RATE OF PASSAGE IN THE RABBIT DIGESTIVE TRACT : INFLUENCE OF MARKER DOSING TIME, ILEAL CANNULATION AND MARKER TYPE

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ABSTRACT : A control group of 5 adult rabbits and a group of 4 ileo-cannulated rabbits, fed ad libitum were used to assess the effect of marker dosing time, marker type (Ytterbium vs Cerium labelled cell-wall) and the effect of the ileal cannulation, on measurement of passage rate (RPM). The passage rate of particles labelled with $^{169}$Yb was equal to those labelled with $^{144}$Ce, and allowed the comparison of RPM obtained with these two markers given at different times to the animals.

In our experimental conditions, over 70% percent of the animals showed no hard faeces excretion between 10:00 and 16:00, because of soft faeces excretion during this period. The total mean retention time (MRT) did not vary significantly when the marker dosing time took place out of the period of caecotrophy (mean 20.3h). But a 25% reduction in MRT (-5.2h) was observed when marker was given during caecotrophy (at 13:00) compared to a dosing time at the beginning of the caecotrophy (11:00). This decrease in MRT values at 13:00 was partly due to a shorter (-1.5h) minimal transit time (Tm). When the marker was given before caecotrophy (8:00) or at the beginning of the caecotrophy (11:00), a part of the labelled particles could be incorporated directly into the soft faeces and ingested, thus contributing to increase the MRT value and particularly the Tm value. For a dosing time after caecotrophy, the labelled particles spent more time in the caeco-colic segment. In addition, the variability of rate of passage parameters appeared to be lower for a dosing time placed at the beginning of the caecotrophy (1:00).

Rabbits having an ileal cannula showed a slight lower (-15%) feed intake and a higher faecal digestibility (+2 units). No significant difference in transit however was registered between conventional and cannulated animals.

RESUME : Etude du transit digestif chez le lapin : incidence de l'heure d'administration du marqueur, de la cannulation iléale et du type de marqueur

Les effets, sur le transit digestif du lapin, de l'heure de distribution du marqueur, du type du marqueur "Ytterbium vs Cérum" et de l'implantation d'une canule ont été analysés à l'aide d'un groupe de 5 animaux adultes conventionnels et de 4 animaux porteurs d'une canule iléale, nourris à jeûne. Le temps de séjour des particules de fibres marquées avec $^{169}$Yb ne diffère pas de celui mesuré avec des particules marquées avec le $^{144}$Ce, ce qui permet de comparer les valeurs de temps de séjour obtenues avec ces deux marqueurs distribués à des heures différentes.

Dans nos conditions expérimentales, plus de 70% des lapins n'excèdent pas de fèces dures entre 10:00 et 16:00, du fait de l'excrétion des caecotrophes à ce moment. La valeur du temps de séjour moyen total ne varie pas significativement tant que l'heure de distribution du marqueur est en dehors de la caecotrophie (en moyenne 20.3h). En revanche, une baisse de 25% (-5.2h par rapport à la valeur obtenue en début de caecotrophie à 11:00) du temps de séjour est observée lorsque le marqueur est donné pendant la caecotrophie (à 13:00). Cette baisse provient en partie d'un temps de transit minimum plus court (-1.5h). Quand le marqueur est donné avant (8:00) ou en début de caecotrophie (11:00), une fraction des particules marquées peut être directement incorporées dans les caecotrophes et ingérées de nouveau, ce qui conduit à augmenter leur temps de séjour, et en particulier le temps de transit minimum. A l'inverse lorsque le marqueur est donné après la caecotrophie, les particules marquées ont un temps de séjour plus long dans les segments caeco-coliens. Par ailleurs, la variabilité des paramètres du transit semble inférieure lorsque le marqueur est distribué en début de caecotrophie (11:00). Comparés aux animaux conventionnels, les lapins porteurs d'une canule iléale présentent une ingestion plus faible (-15%), associée à une digestibilité faecale supérieure (+2 points). Cependant la cannulation n'a pas affecté significativement les paramètres du transit digestif.

