

RATE OF PASSAGE OF FIBRE PARTICLES OF DIFFERENT SIZE IN RABBIT :  
EFFECT OF THE DIETARY LIGNIN LEVEL.

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ABSTRACT

Rate of passage measurements (RPM) of different cell wall (CW) particle size (  $0.05 < S1 < 0.315$  mm,  $0.315 < S2 < 0.5$  mm,  $0.5 < S3 < 1$ mm,  $S4 > 1$ mm) were investigated in two groups of four rabbits fed with two diets differing mainly by their level of lignin "ADL": control "C"=2.3%, high ADL "H"=7.6%. In order to determine the specific effect of fibre level without an effect on the intake level, the diets were distributed in same quantity (110g/d). CW particles of each classes (S1 to S4) were labelled with  $^{141}\text{Ce}$  Cerium. RPM were performed by following the faecal excretion of  $^{141}\text{Ce}$  for six days.

The mean retention time (MRT) of CW was reduced when the level of ADL increased even under similar feed intake level, but the reduction was slight: 30.2 and 28.8 hours for C and H group).

The higher MRT of fine particles (32.2 to 36.6 h for S4 class) compared to larger one (25 to 29 h for S1 to S3 class) were related with the antiperistaltic activity of the proximal colon. Particles ranging between 0.3 and 1mm showed no differential rate of passage. Similar transit time "TT" between classes of particles (general mean= 7.0 h) suggested that segregation of particles (between S1 and S4) would not occur in small intestine and distal colon of the rabbit.

## INTRODUCTION

An important factor in the digestive physiology of rabbits is caecotrophy, which involves a system of segregation of digesta particles in the proximal colon and a recycling of contents of the caecum in soft faeces. As found by BJÖRNHAG (1972, 1981), the antiperistaltic activity of the proximal colon brought back fluid and fine particles (size inferior to 0.3 mm) to the caecum during the hard faeces excretion period. But, to date, this phenomenon has not been quantified, and, notably, no data is available on the retention time of particles ranging in size ranged between 0.3 and 1 mm.

The dietary level of cell walls (CW) is another main factor affecting digestive transit. However, the mechanism by which fibre has an effect on the rate of passage (RP) of digesta is not yet clear. In Particular, the rate of passage may be affected by the increase in voluntary feed intake associated with high dietary fibre diets, and by the direct effect of the physico-chemical properties of cell walls (LEBAS and LAPLACE, 1977a).

The purpose of this present study was thus to provide further informations on the transit of fibre particles of different sizes "S" (0.05 < S1 < 0.315 mm, 0.315 < S2 < 0.5 mm, 0.5 < S3 < 1mm, S4 > 1mm) for rabbits fed with two diets differing mainly in their level of lignin (ADL). In order to exclude an effect of the voluntary feed intake upon rate of passage, same quantity of each diets was provided.

## METHODS

### Animals and feeding.

Two groups of four male New Zealand White rabbits (live weight 2.70 ± 0.13 kg) were given C or H diet (table 1 and 2) in a pelleted form, at a fixed intake level of 110 g per day (at 8.30 am). Animals were housed in individual metabolism cages under a 12/12 light dark schedule. Diets differed essentially in their content of lignin "ADL" : control level "C" diet and high level of ADL "H" diet. Dietary fiber were analysed using the detergent system and the dietary lignin level was determined by Klason (Acid Detergent Lignin) procedures (table 2) according to ROBERTSON and VAN-SOEST (1981).

