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Utilization of Poly Ethylene Glycol 6000 (PEG) as a faecal marker measured with Near Infra Red Spectrometry (NIRS) in sheep

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SUMMARY – An experiment has been conducted with 9 dry non-pregnant Merino ewes placed in metabolism digestibility crates. They were offered 3 different diets (D1, D2 and D3) according to dehydrated alfalfa levels (200, 250 or 300 g/d) and dehydrated sugar beet pulp levels (100, 150, and 200 g/d) respectively. In addition each ewe was fed molasses straw pellets (MSP) *ad libitum* and 50 g/d of mineral supplement. Within each diet, the 3 ewes were dosed twice a day with PEG in solution form at one of the three final levels: 40, 60 or 80 g/d. After a two-week adaptation period, dry matter intake (DMI) and total faeces (TFDM) were measured daily. After a first week of adaptation, ewes were dosed one of the three PEG levels for 3 weeks and DMI and TFDM records were maintained for 3 more weeks. The determination of PEG in faeces was done by Near Infrared Spectroscopy (NIRS). Calibrations were built by adding PEG in representative faeces samples collected before the first doses. PEG was added as a solution in the range 0-100 g/kg DM (by 5 or 10 g/kg DM step). NIRS calibration was carried out, using partial least-squares regression (MPLS procedure, WINISI), with wavelengths in the 1100-2500 nm range after mathematical pre-processing of spectra (SNV and detrend, 2nd derivative). The standard error of prediction (SEP) was between 2.2 and 2.5 g/kg. Individual recovering rate was on average 98%. Whatever the PEG concentration, we observed that faeces DM content decreased (from 42 to 35%). DMI of MSP for D1, D2 and D3 diets increased (by 120, 145 and 126% respectively) as well as TFDM (by 122, 143 and 133% respectively). Two weeks after PEG dosage was stopped, DMI and TFDM remained stable. Although the effect of PEG on faeces DM content has already been reported in the literature, its effects on DMI and TFDM were never reported. These effects could be related to the high dry matter content of the diet (91% \pm 2 on average). The ease and accuracy of NIRS measurement could make PEG a very interesting faecal marker. Investigations are in progress in order to verify if PEG recovery through grab sampling is as good as total collection, and if the effect of PEG on faecal dry matter is related to dry matter content of intake.

Keywords: Intake, sheep, polyethylene glycol, Near Infra Red Spectrometry.

RESUME – "Utilisation du polyéthylène glycol 6000 (PEG) comme marqueur de l'excrétion fécale mesuré par Spectrométrie dans le Proche InfraRouge (SPIR) chez les ovins". Une expérimentation a été conduite avec 9 brebis non gestantes de race Mérinos d'Arles placées en cage à digestibilité. Elles recevaient 3 régimes différents (D1, D2, D3) selon les quantités distribuées de luzerne déshydratée (200, 250 ou 300 g/j) et de pulpe de betterave (100, 150, ou 200 g/j). Elles avaient en plus des bouchons de paille mélassée (MSP) offerts à volonté ainsi que 50 g/j d'un complément minéral. Après une période d'adaptation aux régimes de 2 semaines, les quantités ingérées de matière sèche (DMI) et les quantités de fèces excrétées (TFDM) ont été enregistrées sur 7 jours. Puis, pour chaque régime, les brebis ont reçu 40, 60 ou 80 g/j de PEG administré sous forme de solution par voie orale 2 fois par jour pendant 3 semaines. Pendant cette période, DMI et TFDM ont été enregistrées quotidiennement. La concentration de PEG dans les fèces a été déterminée par spectrométrie dans le proche infrarouge (NIRS). La calibration a été construite par addition de PEG dans des fèces récoltées la semaine avant le premier dosage. Le PEG a été ajouté sous forme de solution pour obtenir une gamme finale de concentrations de 0 à 100 g/kg MS avec un pas de 5 ou 10 g/kg. La calibration a été faite sur une gamme de longueurs d'onde de 1100-2500 nm, en utilisant la méthode de régression des moindres carrés partielle (procédure MPLS, WINISI), après traitement statistique des spectres (SNV et detrend dérivée seconde). L'erreur standard de la prédiction (SEP) s'étendait de 2,2 à 2,5 g/kg. Le taux de récupération du PEG a été en moyenne de 98%. Quelle que soit la dose infusée la teneur en MS des fèces a diminué (de 42% à 35%) pendant la phase de dosage. Simultanément, dans les régimes D1, D2 et D3 les QI de MSP ont augmenté respectivement de 120, 145 et 126% et les fèces totales de 122, 143 et 133%. Deux semaines après l'arrêt du PEG, DMI et TFDM sont restées stables. L'effet du PEG sur la teneur en MS des fèces a été rapporté dans la littérature, mais pas celui sur les QI et les TFDM. Ce phénomène pourrait être lié à la forte teneur en MS des constituants des régimes (91% \pm 2 en moyenne). La facilité et la précision des mesures par NIRS pourraient permettre l'utilisation du PEG comme marqueur fécal. Des essais complémentaires sont en cours pour mesurer la concentration du PEG dans