INTRODUCTION

The measurement of the passage rate for labelled feed particles, through the analysis of the faecal excretion pattern of a marker, is dependent on several factors such as marker characteristics (POND et al., 1986) ; GONZALEZ et al., 1986 ; FAICHNEY et al., 1989) or the time delay between a meal and the intake of the marker (POND et al., 1989). Rabbits are classically fed ad libitum, and its faecal excretion pattern is dependent on the caecotrophy. Consequently, when passage rate measurements (RPM) are investigated after a marker dose has been given, the result could vary depending on whether the marker dosing time is before or after the caecotrophy period (PIEKARZ, 1963; LAPLACE and LEBAS, 1975). The main purpose of the present work was thus to examine in more details, using a specific cell wall labelling technique, the variations of RPM with several dosing times distributed around the caecotrophy period.

For many years, rare-earth metals (lanthanide family) have been extensively used as transit markers in ruminants or rabbits (ELLIS and HUSTON, 1968 ; FRANÇOIS et al., 1968 ; LAPLACE et al., 1974 ; PONCET, 1976), because of their adsorption characteristics on solid particles. Lanthanides have thus been compared with other markers such as mordanced chromium, and higher transit values have been found in rabbits with Ytterbium labelled fibres compared with mordanced chromium (GIDENNE, 1988). But to date, RPM comparison of fibres labelled with radiolanthanides have not been performed in rabbits, although they have been employed simultaneously for measurements of total and ileo-rectal transit on cannulated animals (GIDENNE, 1994). Thus, in this study, we measured the effect of marker type ($^{169}$Yb vs $^{144}$Ce) and of the ileal cannulation on transit.

MATERIAL AND METHODS

Experimental design

A control group of 5 adult rabbits and a group of 4 ileo-cannulated rabbits were used throughout the experiment. Animals were fed ad libitum and housed in individual metabolism cages under a 12h light (7:0 - 19:00) : 12h dark schedule. The experiment was divided into 6 periods (fig. 1) of one week each, including adaptation to the experimental diets (weeks 0 and 2) and periods of passage rate measurements (weeks 1, 3, 4, 5). The effect of the measurement period (table III) was evaluated by comparing the results obtained on the control group during the weeks 3 and 5, using Yb given at 11:00 The effect of the marker type "Ytterbium vs Cerium" (table IV), was controlled by comparing RPM after giving a dose of Yb at 11:00 followed...
by a dose of Ce labelled particles, to the control group fed the low fibre diet (week 1).

Measurements of passage rate were performed, in the control group fed the control diet (weeks 3, 4, 5), for 5 marker dosing times distributed around the caecotrophy period (figure 2): 8:00 = before caecotrophy; 11:00 = beginning of caecotrophy; 13:00 = during caecotrophy; 16:00 = end of caecotrophy; 21:00 = after caecotrophy.

The influence of the ileal cannulation on transit was assessed for two dietary fibre levels, by comparing values from control and cannulated animals during weeks 1 and 3.

Experimental diets
Two diets were formulated (table I) with the same cell wall (CW) source. The low fibre diet differed from the control one by a proportional reduction of the CW material levels, mainly substituted by starch and soya bean meal. Consequently, only the dietary fibre content decreased without any change in the proportions of the cell wall fractions. The chemical composition of the two diets is given in tables I and II. The nutritive value of these two diets was previously assessed in-vivo by BELLIER and GIDENNE (1996) and is reported in the table II.

Cell wall labelling with $^{159}$Ytterbium or $^{141}$Cerium

Before labelling, the cell wall contents of each diet (control or low fibre diet) was extracted with neutral detergent solution (VAN SOEST and WINE, 1967) and Termamyl 120 L (thermostable amylase, Novo A/S Copenhagen) for 1h at 100°C, then rinsed and filtered on a 50μm sieve. The fibre particles of each diet were separately labelled with either $^{159}$YbCl$_3$ or $^{141}$CeCl$_3$, using a competitive binding technique (ELLIS and BEEVER, 1984). First, the particles were maintained in suspension by magnetic stirring for 24h in 100 ml of an acid solution (pH 2.4) containing the markers and citric acid (competitive ligand) in half molar proportions. Labelled particles were then rinsed with acid tap water, and suspended in 100ml acid water (pH 2.4) for 10 min, and then sieved on a 50μm screen. This operation was repeated three times in order to simulate the acidic in-vivo conditions found in the rabbit stomach, with the aim of eliminating the fraction of the marker only slightly bound on particles. The specific radioactivity of the particles was about 5 KBq g$^{-1}$ DM.

