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A method for estimating dry forage intake by sheep using polyethylene glycol as a faecal marker measured with NIRS

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In experiments based on ruminants' individual dry matter intake (DMI) assessment, several external markers can be used to estimate faecal output when total faeces collection is not possible. However, preparation of the markers to be administered and analytical procedures used for marker content determination are time-consuming thus strongly limiting the number of animals involved in the experiments. In this paper, polyethylene glycol (PEG, molecular weight 6000 da) was tested as a faecal marker. Four trials were conducted on dry, non-lactating ewes kept in digestibility crates that allowed individual measurements. The overall experiment was designed to assess the major factors that could lessen the effectiveness of this method, assuming that the use of grab samples of faeces is sufficient. Trial 1 was designed to test two levels of PEG (20 and 40 g/day) administered in two equal amounts. Trial 2 was designed to test the effect of either a single morning (0800 h) dose (20 g/day) or a twice daily administration (0800 and 1600 h) of the same fractionated dose. Trial 3 was designed to test a 20 g/day dose of PEG administered once daily to ewes fed with hays of different qualities: medium (MH) and low (LH). In trial 4, a lower dose of PEG (10 g/day) was administered once a day to ewes fed with fresh oat–vetch forage. It was demonstrated that PEG could be precisely estimated (average prediction error = 3.47 g/kg) with near-infrared reflectance spectroscopy (NIRS). On the basis of the four trials, it has been proved that PEG administration (20 and 40 g/day) did not significantly affect the DMI of ewes fed dry diets (trials 1, 2 and 3), whereas there was an unexpected increase of DMI for ewes fed exclusively with green feed (trial 4) without DM digestibility modification. Providing PEG as a single dose (0800 h) or split into two equal parts (0800 and 1600 h) did not alter the estimated DMI. Considering the interest of grab sampling, there were clear variations of PEG in faeces with higher concentrations observed at 0800 and 1600 h and lower concentrations at 1400 h. Consequently, with PEG (measured with NIRS) administered once and using the grab sampling procedure (morning collection), it is possible to estimate the DMI of dry feeds with good accuracy. For green feeds, more research is needed as the estimated results are still highly variable.

Keywords: polyethylene glycol, near-infrared spectroscopy, faecal marker, intake, sheep

Implications

Several external markers that can be recovered in faeces are used in ruminant studies to estimate dry matter intake (DMI). However, analytical procedures are tedious and time-consuming. Polyethylene glycol (PEG) has been successfully tested as a faecal marker determined with the near-infrared reflectance spectroscopy (NIRS) technique. The present study has confirmed that PEG is a valuable faecal marker to estimate DMI in sheep with good accuracy when measured with the NIRS method. Because it is easily administered and quickly measured with NIRS, PEG can be used to satisfactorily estimate DMI and allows to work on a greater number of animals than other markers.

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Introduction

In ruminants studies based on individual dry matter intake (DMI) assessment, DMI is estimated from total faeces output and DM digestibility (DMD) assessments. The DMD can be estimated using *in vitro* techniques and total faecal output by determining the dilution rate of external markers (Prigge *et al.*, 1981).

Polyethylene glycol (PEG) with high molecular weight (>3000) is generally used as a soluble marker to measure rumen fluid volume and output flow rate from the rumen (Bauman *et al.*, 1971). It has also been used as an external faecal marker to estimate total faecal output (Corbett *et al.*, 1958; Hopson and McCroskey, 1972). However, when PEG is measured with colorimetric or turbidimetric methods, its recovery in faeces has been found to be variable and low

(Corbett *et al*., 1958). These results have been attributed to diet composition (Clark *et al*., 1972), feed digestibility or methodological difficulties (Alexander *et al*., 1969).

More recently, Landau *et al*. (2002) demonstrated that PEG could be determined in goat faeces with the near-infrared reflectance spectroscopy (NIRS) method with good accuracy. Furthermore, Jones and Palmer (2000) showed that PEG addition does not modify *in vitro* DMD. However, they pointed out that PEG binds with condensed tannins (CTs) and may indirectly increase CP digestibility. Compared with other analytical methods, NIRS method only requires the samples to be dried and ground, before scanned, and consequently a high number of samples (a hundred) can be determined daily. The use of PEG in combination with NIRS, allow the use of 10 animals in trials estimating DMI for periods of adequate length, which is difficult (costly and time-consuming analytical methods) to apply with PEG when measured by colorimetric or turbidimetric methods or by other external markers (Corbett *et al*., 1958; Hopson and McCroskey, 1972; Prigge *et al*., 1981).

Considering the potential interest of PEG as a faecal marker and its rapid determination by NIRS, Hassoun *et al*. (2007a) set up a 1000-sample database with wide ranges of PEG content in faeces. In an *in vivo* experiment with sheep (Hassoun *et al*., 2007b), various PEG doses were tested. Regardless of PEG level of administration, the recovery rate was higher than 98%.

With external markers used to estimate total faeces excretion, two sources of errors have to be considered. The first one deals with total recovery of the marker in faeces, meaning that few or no administered marker disappeared until reaching rectal excretion. The second one deals with the daily excretion pattern of the marker that is linked to the grab sampling approach (i.e. faecal samples taken directly from rectum) to estimate total faeces output. Consequently, the marker concentration measured in grab samples must be as close as possible to the marker mean concentration in total faeces. These two potential sources of bias have been studied with chromium sesquioxide (Langlands *et al*., 1963) and PEG (Hopson and McCroskey, 1972; Landau *et al*., 2002). Langlands *et al*. (1963) and Hopson and McCroskey (1972) found that total faeces output was either under- or overestimated due to these two sources of bias according to the administration form of the marker (on paper or into a capsule) and daily administration frequency of the marker (once or twice daily). On the contrary, Landau *et al*. (2002) stated that grab sampling would not bias significantly PEG recovery when goats were given 20g/day PEG.

