats given the higher dose of PEG (**Figure 4b**), but not in those given the lower dose of PEG (**Figure 4a**). The respective average diurnal coefficient of variation for these two groups was 16.7% and 15.3%, lower than that found in steers by previous authors, i.e., 16.4 to 37.8% (*2*). Therefore, it can be assumed that dilution in rumen did not prevent a diurnal pattern of PEG excretion from the gastrointestinal tract. Additional research is needed to elucidate whether the administration of PEG in drinking water consumed freely throughout the day solves this problem. Calculations show that, in goats given 20 g/d PEG, sampling at any one particular hour of the day would not bias PEG recovery significantly. However, pooling of materials collected at different hours of the day into a

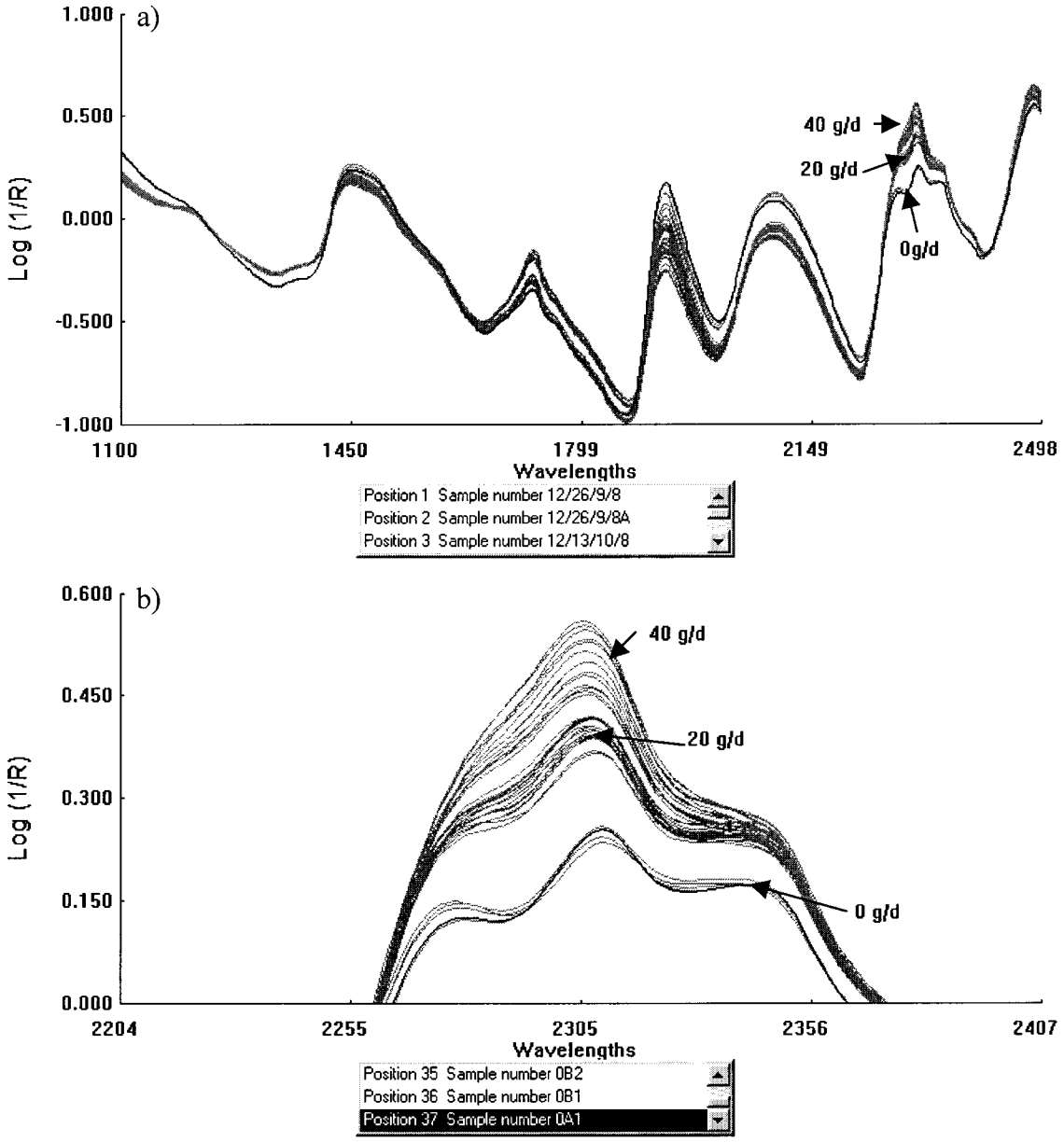

Figure 3. NIR spectra of the feces of a goat dosed successively with 20 and 40 g/d PEG, after standard normal variance and de-trend procedure (*13*). Different spectra feature feces collected at different times of the day. (a) In the whole NIR range; (b) in the 2250–2350 nm range.

