Determination of polyethylene glycol 6000 (PEG) concentration in sheep faeces using near infrared spectroscopy

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Introduction

To assess dry matter intake in grazing ruminants, techniques involving the use of inert markers, such as chromium or ytterbium oxide, have been developed. These markers enable the estimation of total faecal output to be made quite accurately, but their chemical analysis is costly and laborious and the number of assays is therefore limited. Some attempts to use polyethylene glycol (PEG, MW6000) as a faecal marker have been mentioned in the earlier literature [1, 2], but the applications were limited, because the chemical analysis of PEG using turbidimetry was tedious. Recently concentrations of PEG have been estimated in the pharmaceutical industry using near infrared (NIR) spectroscopy [3]. Subsequently Landau *et al*. [4] have used NIR spectroscopy to estimate the concentration of PEG in goat faeces.

A major limitation for the practical use of NIR spectroscopy in this context is that the characteristics of faeces are related to the nature of the diet. Therefore, calibrations based on a unique set of faeces, and therefore indirectly related to a specific diet, are not robust and fail to predict PEG concentrations in animal fed other diets. This was a limitation of the study of Landau *et al*. [4] since the faeces originated from only four animals that were all fed the same diet. The aim of the present experiment was to develop models to predict PEG concentration in the faecal output of sheep fed a wide range of diets.

Materials and methods

Sheep faeces were collected from sheep of three different breeds, INRA 401 (Berichon du Cher x Romanov), Lacaune and Merinos d'Arles, and fed eleven different diets encompassing rangeland vegetation, pasture, silage, and mixed and dehydrated diets. Faeces were dried 48 h at 60°C and ground through a 1 mm sieve. In order to create scales of PEG concentration, dry faeces samples were mixed with solutions containing PEG to obtain final PEG contents from 0 to 200 g.(kg DM)$^{-1}$ in 5 or 10 g.(kg DM)$^{-1}$ steps. The samples containing PEG were then re-dried and ground. A total number of 744 samples were prepared. NIR spectra were collected in reflectance mode in ring cups on a Foss NIRSystem 6500 spectrometer (Foss NIRSystems, Silver Spring, MD, USA). Calibration development was carried out with wavelengths in the 1,100 - 2,500 nm range after mathematical pre-processing of a standard normal variate and detrend procedure on the second derivative of the spectra. The modified partial least-squares method of WinISI software (Win-ISI, Infrasoft International, Port Matilda, PA, USA) was used.

Results and discussion

There was no significant difference in the results between the three breeds of sheep involved in the study.

Calibration equations developed using the whole concentration range from 0 to 200 g.(kg DM)$^{-1}$ had a coefficient of determination (R^2) of 0.99 and a standard error of cross-validation (SECV) of 4.5.

The corresponding ratio of performance to deviation (RPD) (standard deviation divided by SECV) was above 13. Such calibration performance confirms the feasibility of a precise determination of PEG concentration using NIR spectroscopy. At the same time, it also validates the mixing and re-drying procedure used for sample preparation since precise calibrations necessarily originate from samples with accurately known concentrations. The SECV was similar to the results of Landau *et al*. [3] who found values ranging from 3.2 to 4.6 g.(kg DM)$^{-1}$.

In an attempt to minimise possible digestive interactions associated with feeding PEG researchers should aim to feed as lower a concentration as practically possible. We therefore also tested equations developed from narrower concentration ranges (Table 1). The SECV decreased with decreasing range scales, down to values as low as 1 g.(kg DM)$^{-1}$. Naturally, the RPD values decreased at narrower concentration ranges because the standard deviation of a population decreases in proportion to its range.

Table 1. Performance of calibration equations developed over increasing concentration ranges of polyethylene glycol (PEG) to predict the concentration of PEG in sheep faeces (g.(kg DM)$^{-1}$) using NIR spectroscopy.

Concentration of PEG	Population			Calibration				
	n	Mean	SD	SEC	R^2	SECV	RPD	CVE (%)
0 - 20	122	6.6	5.8	0.7	0.983	1.0	5.8	15.0
0 - 40	227	16.0	11.3	1.0	0.992	1.3	8.9	7.9
0 - 60	308	23.8	16.6	1.2	0.995	1.5	11.2	6.3
0 - 80	384	31.7	21.8	1.5	0.995	1.8	12.2	5.6
0 - 100	483	40.2	30.0	1.9	0.996	2.2	13.6	5.5
0 - 120	524	48.6	35.3	2.3	0.996	2.7	13.1	5.5
0 - 140	585	53.8	41.4	2.7	0.996	2.9	14.1	5.5
0 - 160	622	60.4	46.5	3.0	0.996	3.3	14.3	5.4
0 - 180	670	66.5	51.7	3.4	0.996	3.6	14.3	5.5

n: number of samples

SD: standard deviation

SEC: standard error of calibration

R^2: coefficient of determination

SECV: standard error of cross-validation

RPD: ratio of performance to deviation (SD.SECV^{-1})

