Livestock Science 155 (2013) 38–43

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Polyethylene glycol determined by near-infrared reflectance spectroscopy to estimate faecal output in sheep fed fresh permanent grassland forage

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ARTICLE INFO

Article history:
Received 6 November 2012
Received in revised form
19 February 2013
Accepted 24 March 2013

Keywords:
Polyethylene glycol
NIRS
Sheep
Permanent grasslands
Intake
Faeces

ABSTRACT

This study evaluated the feasibility of using different doses of polyethylene glycol (PEG) as an external marker of faecal output in sheep fed permanent grasslands fodder and compared two near-infrared reflectance spectroscopy (NIRS) calibration strategies for determining faecal PEG content. Three levels of PEG (0.25%, 0.75% and 1.5% of total daily intake) were administered to eight wethers, with each level dosed twice daily. Animals were fed forage obtained from two permanent grasslands cut at two dates during the first cycle of growth. Polyethylene glycol recovery rate was higher ($P<0.001$) at the highest dose (0.78) and decreased as dose level decreased (0.61 and 0.30 for PEG levels of 0.75% and 0.25% of total daily intake, respectively). NIRS calibration equations established on PEG data dosed directly on the faecal samples (0.61) gave higher ($P<0.001$) PEG recovery rates than NIRS calibration equations performed on mixtures of faeces with different PEG concentrations (0.49). Finally, faecal output estimates were more accurate ($P<0.001$) when faeces were sampled at 8:00 (0.61) than at 16:00 (0.51). The highest PEG recovery rate (0.88) was achieved using the highest dose on morning samples when PEG content was estimated by NIRS using turbidimetric results as reference values. We conclude that the usefulness of PEG as an external marker for estimating faecal output on permanent grasslands is limited at PEG doses lower than 1.5% of intake.

1. Introduction

Dry matter (DM) intake is a key input factor for predicting the feed value of forages (Jarrige, 1989). Forage feed value is key to understand ruminant nutritional status and thus livestock system performance (Ferreira et al., 2004). Knowing DM intake in grazing systems can help improve grazing management and grazing efficiency. As direct measurements are difficult to take at pasture (Burns et al., 1994), individual intake estimates are generally deduced from forage digestibility value and faecal output assessment. Total faecal excretion is usually estimated using external markers, the most common being chromic oxide, ytterbium (Delagarde et al., 2010), n-alkanes (Dove and Mayes, 2005) and high-molecular-weight polyethylene glycol (PEG) (Hassoun et al., 2007). Polyethylene glycol is an organic polymer that is indigestible at molecular weights greater or equal to 3000 (Landau et al., 2002). Its main disadvantage is the irregular recovery rates obtained at different doses (Hopson and McCroskey., 1972). Another disadvantage of using PEG as a marker is its influence on digestibility coefficients when the diet contains tannins (Decandia et al., 2000) or the increase in faecal water content (Hassoun et al., 2007) due to the

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http://dx.doi.org/10.1016/j.livsci.2013.03.022

effect of increasing osmotic pressure that limits water reabsorption (Schiller et al., 1988). This effect generally occurs when animals are administered high PEG doses, but PEG dose in the faeces needs to be high enough to be detectable with good accuracy. Landau et al. (2002) suggested a level of 3–5% of dry matter. Polyethylene glycol was traditionally measured using a turbidimetric method, but it proved complex, time-consuming, and tedious to perform (Landau et al., 2002). The fact that PEG can now be identified by near-infrared reflectance spectroscopy (NIRS) has sparked renewed interest in using PEG as an external marker. Landau et al. (2002) developed calibration equations to predict faecal PEG content by creating artificial mixtures of different PEG and faeces contents. This strategy could be able to detect PEG content in faeces when administered to animals in smaller doses than the 3–5% proposed by Landau et al. (2002).