Table 2. Actual Values and NIRS-Calculated Estimates of Fecal Output (g/d) and the Recovery of PEG (g/d) in Goats Given PEG at Doses of 20 or 40 g d^{-1} (Means of Four Goats ± SE)

	fecal output		
PEG dose	actual	NIRS estimate	PEG recovered
20 g d^{-1}	317 ± 25	313 ± 17	20.3 ± 0.7
40 g d^{-1} [a]	366 ± 18	310 ± 13	45.5 ± 1.1

[a] Actual and NIRS-estimated values for fecal output differ at $P < 0.05$.

composite sample is a widely established procedure in tracer-aided studies of digestion (*1*), and seems to be recommendable for PEG as well.

Actual values of fecal output and PEG recovery, and their PEG-calculated estimates, are shown in **Table 2**. Actual and NIRS-estimated values for fecal output did not differ, and the recovery of PEG was accurate (20.3 ± 0.7 g/d) in goats dosed with PEG at 20 g/d. In contrast, fecal output was underestimated, and PEG was overestimated ($P < 0.05$), when goats were given 40 g/d of PEG. The higher dose of PEG possibly affected water metabolism and prevented excretion of PEG at a steady rate during the last phase of the experiment, biasing estimates of PEG recovery on a daily basis.

PEG concentration in the feces of goats dosed with 20 g/d of PEG was in the 6−7% range. The analytic ability of NIR instruments is good enough to detect PEG at concentrations 3−5% of DM. Therefore, an optimal dose of PEG as marker of fecal output in goats kept at maintenance level is approximately 15 g/d. Additional studies are needed to assess the optimal dose of PEG in more productive animals, fed at 3- or 4-fold maintenance level. The diets given in the present study did not contain tannins. Because PEG is known to interfere with dietary tannins (*18*, *19*) and was found unsuitable as a marker for rumen volume determination by turbidimetry when animals were fed diets with tannin-containing cottonseed husks (*21*), the validity of PEG as a tracer of fecal output has to be assessed in animals fed tannin-containing diets.

The accuracy of PEG recovery in our study was superior to that, measured by turbidimetry, of PEG in rumen content (*21*)

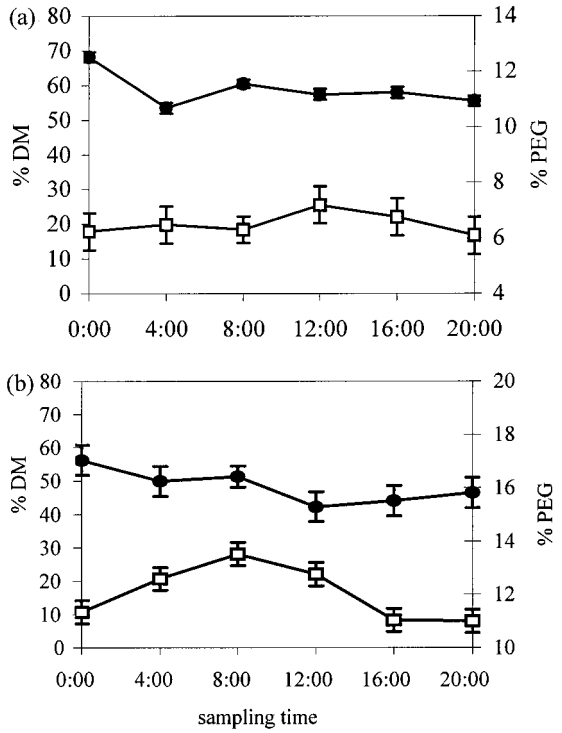

Figure 4. Concentrations of dry matter (●, left *Y*-axis) and PEG (□, right *Y*-axis) in the feces, sampled at different times, of goats given daily PEG doses of 20 g (a) or 40 g (b); means of 4 goats and SE.

or total gastrointestinal tract (*2*) in cattle, but not to the recovery of PEG infused to the gut of humans (*5*). However, when comparing these analytical methods, one must remember that NIR requires only drying, grinding (samples), and weighing (calibration curve), whereas the turbidimetric method requires weighing the samples, diluting with water, adding $BaCl_2$ and $Ba(OH)_2$, mixing, adding $ZnSO_4$, shaking, filtering, adding gum arabic solution to the filtrates, mixing, adding TCA−$BaCl_2$ mixture, mixing again, and reading optical density.

Our data suggest that PEG determination by NIRS-aided analysis, is simple, accurate, and much less laborious than its determination by turbidimetry. PEG can be used as an indigestible marker to determine fecal output in goats, with the condition that PEG doses are not too high. However, this also has to be ascertained in goats fed diets containing tannin and in goats under conditions of high feeding level.

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