les fèces prélevées par voie rectale et pour vérifier si l'effet du PEG sur la teneur en MS des fèces est lié à celui de la teneur en eau du régime (herbe).

Mots-clés : Ingestion, ovins, polyéthylène glycol, spectrométrie dans le proche infrarouge.

Introduction

In order to evaluate individual intake at pasture with faecal markers, one needs to know the dry or organic matter digestibility of intake and total faeces. Digestibility can be estimated through various relationships between digestibility and chemical composition of herbage or faeces (Penning, 2004). Total faeces can be measured with faecal bags (Penning, 2004) or with indigestible markers such as Chromium (Dove *et al.*, 2000), Ytterbium (García *et al.*, 2003) or alkanes (Mayes *et al.*, 1986). These markers give quite accurate results but are either difficult to prepare and the chemical or physical analysis are expensive, time consuming and need a lot of chemicals. It has been recently reported (Landau *et al.*, 2002) that polyethylene glycol (PEG) could be used as faecal marker in goat and determined by Near Infra Red Spectrometry (NIRS) in the faeces. Furthermore, we recently presented that PEG content could be determined with NIRS over a wide range (i.e. 0-200 g/kg DM) on faeces collected from different diets (Hassoun *et al.*, 2005). The interest of such a marker is that it is rapidly prepared (soluble form) easily given to animals and rapidly determined by NIRS. Also, it does not require using chemicals for extraction.

Material and methods

An experiment has been conducted in south of France, in the Frejorgues experimental farm of INRA. Nine dry non pregnant Merinos ewes (49 kg \pm 2.9) were placed in metabolism digestibility crates. They were fed 3 different diets (D1, D2 and D3) according to dehydrated alfalfa levels (200, 250 or 300 g/d) and dehydrated sugar beet pulp (SBP) levels (100, 150, and 200 g/d) respectively. In addition, each ewe was *ad libitum* given molasses straw pellets (MSP) and 50 g/d of mineral supplement. Ewes were kept on the same diet, in the crates, for a 2 weeks pre-experimental period (adaptation), the experimental period (3 weeks) and the post-experimental one (2 weeks). During the experimental period, for each diet three levels of PEG (MW6000, Renex, ICI CC&P, Chocques, France), were given to one the three ewes. PEG was given twice a day in a solution form in order to obtain one of the three final levels: 40, 60 or 80 g/d/ewe. During the whole trial (i.e. 7 weeks) DMI and TFDM were daily recorded.

Faeces were sampled every day for dry matter determination (48 h, 60°C). The last week of PEG and the following two weeks, dry faeces samples were individually ground (with hammer mill and 1mm sieve) and stored pending PEG content determination. The determination of PEG in faeces was done by NIRS. Calibrations were built by adding PEG in representative faeces samples collected before the first doses. PEG was added as a solution in the range of 0-100 g/kg DM (by 5 or 10 g/kg DM step). NIRS calibration was carried out, using partial least-squares regression (MPLS procedure, WinISI; ISI, 1999), with wavelengths in the 1100-2500 nm range after mathematical pre-processing of spectra (SNV and detrend, 2nd derivative).