The present experiment aimed to measure daily total PEG excretion in faeces, the diurnal excretion pattern in grab samples and to test three levels of PEG and two methods of administration in order to conclude on the usefulness of the PEG as external marker in forage intake studies.

Material and methods

Four trials were conducted. The first three trials were conducted at the Montpellier-Vauguières Experimental Unit (INRA–PHASE, UMR SELMET) in the south of France, and trial 4 at the La Fage experimental farm unit (INRA–GA–SAGA) in a semi-mountainous area. All trials were conducted indoors in January/February 2005 (trial 1), March 2006 (trial 2), October/November 2006 (trial 3) and April 2007 (trial 4). All experimental procedures were approved by the current French Animal Ethics Committee at the time that each trial was performed.

Trial design

For all trials, each adult dry and non-pregnant ewe was previously orally drenched against internal parasites with 14 ml of Supaverm® (Jansen-Cilag, Issy-les-Moulineaux, France) (trials 1 to 3) or 18 ml of Panacur® 2.5% (Intervet, Beaucouzé, France), and placed in individual metabolism crates. Within trials, groups were balanced on a BW basis.

Trial 1 was conducted on two groups of Merinos d'Arles (MA) (48 ± 3 kg). It consisted of a 14-day control period, followed by two measurement periods of 2 weeks. The control period consisted of 1 week of adaptation, followed by a 5-day DMD measurement period. The measurements periods consisted of 2 weeks during which 20 g/day (PEG_{20}) and 40 g/day of PEG (PEG_{40}) were administered as a solution in two equal parts (0800 and 1600 h) to five (PEG_{20}) and four (PEG_{40}) ewes. During the 1st week, the daily kinetics of the appearance of PEG in total faeces was recorded. In the 2nd week, PEG recovery was measured daily on total faeces output. The excretion pattern between 0800 and 1800 h was defined through grab samples collected every 2 h for 5 consecutive days. The diet DMD was measured over the same period. The PEG administration was then stopped and the kinetics of PEG disappearance in total faeces output was recorded for 1 week. Diet DMD was measured the 2nd week for 5 consecutive days

Trial 2 was conducted on two groups of four MA each (51 ± 5 kg). It was performed according to a cross-over design with two periods of 2 weeks, including 1 week of transition, followed by 1 week of measurement. Two groups of four ewes received 20 g/day of PEG in solution during the two periods. The first group (PEG_{single}) received a single dose at 0800 h (period 1) and twice daily in split doses (period 2) at 0800 and 1600 h. The reverse procedure was applied for the second group (PEG_{twice}). The PEG recovery, diurnal kinetics of excretion and the DMD of the diet were measured during the 2nd week of each period for 5 consecutive days.

Trial 3 was conducted on two groups of five MA each (51 ± 2 kg) successively fed two Crau perennial meadow hays of different qualities. The control group (C3) was not dosed with PEG, and the experimental group (E3) received a dose of 20 g/day of PEG administered once a day (0800 h) on a daily basis. The trial consisted of a 2-week adaptation period, followed by 1 week for DMD determination with the medium quality hay (MH) and a 1-week adaptation period for PEG and one measurements week. A 1-week adaptation period was then used to adapt the animals to the low quality hay (LH), followed by one measurements week. The PEG recovery was measured on total faeces collected for 5 consecutive days, and grab samples were simultaneously collected at 0800 h.

Table 1 *Chemical composition of the feeds provided in trials 1, 2, 3 and 4*

	DM (%)	OM (g/kg DM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	CT (g/kg DM)
ALF (trial 1)	90.3	972	155	485	326	nd
SBP (trial 1)	89.4	920	80	503	262	nd
STR (trial 1)	90.7	941	36	810	481	nd
Hay (trial 2)	90.3	909	109	650	334	nd
Hay − MH (trial 3)	89.7	914	105	539	327	nd
Hay − LH (trial 3)	89.8	923	66	575	355	nd
Oat−Vetch (trial 4)	15.3	896	168	423	206	1.7

DM = dry matter; OM = organic matter; NDF = neutral detergent fibre expressed exclusive residual ash; ADF = acid detergent fibre expressed exclusive residual ash; CT = condensed tannins; ALF = dehydrated alfalfa; SBP = dehydrated sugar beet pulp; STR = molassed straw pellet; MH = medium-nutritive value hay; LH = low-nutritive value hay; nd = not determined.

Trial 4 was conducted on two groups of five dairy Lacaune ewes each (79 ± 17 kg). The control group (C4) received fresh oat-vetch forage without PEG and the experimental group (E4) received the same forage plus 10 g/day of PEG in a daily single dose (0800 h). The trial consisted of a 2-week adaptation period including PEG adaptation, followed by a 10-day measurement period (total and grab PEG recovery, DMD determination). Grab samples were collected at 0800 h.