### Cell wall labelling with <sup>141</sup>Cerium

Before labelling, cell wall material for each diet (batch of 25 g of pellets) was extracted with neutral detergent solution (VAN SOEST and WINE, 1967) and Termamyl (thermostable amylase) for one hour at 100°C, then rinsed and filtrated on a 0.05mm sieve. Particles were labelled with <sup>141</sup>Cerium-chloride using a competitive binding technique (ELLIS and BEEVER,

1984). First, particles were maintained in suspension by magnetic stirring for 24 hours in 100 ml of an acid solution (pH=2.4) containing CeCl<sub>3</sub> and citric acid (competitive ligand) in half molar proportions. Labelled particles were then rinsed with tap water. Before drying, particles were sieved to obtain four classes of size "S": 0.05 < S1 < 0.315 mm, 0.315 < S2 < 0.5 mm, 0.5 < S3 < 1mm, S4 > 1mm. Specific radioactivity of labelled cell wall particles was about 5 KBq/g DM (0.14 µCi/g DM).

Table 1. Ingredients of the experimental diets (g/Kg).

	Diet C Control	Diet H High ADL
Wheat	389	350
Wheat bran	40	0
Lucerne meal	78	233
Wheat straw	30	0
Beet pulp	100	40
Sunflower hull	0	118
Soja bean hull	150	0
Sunflower meal	0	120
Soya bean meal	154	80
Vitamins and mineral	59	59

Table 2. Chemical composition of the experimental diets (g/Kg dry matter).

	Diet C Control	Diet H High ADL
Crude protein (Nx6.25)	190	184
Starch	250	239
Crude Fibre	137	196
Neutral Detergent Fiber	317	367
Acid Detergent Fiber	159	221
Acid Detergent Lignin	23	76

Rate of passage measurements.

Labelled particles of the four classes of size were administered to each rabbit following a double latin square design: 2 levels of ADL X [(4 levels of S) X (4 rabbits)]. The experiment was conducted over four

consecutive weeks, each measurement period of passage rate lasting 6 days. Labelled particles ( $\approx 0.15$  g) were placed in a syringe, suspended in 4 ml water and then given (between 9 and 9.30 am) by oesophageal intubation. The dosing procedure took less than 30 seconds for each rabbit. After dosing, the fecal excretion was fractionated (42 samples for 6 days, see fig 1) using an automatic fecal sampler adapted to the metabolism cages. Each day the fecal collections were dried, the total quantity of faeces was then analysed for  $^{141}\text{Ce}$  content in a gamma spectrometer.

#### Calculation of rate of passage parameters.

The calculation of the Mean Retention Time (MRT) is well-adapted to the discontinuous fecal excretion of the rabbit:  $M.R.T = \Sigma M_i \cdot t_i / \Sigma M_i$ , when  $t_i$  was the time elapsed between dosing and the  $i^{\text{th}}$  defecation and  $M_i$  was the quantity of marker excreted in the  $i^{\text{th}}$  defecation (FAICHNEY, 1975). Then, MRT reflect the mean digesta retention time in the whole digestive tract. It include the transit time (TT), which is the elapsed time between dosing and the first appearance of the marker in faeces. TT reflect the retention time of digesta without a delay time in the large compartments, so it would represent the RP in the tubular segment of the tract, i.e. small intestine and distal colon.

In addition, we calculated an index "Ecp" which was more specific for rabbit physiology. That is the quantity of marker (in percent of the total amount: CumEx, fig. 1) excreted between dosing and the following phase of caecotrophy. This was an estimation of the quantity of marker potentially recycled in soft feces, and then it was an estimation of the effect of caecotrophy upon the rate of passage.

#### Statistics

Analyses of variance were conducted by the GLM procedure of SAS (1985) using a split-plot design for determining diet and particle size effect.