Results and discussion

After the adaptation periods, DMI of MSP was the same for the three diets (0.78 \pm 0.17 kg). Then, when sheep received PEG, DMI of MSP increased up to 0.94 \pm 0.04, 1.1 \pm 0.09, 0.98 \pm 0.12 kg DM for D1, D2 and D3 respectively and remained stable for the next two weeks. As a consequence, TFDM increased from 0.66 \pm 0.09 to 0.80 \pm 0.07 kg, to 0.94 \pm 0.09 and to 0.89 \pm 0.08 kg for D1, D2 and D3 respectively and, after the two weeks without PEG, returned to: 0.73 \pm 0.07, 0.88 \pm 0.07, 0.83 \pm 0.07 kg. Also we observed that DM content of the faeces decreased during the PEG period and increase after PEG has been stopped (Fig. 1). PEG content measured with NIRS allowed to determine the individual recovery rates calculated with TFDM for 7 days by ewe. The recovery rate was very good whatever the PEG level: 99 \pm 16, 98 \pm 8 and 97 \pm 9% respectively for 40, 60, 80 g/d levels of PEG infusion. These results are in very good agreement to with the values reported by Landau *et al.* (2002).

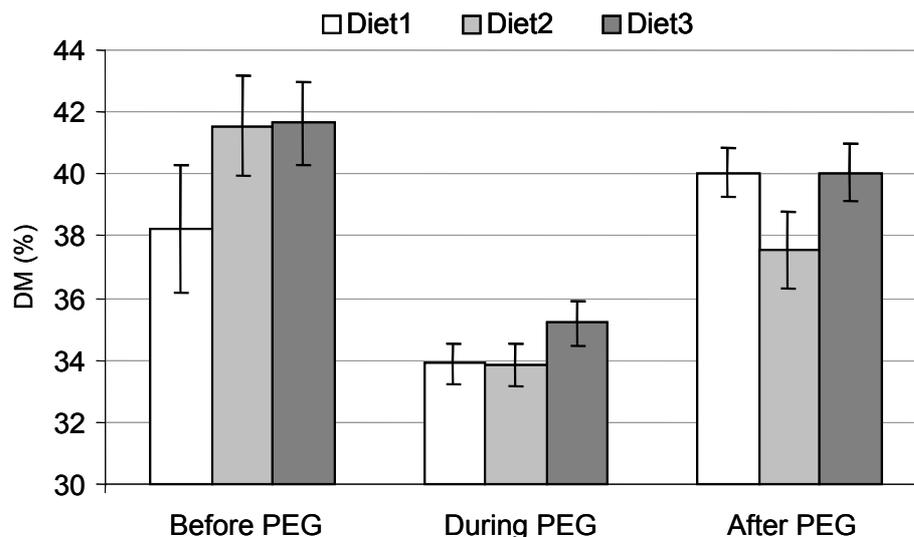


Fig. 1. Effect of PEG6000 on the dry matter content of faeces (DM).

The effect of PEG on the intestinal transit, accelerating faeces excretion, can be explained by the very high DM content of diets (mean 91% \pm 2). This phenomenon can be due to the effect of PEG which increases the osmotic pressure limiting the water re-absorption (Schiller *et al.*, 1988). Landau *et al.* (2002) also observed such low DM content of goat faeces. As with Landau's results, the magnitude of this effect can even be important because of the high DM content of the diets (i.e. > 90). Consequently, in these conditions, the effect of PEG previously suggested by Schiller *et al.* (1988) would have been enhanced. This is a problem for a marker because it modifies the apparent digestibility of the diet. We believe, however, that this effect would be much lower with diets with a higher water content. The high recovering rate of PEG is in agreement with previous results. Although it must be verified in other nutritional situations, it is an interesting result.

Conclusions

Although the effect of PEG on faeces dry matter content has already been reported in the literature, its effects on DMI and TFDM were never reported. This experiment confirm, as previously shown on goat, that PEG can be used as faecal marker because it is indigestible, not metabolised and non toxic, together which is confirmed by the good recovery rate in the faeces. Furthermore, the NIRS determination of faecal PEG is quite accurate. However, its effect on faeces DM content has to be checked with diets of a higher water content (i.e. herbage). We are, by now, verifying that the sampling procedure of faeces, i.e. a twice a day collection from the rectum, will give as reliable results as when done with total collection procedure.

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