Diets and feeding

In trial 1, animals were fed 0.3 kg of dehydrated alfalfa pellets and 0.2 kg of dehydrated sugar beet pulp pellets, both provided in two equal parts at 0900 and 1600 h. In addition, they were fed 1.5 kg of molassed wheat straw pellets once a day at 0900 h. In trial 2, animals were fed a 5-year-old semi-mountainous meadow hay harvested in July 2004. The hay was chopped into 5-cm-long pieces and given for *ad libitum* intake (allowing 10% to 15% refusal on the DM basis) in two equal meals as in trial 1. In trial 3, animals were fed hay for *ad libitum* intake (allowing 10% to 15% refusal on the DM basis), chopped into 5-cm-long pieces. In trial 4, animals received fresh oat-vetch cut every day and given for *ad libitum* intake (allowing 20% refusal on the DM basis). Fresh water was freely available to all of the animals in all of the trials. The chemical composition of the feeds is presented in Table 1.

Chemical analysis

All samples (feed and faeces) were ground through a 1-mm sieve. Total mineral content was determined by ashing in a muffle furnace for 5 h at 550°C. Total nitrogen was determined using the Kjeldahl procedure. Cell wall fractions (NDF, ADF) were determined sequentially according to the method of Goering and Van Soest (1970) with an amylolytic and protease pre-treatment. Cell wall fractions are expressed exclusive of residual ash. The CT (trial 4) were determined using the vanillin method (Burns, 1971).

DMI and digestibility

Individual DMI was measured daily in the four trials. Refusals were recorded every morning before the first new meal. Offerings and refusals were sampled every day for DM content determination (48 h, 60°C). During the measurement periods, total faeces were collected, weighed and sampled for DM determination (48 h at 60°C) in order to calculate total faecal output. Apparent DMD, based on the individual average DMI and faecal output, was calculated on 5 to 7 consecutive days, depending on the trial.

PEG preparation, administration and NIRS measurement

The PEG used was PEG 6000 (molecular weight 6000 da, Panreac Qimica SA, Barcelona, Spain). It was administered in solution form with plastic syringes. The PEG solution, with a concentration of 333.33 g/l, was prepared a few days before in the laboratory with double distilled water. In order to precisely determine the quantity of PEG administered, each syringe was weighed to the nearest 0.1 g before and after administration. The concentration of PEG in faeces was estimated with the NIRS method. Calibration databases were built by adding known amounts of PEG in faeces samples collected before the first doses, in order to build PEG + faeces databases similar to collected samples. The PEG was added to faeces in solution within a range of 0 to 100 g of PEG/kg DM (by 5 or 10 g of PEG/kg DM steps). The PEG + faeces mixtures were dried (50°C until constant dry weight) and ground through a 1-mm sieve, similarly to the procedure applied for faeces preparation during trials.

The samples were scanned on a monochromator NIR spectrophotometer (NIRS 6500, Foss NIRSystems, Silver Spring, MD, USA). Measurement was done in reflectance mode in small circular cups (diameter: 50 mm) with quartz glass. Spectral data were collected every 2 nm from 400 to 2500 nm. Samples were scanned in duplicate (two different cup fillings) and spectra were averaged. Spectra were added to our existing database (Hassoun *et al.*, 2007a), leading to a global database of 527 samples of faeces samples collected from various origins (sheep breed and diets). The NIRS calibration was carried out using partial least squares regression (MPLS procedure, WinISI software, Infrasoft Int., Port Matilda, PA, USA). Only wavelengths in the 1100 to 2500 nm range were used, because of the unstability of models built with visible wavelengths. Mathematical pre-processing was applied to spectra with detrending and normalization (SNV) of data, and use of the second derivative calculated on five consecutive points with a smoothing also on a gap of five points. During the calibration process, prediction outliers

(studentized residual $T > 2.5$) were discarded. Cross-validation of NIR equations was performed during the calibration process, dividing the database into four groups of samples of which three were used for calibration and the fourth for validation. Criteria for the evaluation of the prediction models are SEC (standard error of calibration) and SECV (standard error of cross-validation), which is an estimate of precision that can be expected in routine analysis. The RPD (=s.d./SECV) was calculated as an indicator of the quality of the models (Williams and Sobering, 1993). The Mahalanobis distance was used to compare spectra with the database and identify spectral outliers following Shenk and Westerhaus (1991).

PEG recovery and PEG content in grab samples

The PEG recovery (REC) was calculated with the formula: $REC = PEG_T$ (g/kg DM) $\times$ FT (g/kg DM)/PEG_{in} (g/day), where PEG_T is the PEG content estimated with NIRS in total faeces (FT) and PEG_{in} the daily amount of PEG intake. The REC was calculated daily for each ewe. Faecal grab samples were collected at the rectal level the same day that total faeces were collected for REC measurement. The PEG content of grab samples (PEG_g) collected at 0800 h (trials 1 to 4) and 1600 h (trials 1 and 2) was determined with NIRS. It was compared with PEG_T as a percentage:

$$(PEG_g / PEG_T) \times 100.$$

Estimated faecal output and DMI

Faecal output was calculated including the correction of the two sources of bias values (total and grab sample recovery) or without correction (i.e. assuming that PEG recovery was not different of 100%). Each bias was calculated within trial and factor of variation when applied or averaged for the four trials. Estimated faecal output, corrected (F_c) with the within trial biases or averaged trial biases or not corrected (F_{nc}), was calculated as follows:

$$F_c\,(\text{kg DM}) = [PEG_{in} \times REC \times (1000 - PEG_g8 / REC_g) / (PEG_g8 / REC_g)] / 1000$$

$$FEC_{nc}\,(\text{kg DM}) = [PEG_{in} \times (1000 - PEG_g8) / PEG_g8] / 1000$$

where PEG_g8 (g/kg DM) is the PEG content measured in grab samples collected at 0800 h and REC_g the PEG recovery amount measured in grab samples.