### RESULTS AND DISCUSSION.

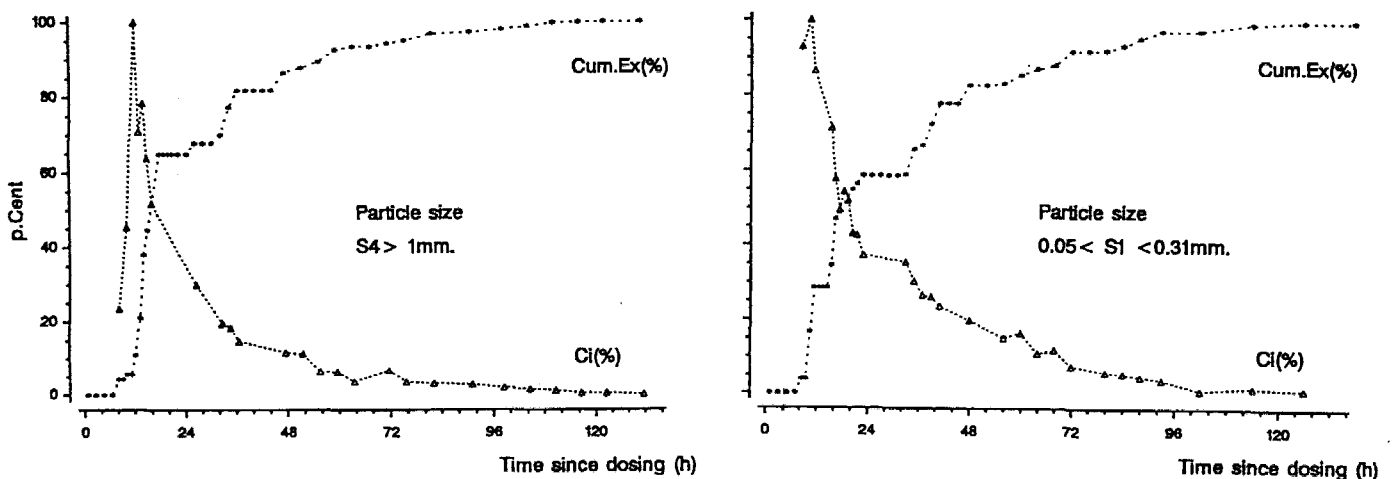
#### Effect of the level of lignocellulose on dry matter digestibility and rate of passage measurements.

The two experimental diets included common raw materials (table 1), they were similar in crude protein and starch level (table 2) and differed mainly in their level of acid-detergent lignin "ADL": lignin content was three time higher in the H diet compared to the control 'C', consequently lignocellulose "ADF" content of H diet was 40% greater than the C diet.

The feed intake level was fixed at 110 g/d corresponding to -20% and -27% of the voluntary feed intake for C and H diet respectively. As the feed intake level was the same for the two diets, we supposed that the fibre level effect was essentially due to a physico-chemical effect of the CW on the motility of digestive organs.

The mean retention time (MRT) was significantly reduced when the level of ADL was increased (table 3) without significative interaction with the particle size effect. However, this decrease in MRT was slight ( 30.2 vs 28.8 h for C and H group repectively), whereas the diet effect on the Ecp index was not significant (63.0 vs 61.0%). An example of a classical fecal excretion of the marker in rabbit was presented in figure 1. The incidence of the first phase of caecotrophy was clearly observed between 20 h and 35 h post-dosing. The  $^{141}\text{Ce}$  concentration ( $C_i$ ) dropped by more than 50% between the peak of concentration and the first phase of caecotrophy (fine particles, S1), and then increased because a quantity of marker was recycled with the soft faeces. This phenomenum was less evident when dosing with large particles "S1", indicating a lower incidence of caecotrophy. On the other hand, the level of ADL significantly reduced the transit time "TT", but only for particle sizes over 1 mm (significant interaction with the particle size effect).

Figure 1. Fecal concentration ( $C_i$ ) and excretion (CumEx) of the marker ( $^{141}\text{Ce}$ ) in faeces according to time since dosing.



Rabbit n°2 ( C diet): TSM=26.9 and 31.9 h respectively for S4 and S1 particle size.

$C_i$  (%): marker concentration as a percent of the maximum concentration.

CumEx (%): cumulative quantity of marker excreted in faeces as a percent of the total excretion.

It has been pointed out that a low feed intake level increases the digesta retention time (GIDENNE et al, 1987), thus values observed here were relatively high (general mean MRT = 29.4 h) compared to values generally observed in ad-libitum fed rabbits (15 to 22 h depending of CW level).