Rate of passage measurement

The passage rate for fibre particles was measured by following the faecal excretion of a dose (100 mg) of rare-earth labelled fibre particles (GIDENNE, 1994). The dose was introduced in a modified 1ml syringe, and given in the back of the throat of the animal (without oesophageal intubation) for some seconds. Then, the faecal excretion was divided into 36 samples (respect to the circadian rhythm), over 4 days, by means of an automatic faecal sampler (API, Castanet, France) adopted for use in rabbit metabolism cages. After drying, the faeces were directly analysed for their marker content in a gamma spectrometer (Packard Instrument, model 5530, Downersgrove, IL, USA).

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Table I : Ingredients (g kg$^{-1}$) of the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>control C</th>
<th>low-fibre LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated alfalfa meal</td>
<td>215</td>
<td>140</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>132</td>
<td>65</td>
</tr>
<tr>
<td>Wheat straw (ground)</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Wheat</td>
<td>426</td>
<td>562</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>133</td>
<td>175</td>
</tr>
<tr>
<td>Minerals and vitamins</td>
<td>24</td>
<td>28</td>
</tr>
</tbody>
</table>

Table II : Determined analysis and nutritive value of the experimental diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>control C</th>
<th>low-fibre LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical composition (g kg$^{-1}$ DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>903</td>
<td>898</td>
</tr>
<tr>
<td>Crude protein</td>
<td>183</td>
<td>194</td>
</tr>
<tr>
<td>Starch</td>
<td>342</td>
<td>396</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>133</td>
<td>92</td>
</tr>
<tr>
<td>Neutral Detergent Fibre</td>
<td>321</td>
<td>230</td>
</tr>
<tr>
<td>Acid Detergent Fibre</td>
<td>158</td>
<td>106</td>
</tr>
<tr>
<td>Acid Detergent Lignin</td>
<td>36</td>
<td>23</td>
</tr>
</tbody>
</table>

| Nutritive value (1)       |           |              |
| Digestible energy (MJ kg$^{-1}$ DM) | 121     | 141          |
| Digestible crude protein (g kg$^{-1}$ DM) | 147     | 160          |

(1) measured in vivo by BELLIER and GIDENNE (1996).

(1) Minerals (%): Cu (0.35), Zn (0.77), Mn (0.33);

Vitamins (IU/kg): A (1,800000), D3 (200000), E (33 g/kg);

Riboflavin (132 g/kg)
The digesta mean retention time "MRT" was algebraically calculated by numerical integration of the quantity of marker excreted in the faeces: \[ MRT = \frac{\sum M_i}{t} \] where \( t \) was the time that had elapsed between marker administration and the \( i \)th defecation and \( M_i \) was the quantity of marker excreted. MRT included the minimal transit time "TTm", which is the time that elapsed between marker administration and the first marker appearance in the faeces. TTm reflected the retention time of digesta when there was no delay in the mixing compartments. Thus, it represented an evaluation of the passage rate in the tubular segment of the tract, i.e. mainly in the small intestine and also in the distal colon (GIDENNE, 1994). In addition, we calculated an index "Ecp", which was more specific of rabbit physiology. Ecp was the quantity of marker (as a percentage of the total administered) excreted between dosing and the following phase of caecotrophy. This provided an estimation of the quantity of marker potentially recycled in soft faeces, and also reflected the potential effect of caecotrophy upon rate of passage.

Biochemical Analysis
Chemical analysis of the feed was performed on duplicate samples. Dry matter (DM) was determined by drying at 103°C for 24h. Nitrogen was measured by a KJELDHAL procedure and converted to crude protein (CP) using the factor 6.25. Neutral and acid detergent fibre residues (NDF, ADF) were determined according to the procedure of Van Soest using an amylolytic pretreatment (VAN-SOESt et al., 1991). Starch was gelatinised by autoclaving and then hydrolysed enzymically and the resultant glucose was measured by using the hexokinase (EC 2.7.1)-glucose-6-phosphate dehydrogenase (NAD) (EC 1.1149) system (Boehringer Mannheim).

Statistical analysis
The results were subjected to variance analysis according to the general linear model (GLM) procedure of the Statistical Analysis System Institute (SAS, 1988). The effects of the measurement period (table III) and that of the marker type (table IV) were analysed by a two way variance analysis to estimate variations from the main factor (marker or period) and interindividual variations. As the effect of the measurement period was significant and dependent on the feed intake, the effect of the marker dosing time (table V) was estimated through a covariance analysis using the daily intake (g/d/kg LW) within each rabbit as a covariate. Consequently

Table III: Effect of the period of measurement on feed intake, dry matter digestibility and rate of passage.