Estimated DMI using F_c calculated with each trial biases (DMI_c), averaged trial biases (DMI_{avc}) or using F_{nc} (DMI_{nc}) were calculated as follows:

$$DMI_c \text{ or } DMI_{avc}\,(\text{kg/d}) = F_c/(1 - DMD/100)$$

$$DMI_{nc}\,(\text{kg/d}) = F_{nc}/(1 - DMD/100))$$

Statistical treatment of results

In all trials, DMI, DMD and faecal DM content (FDM_c) were compared between groups (PEG_{20} and PEG_{40}, PEG_{single} and PEG_{twice}, C3 and E3 and C4 and E4, for trials 1, 2, 3 and 4, respectively) at each period: before PEG (trials 1 to 3), with PEG (trials 1 to 4) and after PEG (trial 1). Data were averaged per period for each ewe. Consequently, because of the low number of ewes per treatment or period, the non-parametric Mann and Whitney *U*-test (Siegel and Castellan, 1988; Sprent, 1992) was used to compare two independent samples. The REC was also compared at each measurement period between PEG_{20} and PEG_{40} (trial 1), PEG_{single} and PEG_{twice} (trial 2) and E3 MH and E3 LH (trial 3), with the same non-parametric test. Because non-statistical intra-trial differences were found, the REC between trials was compared adding trial 4, with the non-parametric Kruskal–Wallis test for multiple independent samples (Siegel and Castellan, 1988; Sprent, 1992). The PEG content measured on grab samples at 0800 h, 1600 h or averaged was compared with PEG_T with the non-parametric Wilcoxon test for paired samples because sample numbers were small, from four to eight (Siegel and Castellan, 1988; Sprent, 1992). For the same reason, actual DMI were compared with DMI_c, DMI_{avc} and DMI_{nc}, respectively, with the non-parametric Wilcoxon test for paired samples. All statistical analyses were performed using Statistica v9.1 for Windows (Statsoft 2010, www.statsoft.fr).

Results

NIRS calibration for PEG content

The calibration itself had an $R^2 = 0.99$ and a residual standard error (SEC) of 3.05 g/kg. No major bias was observed in the calibration process, and there were only 13 outlier samples, that is, 2.5% of the calibration database. The validation of the calibration model by cross-validation resulted in a standard error (SECV) of 3.47 g/kg, which is quite accurate for such a measurement. The RPD value was 9.1.

When applying the calibration to our experimental samples, it appeared that all samples to be predicted were inside the calibration database range. Only seven samples had an H (Mahalanobis) distance higher than 3, which was perfectly satisfactory.

Intake, digestibility and faecal DM content

The average DMI, FDM_c and DMD for trials 1 to 4 are presented in Table 2. In trial 1, DMI and DMD were not significantly different ($P > 0.05$) before PEG (control period), with 20 or 40 g of PEG, or after when PEG was stopped for 1 week (Table 2). The FDM_c was not significantly different between PEG_{20} and PEG_{40} when PEG was administered. However, it decreased compared with the first period, from 36.4% to 34.1% for the PEG_{20} group, and from 35.3% to 32.8% for the PEG_{40} group. In contrast, FDM_c was significantly different ($P < 0.05$) between PEG_{20} and PEG_{40} measured 1 week after PEG was stopped. During this period, the FDM_c of PEG_{20} group increased and recovered its initial FDM_c value (before the PEG period). On the other hand, the FDM_c of PEG_{40} did not change and remained at the same value as during PEG administration.

In trial 2, there were no significant differences between single or split doses on any parameters (Table 2). The FDM_c

Table 2 *Mean and relative standard deviation (values in parentheses) of DMI, FDM_c, DMD and PEG recovery rate (REC) measured in the four trials*

Trial	Group	Dose (g/day)	Frequency	Period	DMI (kg/day)	DMI (g/kg$W^{0.75}$)	FDM_c (%)	DMD (%)	REC (%)
1	PEG_{20}			Before PEG	1.48 (3.0)	81 (5)	36.4 (1.9)	41.9 (7.4)	
1	PEG_{40}			Before PEG	1.43 (3.5)	79 (9)	35.3 (2.2)	40.3 (3.7)	
1	PEG_{20}	20	Split	With PEG	1.52 (8.6)	82 (9)	34.1 (3.5)	37.8 (4.3)	109.2^a (2.0)
1	PEG_{40}	40	Split	With PEG	1.57 (6.1)	85 (8)	32.8 (1.3)	36.3 (2.9)	108.0^a (2.6)
1	PEG_{20}			After PEG	1.57 (7.3)	84 (8)	37.3^a (4.4)	42.3 (4.6)	
1	PEG_{40}			After PEG	1.60 (8.0)	85 (9)	33.0^b (2.1)	41.2 (1.6)	
Significance					ns	ns	0.019	ns	
2	PEG_{single}	20	Single	1	1.10 (12.9)	56 (18)	39.0 (0.4)	62.7 (3.5)	97.0^b (4.9)[1]
2	PEG_{twice}	20	Split	1	1.18 (16.1)	65 (15)	36.1 (1.7)	62.3 (4.4)	95.5^b (7.7)[1]
2	PEG_{single}	20	Single	2	1.28 (3.9)	68 (5)	35.8 (2.2)	62.3 (1.5)	
2	PEG_{twice}	20	Split	2	1.20 (6.8)	59 (15)	38.2 (1.9)	62.9 (3.8)	
Significance					ns	ns	ns	ns	
3	C3			Control-MH	1.24 (16.7)	65 (18)	46.6 (4.3)	63.9 (4.9)	
3	E3			Control-MH	1.20 (8.3)	63 (8)	45.8 (5.8)	63.6 (2.1)	
3	C3			Exp-MH	1.14 (15.9)	61 (18)	48.1 (3.0)	61.3 (2.0)	
3	E3	20	Single	Exp-MH	1.14 (4.8)	59 (7)	42.1 (1.1)	59.7 (4.9)	95.4^b (2.0)
3	C3			Exp-LH	1.30 (9.4)	66 (11)	45.2 (2.1)	55.9 (1.8)	
3	E3	20	Single	Exp-LH	1.24 (4.4)	64 (6)	40.8 (1.1)	57.0 (2.1)	98.4^b (1.7)
Significance					ns	ns	ns	ns	
4	C4			–	0.74^b (22.6)	31^b (14)	56.5^b (2.7)	80.4 (2.0)	
4	E4	10	Single	–	1.40^a (31.5)	55^a (27)	37.0^a (5.5)	79.8 (3.1)	87.5^b (10.7)
Significance					0.028	0.047	0.022	ns	0.0004