Previous studies on the effect of the level of dietary fibre have generally been performed on ad-libitum fed rabbits. Then, MRT values were largely reduced for adult (GIDENNE et al 1990) or growing rabbits (LEBAS and LAPLACE, 1977b; FRAGA et al, 1984; GIDENNE et al, 1991) when fibre level increased. This effect originated essentially in a decreased MRT in the caeco-colic part of the tract (GIDENNE et al, 1990). But, the slight variations of MRT observed here when comparing C and H diets suggests that the effect of the dietary fiber level observed previously, originates largely from variations in voluntary feed intake.

Table 3. Mean retention time (MRT), transit time (TT) and Ecp measurements of cell wall particles.

Class of particle size "S" in mm	M.R.T (hours)		TT (hours)		Ecp index (%)	
	Diet C	Diet H	Diet C	Diet H	Diet C	Diet H
S > 1	26.0 <sup>a</sup>	25.4 <sup>a</sup>	9.2	3.9	69.2 <sup>a</sup>	64.0 <sup>a</sup>
1 > S > 0.5	28.8 <sup>a</sup>	27.9 <sup>a</sup>	7.3	7.0	65.9 <sup>a</sup>	62.9 <sup>a</sup>
0.5 > S > 0.31	28.7 <sup>a</sup>	28.9 <sup>ab</sup>	6.8	7.6	61.4 <sup>ab</sup>	57.9 <sup>a</sup>
0.31 > S > 0.05	36.6 <sup>b</sup>	32.2 <sup>b</sup>	8.1	6.1	58.4 <sup>b</sup>	60.5 <sup>a</sup>
S.E.D <sup>1</sup>	1.17		0.81		2.57	
S.E.D <sup>2</sup>	2.53		1.04		4.79	
Statistical significance:						
Diet effect	0.036		0.041		N.S	
Particle size effect	0.020		N.S.		0.028	
Interaction	N.S		0.048		N.S	

<sup>a,b</sup> : Within a column, mean values (n=4) with different superscript were significantly different (P<0.05).

SED<sup>1</sup>, standard error of differences between means for rabbits receiving the same diet (13 df); SED<sup>2</sup>, standard error of differences between means for rabbits receiving different diets (6 and 13 df).

Fibre particle size effect on rate of passage measurements.

Depending on the parameter considered, the effect of the size of fibre particles on their rate of passage was not similar. The effect of fibre particle size was significant whichever parameter was considered, except for transit time (table 3). TT represent the digesta retention time in the tubular part of the tract (mainly in the small intestine). As TT values for different classes of particle size did not differ significantly, it could be assumed that segregation of fibre particles (ranging between 1mm and 0.05mm) would not occur along the small intestine and distal colon.

MRT values differed significantly between the finest particles and others classes (table 5). This reflects the anti-peristaltic activity of the proximal colon which delay the transit of the fine particles; a higher retention time of fine particles (< 0.075mm) compared to large one (>0.3mm) were also found in adult rabbits (fed ad-libitum) by SAKAGUCHI AND HUME (1990) and by UDEN and VAN-SOEST (1982). However, we did'nt find in our study any selective retention of digesta particles for sizes ranging between 0.3mm and 1mm, whatever the RP parameter considered.

Values of the Ecp index also indicated that fine labelled particles were delayed in the tract. But this effect was observed only for the control group: about 70% of the largest particles were excreted in about 20h (before the first period of caecotrophy) compare to 58% for the finest particles.

#### CONCLUSION

A limited feed intake level induced a relatively high mean retention time even for a diet containing a high level of lignified cell wall.

The slight effect of cell wall level observed here for a fixed feed intake, suggested that the intake level plays an important role in the determinism of digesta rate of passage.

The segregation of fine particles (<0.315mm) in the distal part of the rabbit digestive tract was confirmed, whereas particles ranging between 1 and 0.3mm appeared to move together. On the other hand, fibre particles seemed to move in the small intestine and distal colon without selective retention time according to the particle size.

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