<table>
<thead>
<tr>
<th>Period</th>
<th>Live weight (g)</th>
<th>Feed intake (g·kg⁻¹ LW)</th>
<th>DM digestibility (%)</th>
<th>Mean Retention Time (h)</th>
<th>Minimal Transit Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 3</td>
<td>4501</td>
<td>34.2 a</td>
<td>67.0</td>
<td>20.1</td>
<td>5.9</td>
</tr>
<tr>
<td>week 5</td>
<td>4582</td>
<td>28.6 b</td>
<td>67.4</td>
<td>23.9</td>
<td>7.3</td>
</tr>
<tr>
<td>SEM</td>
<td>11</td>
<td>0.55</td>
<td>0.34</td>
<td>1.04</td>
<td>0.23</td>
</tr>
<tr>
<td>P level</td>
<td>0.0066</td>
<td>0.001</td>
<td>0.196</td>
<td>0.063</td>
<td>0.0135</td>
</tr>
</tbody>
</table>

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Table VI: Effect of the ileal cannulation and fibre level on feed intake, dry matter digestibility (DMD) and on the rate of passage.

<table>
<thead>
<tr>
<th></th>
<th>Effect of Ileal cannulation</th>
<th>Effect of Dietary fibre level</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional</td>
<td>Cannulated</td>
<td>Control</td>
</tr>
<tr>
<td>Intake (g/d/kg LW)</td>
<td>35.2</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>DMD (%)</td>
<td>69.2</td>
<td>71.7</td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>23.7</td>
<td>25.2</td>
<td></td>
</tr>
<tr>
<td>TTM (h)</td>
<td>6.3</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Ecp index (%)</td>
<td>67.2</td>
<td>67.5</td>
<td></td>
</tr>
</tbody>
</table>

The results of the dosing time effect were presented as least square means. The effect of the cannulation (table VI) was determined according to a split plot design where the error term was the mean square of the inter-individual variations within rabbit group.

RESULTS

The experiment was conducted over 4 successive periods of passage rate measurement (RPM). The effect of the period on transit was controlled by repeating a RPM two weeks later (fig. 1) on the same animals (table III). The mean retention time was 3.8 h longer (+19%) in the week 5 compare to the week 3 (P=0.063), and the minimum transit time increased significantly by 1.4h. In parallel the feed intake decreased significantly by 16%, whereas the dry matter digestibility remained unchanged. Thus, when the statistical analyses included the feed intake (g/d/kg LW) as a covariate, the effect of the period was not significant for TTM (P = 0.44) and MRT (P=0.12).

In order to reduce the duration of the experiments, two markers $^{166}$Yb and $^{141}$Ce were used to evaluate the influence of the marker dosing time, thus the possible effect of the type of marker on RPM was also assessed (table IV). No change in Total MRT or TTM was observed, indeed allowing us comparison of RPM measurement using Yb or Ce indifferently.

Under our experimental conditions, the hard faeces excretion generally occurred for two-thirds of the animals between 18h and 8h (fig. 2). Over 70% percent of the animals did not excrete hard faeces between 10h and 16h, thus indicating soft faeces production at this time. The total MRT did not vary significantly when the marker dosing time took place out of the period of caecotrophy (table V). But a 25% reduction in MRT (-5.2h) was observed when the marker was given during caecotrophy (13h) compared to a dosing time at the beginning of the caecotrophy (11h). This decrease in MRT values at 13h was partly due to a shorter time delay or minimal transit time (-1.5h). On the other hand, when dosing time was at the end or after the caecotrophy period (16h and 21h resp.) the quantity of marker excreted before the following caecotrophy "Ecp" was significantly reduced, due to a shorter time delay till caecotrophy (on the day following marker dosing). In parallel, we observed a significantly lower TTM for a dosing time at 16 or 21h compared to values obtained when the marker was given at 8h.

Compared to conventional animals, the cannulated rabbits had a slight lower feed intake (table VI), and a slightly but a significantly higher DM digestibility. No significant difference was however observed for any RPM parameters between the cannulated and conventional rabbits. Conversely, the reduction of the dietary fibre level resulted in an increase of the DM digestibility and of the rate of passage.