DMI = dry matter intake; FDM_c = faecal dry matter content; DMD = dry matter digestibility; PEG = polyethylene glycol; MH = medium-nutritive value hay; LH = low-nutritive value hay.
Within trial and period for DMI, FDM_c and DMD, values with different superscript letters in a column are significantly different ($P < 0.05$). For REC values with different superscript letters in the column are significantly different ($P < 0.05$).
[1]Average REC values per frequency.

tended to be lower in both periods for ewes that first received PEG as a split dose and then as a single dose (36.1% and 35.8%) compared with the other group (39.0% and 38.2%), as presented in Table 2.

In trial 3, during the control period (Table 2), one ewe in the C3 group had a mouth problem and its results have been discarded for this period. After 3 days, the ewe recovered normal intake and had no more problems until the end of the trial. During the control period with MH hay, both DMI and DMD were not significantly different ($P > 0.05$) between the C3 and the E3 group (Table 2). Also, FDM_c was not significantly different ($P > 0.05$) between C3 and E3. When PEG was administered to group E3 fed with MH or LH, DMI and DMD were not significantly different ($P > 0.05$) compared with the C3 group (Table 2). Only FDM_c tended to be lower ($P = 0.06$) for E3 with MH but not with LH ($P = 0.31$).

In trial 4, during the 1st-week adaptation period, two ewes (one in each group) were replaced after 4 days because they did not adapt to the crates. This was the only problem that occurred. The DMI of E4 was significantly higher ($P < 0.05$) than the control group C4 (Table 2). In contrast, DMD was not significantly different ($P = 0.84$) between C4 and E4. The FDM_c was significantly lower ($P < 0.05$) for the E4 compared with the C4 group: 37.0% *v.* 56.5%, respectively.

PEG recovery, daily and diurnal excretion pattern

The recovery (REC) measured in the total faeces collected over 7 (trial 1), 5 (trials 2 and 3) or 9 days (trial 4) are summarized in Table 2. The REC obtained in the first three trials ranged between 95% and 109%, with a similar variability of 6.2% to 8.9%. In trial 4, a lower mean value was obtained (88%) with a higher variability of 24%. No significant effect ($P < 0.05$) of the treatments was observed: dose, frequency and hay in trials 1, 2 and 3, respectively. However, when comparing trials, REC in trial 1 was significantly higher than in trial 2 ($P < 0.05$), trial 3 ($P < 0.001$) and trial 4 ($P < 0.001$). The three other trials were not significantly different ($P > 0.05$).

Faecal PEG content measured on grab samples (PEG_g) collected at 0800 h (PEG_g8) and 1600 h (PEG_g16) or pooled (PEG_g8-16) were compared with PEG_T. Values and statistical results are summarized in Table 3. In all trials, PEG_g8 values were not significantly different ($P > 0.05$) compared with PEG_T, except in trial 1 when 20 g/day of PEG was administered ($P = 0.04$), and in trial 2 when 20 g/day of PEG was administered as a single dose ($P = 0.03$). The values of PEG_g16 and PEG_g8-16 were significantly different ($P < 0.05$) from those of PEG_T in trial 2 but not in trial 1. The value of PEG_g8 represented more than 90% of PEG_T, except in trial 2 when PEG was administered in a split dose (79%). Average PEG_T values measured in the four trials are summarized in Table 3. Values ranged from 22.0 to 43.0 g/kg DM with a similar standard deviation (s.d.) of 2.2 to 8.6, which gives a relative s.d. ranging from 5% to 28% (results not shown). Trial 4 gave the highest s.d. and relative s.d. The PEG_T was followed from the 1st day until 1 week after the last

Table 3 *Average PEG content measured in grab samples collected at 0800 h (PEG_g 8), 1600 h (PEG_g16) or averaged (PEG_g8–16), and in total faeces (PEG_T), statistical result (P-value of the Wilkinson test) for the comparison of means*

Trial	Group	PEG_g8 (g/kg DM)	PEG_g16 (g/kg DM)	PEG_g8–16 (g/kg DM)	PEG_T (g/kg DM)	PEG_g8 *v.* PEG_T *P*	PEG_g16 *v.* PEG_T *P*	PEG_g8–16 *v.* PEG_T *P*
1	PEG_{20}	19.0	19.9	19.5	22.0	0.04	0.22	0.04
	PEG_{40}	39.7	38.2	39.0	40.8	0.46	0.14	0.27
2	PEG_{single}	44.8	30.4	37.6	41.5	0.03	0.01	0.01
	PEG_{twice}	39.8	28.1	34.0	43.0	0.12	0.01	0.01
3	E3MH	37.9	–	–	40.7	0.37	–	–
	E3LH	35.3	–	–	37.4	0.33	–	–
4	E4	33.7	–	–	31.1	0.5	–	–

PEG = polyethylene glycol; MH = medium-nutritive value hay; LH = low-nutritive value hay.