A potential factor of error in PEG-based faecal output estimates is diurnal variation in faeces, which is dependent on PEG dose administered to the animals (Hopson and McCroskey, 1972; Landau et al., 2002). There may be limits to use of PEG as an external marker for predicting the faecal output of animals fed permanent grassland forage as permanent grasslands feature species that may contain compounds able to interact with PEG and diminish its efficiency as an external marker. The objective of this study was to evaluate different doses of PEG as an external marker of faecal output in sheep fed permanent grassland. Furthermore, we compared two NIR calibration strategies and evaluated the effect of collection time and PEG dose level on the accuracy of the faecal output predictions.

2. Materials and methods

2.1. Forages

Animals were offered fresh forage from two extensively-managed permanent grasslands of different floristic composition. Average botanical composition was 95% grasses and 5% forbs on one sward *versus* 79% grasses and 21% forbs on the second sward. Both swards were cut at two phenological stages (end of pre-bloom/early flowering and end of flowering/seed setting of cocksfoot respectively) in the first growth cycle, estimated based on the method given by Andrieu et al. (1989) who suggested classifiying grasses found in a metre row based on maturity stages. To simplify this approach we focused on the cocksfoot. The grasslands were located at Saint-Genes-Champanelle (Puy-de-Dôme, France, 3°1′ E, 45° 43′ N), at 870 m above sea level.

2.2. In vivo trials

In vivo digestibility and voluntary intake were determined using 24 two-year-old Texel wethers (mean liveweight: 60 kg). Before starting the experiment, the animals were blocked ($n=6$) according to their bodyweight (BW) and randomly assigned to the treatments. Treatments consisted of each grassland, with forage offered at maintenance level (40 g DM/kg $BW^{0.75}$) or at *ad libitum* level. The amount of forage offered to animals fed *ad libitum* was adjusted daily on the basis of the previous day's intake. A refusal of 10% of offered quantity was allowed. The digestibility trials were carried out in metabolism crates over a period of 20 days, where the first 15 days were devoted to diet adaptation and the last five days to data collection (daily offer and faeces). After each data collection period, animals were allocated to individual cages to rest until the next measurement period.

The offered diet consisted of forage cut daily from each plot and chopped to a length of 5–7 cm. It was offered twice a day, at 8:00 and 16:00. All animals had free access to water and vitamin-mineral blocks throughout the experimental period.

During the digestibility trial, animals were orally dosed with a drenching gun daily with three doses of PEG (MW 4000, Renex, ICI CC&P, Chocques, France) dissolved in 50 ml of water. Polyethylene glycol solutions were administered twice daily (8:00 and 16:00) to obtain three final doses, *i.e.* 0.25%, 0.75% and 1.5% of total daily intake. Each dose was administered to two sheep chosen at random from within grassland and intake level.

2.3. Samples

During data collection periods, total faeces were collected from each wether over all five days of each experimental period. In addition to the total collection of faeces, individual faecal samples were taken twice daily (at 8:00 and 16:00) on each day of each experimental period. To assess diurnal variations in PEG excretion, faecal samples were collected and weighed every 2 h between 8:00 and 20:00 during the last 24 h of each experimental period.

All samples collected were divided into two subsamples. One subsample (about 50 g) was frozen and stored at −20 °C until PEG analysis, and the second subsample (about 15 g) was oven-dried at 60 °C for 72 h then ground through a 0.8 mm screen and used for NIRS spectra analysis.

2.4. Analysis, spectra and calibration strategies

Dried and ground faecal samples were placed in a 50 mm-diameter ring cup and scanned in reflectance mode at 2 nm intervals from 400 to 2500 nm using a Foss NIRSystems model 6500 scanning VIS/NIR spectrometer (Foss NIRSystems, Silver Spring, MD). Spectra and reference values were recorded on NIRS3 software (Infrasoft International, Port Matilda, PA). Each spectrum was time-averaged from 32 scans. A reference scan (using the internal ceramic reference tile) was performed before and after each sample Reflectance (R) values were converted into absorbance (A) values using the formula $A=\log(1/R)$.

Spectra and PEG data were used to establish calibration models *via* two approaches: For the first calibration strategy (dPEG), 162 spectra were selected from a total population of 684 faecal samples obtained in digestibility trials using the Shenk and Westerhaus (1991) procedure. Briefly, selected samples were the representative samples from a larger set working to the assumption that only one sample is required to represent all samples in a