DISCUSSION

The passage rate of particles labelled with Yb was equal to those labelled with Ce, allowing to compare the RPM measurements obtained with these two markers given at different times to the animals. In general terms, this emphasised the convenience of using rare-earth metal markers for simultaneous measurements of transit, for example when several raw materials in a single diet are compared (Gidenne et al., 1987) or to measure simultaneously the total and ileo-colic transit (Gidenne, 1994).

Caecotrophy is the predominant specificity of rabbit digestive physiology, involving the excretion of two types of faeces (caecotrophes and hard faeces), and the exclusive ingestion of caecotrophes (Hörnicke 1981, Gallouin 1983). This dual faecal excretion originates in the motility pattern of the proximal colon. During caeco-trophy, caecal digesta are almost "directly" incorporated into soft faeces. During the hard faeces excretion period, the proximal colon alterns peristaltic and antiperistaltic motility, the latter returns fluid and fine particles (under 300μm) towards the caecum (Björnhag, 1981). Consequently a higher proportion of large particles is incorporated in hard faeces (Jilge, 1982) than in caecotrophes. Thus, when labelled particles passed through the colon after the caecotrophy period (dosing time at 16:00 or 21:00), the fine labelled particles are brought back to the caecum, consequently increasing their retention time (Gidenne, 1993), and leading to higher MRT values. In contrast Laplace and Lebas (1975) observed a lower retention time when the marker was given after caecotrophy. However, their labelling technique differed from our trial, as Cerium is directly put on a pellet, without controlling its fixation on particles.

On the other hand, when marker was given before (8:00) or at the beginning of the caecotrophy (11:00), a fraction of
the labelled particles arrived in the colon during caecotrophy, and could be incorporated rapidly in soft faeces and recycled. This contributed to increase the MRT value and the time delay for the first appearance of marker in hard faeces (TTm). Conversely, the low TTm values, for a marker dosing time placed after caecotrophy, could be attributed to a direct incorporation of the labelled particles in hard faeces. This was in agreement with the study of LAPPLACE and LEBAS (1975), who showed that retention time in growing rabbits was increased when the marker was administered just at the end of the caecotrope period, compared to a dosing time during caecotrophy. In addition, when the dosing time took place after caecotrophy, the Ecp index was lower as the delay between first appearance of marker in faeces and the following caecotrophy was shorter: the delay was around 12 and 8h respectively for a dosing time at 16:00 and 21:00, and it was around 19 and 16h for a dosing time at 8:00 or 11:00. More globally, the comparison of values obtained for a dosing time after (16:00 or 21:00) or before (8:00) caecotrophy revealed that MRT remain unchanged, whereas TTm decreased. This suggested a longer retention in the mixing compartments (mainly the caecum) for labelled particles given after caecotrophy (MRT-TTm = 15h), compared to those given before caecotrophy (MRT-TTm = 12.4h), which is due to the antiperistaltic motility of the proximal colon outside the caecotrophic period. On the other hand, the variability expressed as variation coefficients (CV %), appeared to be lower for all RP parameters when the marker was given at 11:00. The beginning of caecotrophy may therefore correspond to a period where rabbits are more synchronised than in the rest of the day (JILGE, 1987). Consequently, if the marker is given at this moment the interindividual variability of the RPM could be reduced.

Fitting animals with cannula is suspected to modify the digestive process. For instance, a recent study has reported a slight reduction in soft faeces production level for ileo-cannulated rabbits (GIDENNE et al., 1994). Furthermore, impairment of transit by cannulation has been suggested, more especially in tubular organs such as the small intestine, where the cannula could represent a physical obstacle to the digesta flow. However, to our knowledge this hypothesis has not been tested. Under our experimental conditions, passage rate of labelled particles was not significantly affected by the ileal cannulation, and no interaction with the dietary fibre level was observed. But, the variability of the RP measurements was relatively high, and we cannot exclude that the effect of the cannulation might have appeared significant if a higher number of animals have been used. For instance, dry matter digestibility, a more precise measurement, was affected here by the ileal cannulation, in relation with a lower feed intake.

In conclusion, passage rate values appeared lower when the dosing time took place during caecotrophy. Higher values were found either for a dosing time before caecotrophy (because of marker recycling) or for a dosing time after caecotrophy (because of a higher retention time in the caecum). But the precision of the measurements seemed to be higher when the marker was given at the beginning of the caecotrophy, possibly because the individual biorhythm of the animals may be more synchronised at this moment. In addition, Ytterbium or Cerium could be used indifferently for RP measurements, and ileal cannulation did not seem to greatly impair the transit of digesta.

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