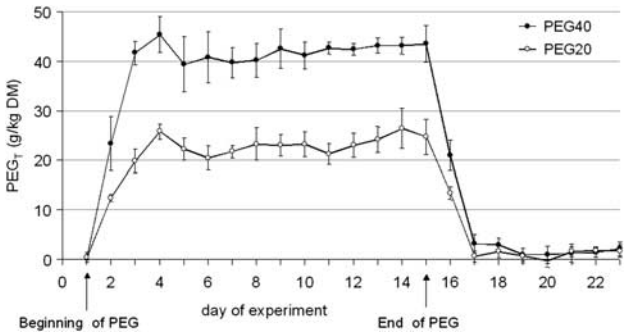

Figure 1 Evolution of PEG content (PEG_T) in total faeces during the experiment for groups dosed with 40 (PEG_{40}) or 20 (PEG_{20}) g of PEG per day (trial 1). PEG = polyethylene glycol.

PEG administration in trial 1. The excretion pattern is presented in Figure 1.

Once PEG was administered, PEG_T rapidly increased over the first 2 days and then remained quite stable although markedly different between doses, until the last day for both 20 and 40 g/day doses. When PEG administration was stopped, PEG_T decreased as quickly as it had increased at the beginning. No PEG was recorded 2 to 3 days later. Although the values observed on subsequent days were not exactly null (1.1 and 1.3 g/kg DM for PEG_{20} and PEG_{40}, respectively), they ranged within the NIRS standard error of prediction: SECV = 3.47 g/kg DM.

The diurnal excretion pattern was measured on grab samples in trial 1 (Figure 2a) and trial 2 (Figure 2b). When PEG was administered twice a day in trial 1, PEG_g decreased from 0800 h to 1400 h, and then increased until 1800 h. The PEG_g value at 0800 h and at 1800 h represented the highest daily values in trial 1. The PEG_g value decreased and increased even more rapidly for PEG_{40} than for PEG_{20} (Figure 2a). In trial 2, regardless of the frequency of the dosed PEG, the highest PEG_g was observed at 0800 h and then decreased until 1400 h, like in trial 1, and remained almost identical at 1600 h. The PEG_g then increased in both groups but more quickly when administered as a single dose, almost reaching the initial value (i.e. at 0800 h), whereas with the split dose it remained lower (Figure 2b).

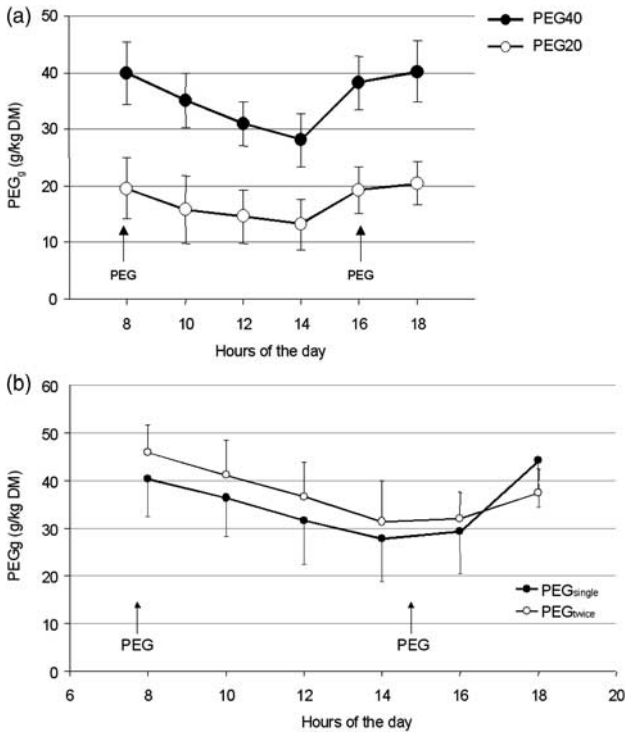

Figure 2 Kinetics of PEG content (PEG_g) in grab faeces samples for groups dosed (a) with 40 (PEG_{40}) or 20 (PEG_{20}) g of PEG per day (trial 1) and (b) daily with 20 g of PEG administered once at 0800 h (PEG_{single}) or split in two amounts at 0800 and 1600 h (PEG_{twice}; trial 2). PEG = polyethylene glycol.

Estimated DMI

Actual DMI compared with each estimated values of DMI (Table 4) are not statistically different ($P > 0.05$). The coefficient of variation (CV) of actual DMI in trials 1, 2 and 3 ranged from 4% to 12%. In trial 4, the CV reached 32%, respectively. The estimated values had a CV ranging from 5% to 22%, but was higher once again (48%) in trial 4.