CVE: coefficient of variation of error (SECV.Mean^{-1})

Conversely, the coefficient of variation of error (SECV.Mean^{-1}) increased with decreasing range scales (Figure 1), particularly below the 0 to 60 g.(kg DM)$^{-1}$ range scale. This is an important point because marker concentration is a variate used to calculate digestibility. If the experimental marker concentration is decreased, while maintaining analytical precision, then the error on digestibility estimation is increased. That is the error of estimating digestibility is linked to relative error and not to absolute error. In the present case it is advisable to use PEG doses leading to concentrations of PEG in faeces in the 0 to 80 g.(kg DM)$^{-1}$ range in order to use a calibration equation with the best compromise between as low a range as possible and no decrease in performance.

A complementary calibration trial was performed by applying the calibration to ten series of samples (545 samples) and predicting the eleventh series (200 samples), in order to obtain an estimation of the standard error of prediction (SEP) in a situation where the prediction equation was applied to a different set of faeces. The SEP value obtained was 2.5 g.(kg DM)$^{-1}$ for concentrations of PEG in faeces in the 0 to 100 g.(kg DM)$^{-1}$ range. This value is very close to the calculated SECV. It is concluded that the gathering of several series of faecal samples of different characteristics in a

database can allow the prediction of PEG in a different series derived from different sheep fed different diets. This is particularly important for the practical use of calibrations in the future.

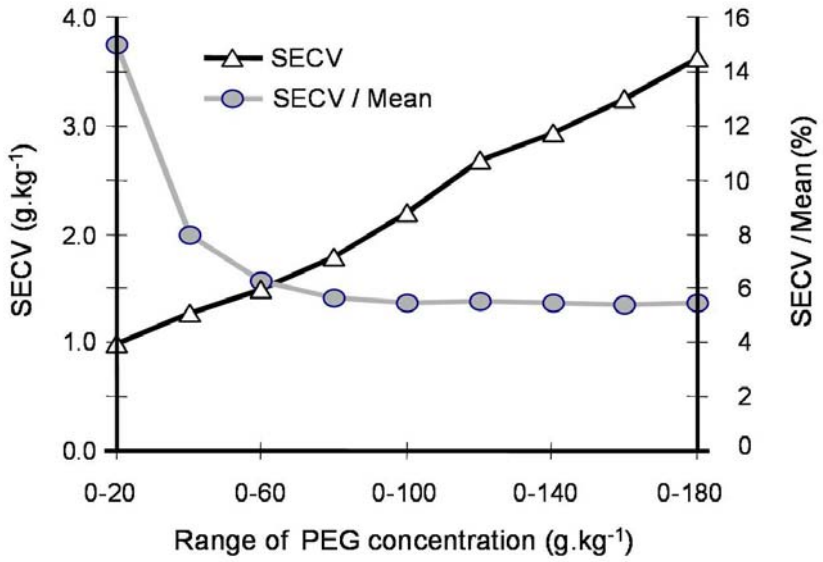

Figure 1. Plot of the range in PEG concentration in sets of faecal samples against the standard error of cross-validation (SECV) and the coefficient of variation of error (SECV.Mean^{-1}).

Before using this technique in routine determinations, two important checks will have to be made. The first one is to determine the real effect of PEG on digestion, since PEG has a water retaining capacity which could, in certain conditions, increase the dry matter content of faeces and modify digestibility. The second is the interaction between PEG and tannins as PEG is known to bind to tannins [5]. This property could interfere with the digestion processes for diets containing significant amounts of tannins and bias the results.

Conclusion

The use of NIR spectroscopy is a very precise method to predict PEG content in sheep faeces, with SECV values as low as 1 g.(kg DM)$^{-1}$. The precision levels obtained allow the use of PEG as a marker for the prediction of dry matter intake. For an expected daily intake in the range of 600 to 3,800 g dry matter and a digestibility in the range 40 to 80%, the daily dose of PEG, considering the present results, could be limited to 10 to 20 g per sheep. Such a quantity is likely to have a minimal interaction with the digestion process.

References

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