Discussion

The precision of NIRS calibration was high, with a low measurement error (3.47 g/kg) and high R^2 and RPD values. The analytical precision is of extreme importance in studies

Table 4 *Average and relative standard deviation (values in parentheses) of actual DMI and estimated values with each trial recovery bias (DMI_c) or average recovery bias of the four trials (DMI_{avc}) included or without (DMI_{nc}) recovery bias. Results were not significantly (P>0.05) different.*

		DMI (kg/day)			
Trial	Factor	DMI	DMI_c	DMI_{avc}	DMI_{nc}
1	PEG_{20}	1.51 (10)	1.60 (10)	1.49 (10)	1.53 (10)
	PEG_{40}	1.55 (4)	1.67 (5)	1.54 (5)	1.58 (5)
2	PEG_{single}	1.19 (12)	1.24 (21)	1.31 (22)	1.35 (22)
	PEG_{twice}	1.19 (11)	1.21 (12)	1.12 (12)	1.15 (12)
3	E3MH	1.14 (6)	1.19 (14)	1.29 (14)	1.32 (14)
	E3LH	1.20 (7)	1.20 (15)	1.30 (15)	1.33 (15)
4	E4	1.39 (33)	1.53 (48)	1.59 (49)	1.63 (49)

DMI = dry matter intake; PEG = polyethylene glycol; MH = medium-nutritive value hay; LH = low-nutritive value hay.

with markers because a small error in the quantification of markers in faeces has a strong effect on the calculation of feed intake. For this reason, it is essential to update the calibration database at each new experiment with some faecal samples obtained before PEG treatments and enriched *in vitro* with PEG in order to obtain concentrations within the range of those expected during the experiment.

The fact that recovery rates of PEG were close to 100% in the first three trials confirms that there was no major bias in PEG estimation. It validates the experimental calibration protocol, based on PEG addition in faeces, which was used to build and update the calibration database. This is also confirmed by the fact that the spectra of experimental samples were well fitted to the calibration database, that is, no major change was observed between PEG added *in vitro* and PEG having transited *in vivo*.

The estimation of PEG concentration in faeces by NIRS proved to be very efficient. As no alternative reference analysis method exists for quick PEG determination, it would have been impossible to measure PEG concentration in several hundred samples, as required in this type of studies. The ease of NIRS analysis makes it extremely convenient for intake studies, making it possible to analyse a very high number of samples at a low cost in terms of time and money.

Previously, PEG was used as faecal marker to estimate faecal output (Hopson and McCroskey, 1972; Landau *et al*., 2002). None of these studies reported any metabolism, intake or digestibility modifications due to PEG. In the present four trials, no effect of PEG was observed on DMI and DMD, except in trial 4 where E4 ewes ate twice as much as C4 ewes, 1.4 *v.* 0.74 kg DM, respectively. The PEG is known to alleviate the negative effect of CT on intake and digestibility (Silanikove *et al*., 2001; Bhatta *et al*., 2004). The CT content of oat-vetch in trial 4 was low (1.5 g/kg) compared with the values having a negative effect (i.e. >50 g/kg DM) on voluntary intake reported by Frutos *et al.* (2004). Therefore, it seems that PEG interaction with CT was not responsible for the higher DMI observed in trial 4. No valid explanation was found to interpret these results.

In all trials, FDM_c significantly decreased for ewes dosed with PEG: −2.5 points (trial 1), −4 to −5.6 points (trials 2 and 3) and −19.5 points in trial 4. Landau *et al*. (2002) and Teeter and Owens (1983) observed with goats and steers, respectively, that FDM_c tended to decrease with PEG. This FDM_c decrease was also observed with other water soluble markers (Teeter and Owens, 1983). In sheep species, water is absorbed throughout the gut, from the small intestine to the rectum (Grovum and Hecker, 1973; Grovum and Williams, 1973). Consequently, the digesta become drier from the reticulo-rumen to the rectum. The DM content of digesta in the caecum and proximal colon is low and progressively increases until the terminal colon and rectum (Hecker and Grovum, 1971). Most of the water is absorbed at the caecum and proximal colon because digesta are retained for a relatively long time compared with the other sections of the colon (Hecker and Grovum, 1971).

It is known that PEG has no effect on water movement at the rumen level and does not interfere with the physiological functions of ruminal microorganisms (Sperben *et al*., 1953, quoted by Sinha *et al*., 1970). As the intestine cannot secrete free water (Lord, 1999), the lower FDM_c observed with PEG addition is probably due to the osmotic effect of PEG (Schiller *et al*., 1988), which sequesters water in the intestine, inhibiting water absorption (Davis *et al*., 1980). The osmotic effect of the PEG is probably higher at the caecum and proximal colon level. Considering the great difference observed in DMI but not in DMD in trial 4, Blaxter *et al*. (1956) observed that DMD (79.1% to 80.3%) was not modified when sheep were fed dry grass offered in a long form at 600 g or 1200 g/day. In the same time, they observed that DM content of the faeces tended to decrease (−3.7 points) from 42.3% to 38.6% when DMI increases. Grovum and Williams (1973) observed that the quantity of water reaching the proximal colon is higher for sheep given 1200 g/day of lucerne chaff than those given 400 g/day but water absorption from the large intestine is also higher. They observed the same water content of digesta in the rectum and concluded that the level of DMI does not influence the water content in the rectum. These results can explain partly why in trial 4 the DM content of the faeces in E4 is lower as more water reaching the colon is not fully absorbed because of the osmotic effect of PEG.

These results could be elucidated in offering two levels the same forage (fresh and as hay) at two levels of offered with or without PEG.

The REC was high in the first three trials, ranging from 95% to 109%. The same PEG recovery ranges (96% to 113%) were measured indoors with cattle (Corbett *et al*., 1958; Teeter and Owens, 1981), sheep (Hassoun *et al*., 2007b; Caja *et al*., 2009) and goats (Landau *et al*., 2002). In trial 4, REC was lower (87.5%), with an average range of 74.5% to 102.3%, as reported by Caja *et al.* (2009) when ewes were allowed to graze Italian ryegrass for 6 h (REC = 81.6%) in addition to being fed dry food indoors, compared with ewes exclusively fed

indoors (REC = 101%). However, it was demonstrated that PEG with a high molecular weight (>3000) is not absorbable in the intestines (Schiller *et al.*, 1997) and not found in the urine (Teeter and Owens, 1981 and 1983). The REC results of trial 4 may be attributed to the low PEG level (10 g/day) or to the nature of the diet (fresh forage with low DM content), but this remains to be clarified.

Marker excretion must be as stable as possible from day to day. In trial 1, daily PEG excretion was rather constant over the 14-day trial. In trial 1, PEG reached its maximum level in faeces 2 days after the first administration with a rapid steady state. Moreover, PEG rapidly disappeared 3 to 4 days after the last PEG administration (Figure 1). These results are in agreement with Corbett *et al.* (1958) and Hopson and McCroskey (1972) who observed a total PEG recovery within 2 to 3 days with steers dosed with 50 to 400 g/day of PEG. The pattern of PEG excretion is similar to other water-soluble markers (Cr-EDTA, Co-EDTA) excreted within 48 h after the first administration (Aharoni *et al.*, 1999). The diurnal excretion pattern in trials 1 and 2 showed a curvilinear excretion with two high PEG content values at 0800 and 1800 h, and one lower value at 1400 h (i.e. 6 h after the morning dose). Corbett *et al.* (1958 and 1959) found the same diurnal excretion pattern of PEG on cows dosed once or twice daily, with the lowest value observed 6 to 8 h after the morning dose and the highest at 0800 and 1800 h. Hopson and McCroskey (1972), administrating various PEG levels in a single dose (0800 h) or twice daily (0800 and 1630 h) to steers, observed a similar diurnal excretion pattern but with a more pronounced difference between the highest and lowest PEG content with a single dose than with a split dose. Landau *et al.* (2002), using 20 and 40 g PEG/day administered in a single dose at 0800 h to goats, obtained the same excretion pattern with 40 g/day that observed in the present experiment with 20g PEG/day administered in a single or split dose (trial 2), but a different excretion pattern with 20 g PEG/day with less hourly variability.

When a marker is used for assessing total faecal output through grab sampling, the marker content must be as close as possible to the mean content in total faeces. Otherwise, marker content of grab samples must be corrected for this systematic bias. The PEG_g8 values, which represented 86% to 108% of the corresponding PEG_T values, were not significantly different from actual PEG_T in all trials, except in trial 1 (PEG_{20}) and in trial 2 when PEG was administered in a single dose (Table 3). Averaging PEG_g8 and PEG_g16 did not reduce this bias. Hopson and McCroskey (1972) obtained slightly higher values (92% to 116%) when PEG content in gathered grab samples (0800 and 1630 h) were compared with PEG in total faeces collection. An average CV of 14% and 13% was found when measured at 0800 and 1600 h, respectively, that is similar to those observed by Landau *et al.* (2002) with 16.4% and 15.3% with 20 and 40 g PEG/day, respectively. The CV was only higher in trial 4 with green forage, with 38%. Similarly, CV calculated on PEG_T ranged from 3% to 19%, except in trial 4 (30%). Considering these results, it seems advisable to collect grab samples only in the morning, which is easier from the practical point of view (less work and animal handling). Our results also reveal that there is no major difference between administering PEG once or twice daily.

The actual DMI variations between ewes in trials 1, 2 and 3 are in good agreement with other values observed under various conditions (Andueza *et al.*, 2011). However, the values were much higher (32%) with fresh green forage than those observed for other species of fresh green forage (Dulphy, 1971). Consequently, if the diet does not contain tannins, the DMI can be estimated with good accuracy, corrected or not with the recovery biases. Landau *et al.* (2002) obtained the same results without any correction.

Conclusions

These results confirm that faecal PEG content can be estimated by NIRS with good accuracy, provided that faeces obtained from sheep fed the diet on which measurements are to be made are included in the general NIRS database for calibration. Because PEG is rapidly excreted, it is recommended that faeces be collected within 1 week after the start of administration. The PEG recovery rate in total faeces is high and comparable with other results found in the literature concerning dry feed. Using PEG as a faecal marker at levels of 20 to 40 g/day does not modify DMD and DMI when sheep are fed dry diets. Surprisingly, PEG modifies the DMI but not the DMD of a fresh forage diet. Further experiments with fresh forage must be conducted because most of the results in the literature deal with dry diets. Consequently, PEG estimated by NIRS can be a good faecal external marker, allowing a rapid determination, no extraction, no use of health hazards for technicians (in contrast with other external marker like ytterbium or alkanes) and no problem of disposal. Briefly, the practical procedure proposed is to dose animals with PEG solution once a day (20 g of PEG/day) and to collect grab faeces samples in the morning for 5 days at the same time the animals are dosed. Collected faeces should be dried (at a temperature not exceeding 50°C) and gathered per animal and per period before being NIRS scanned.

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