IMPACT OF CAECOTROPHY ON RATE OF PASSAGE, INTAKE AND FAECAL EXCRETION PATTERN IN THE GROWING RABBIT.

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ABSTRACT: Rate of passage was measured on 12 rabbits, either allowed to practice caecotrophy (Control period, from 56 to 60 days of age) or prevented from consuming soft faeces by wearing a plastic collar (without soft faeces intake = WSF periods, 63-66 d old and 70-73 d old). Measurements of digestive transit of the solid phase of the digesta were carried out by analysing the kinetics of faecal excretion of a fibre particles labelled with $^{141}$Ce. The excretion pattern of hard and soft faeces, hourly quantified during three complete 24-h cycle during WSF periods, showed that hard faeces excretion averaged $35.3 \pm 3.2 \text{ g/day DM}$, while caecotrophes excretion was meanly $10.7 \pm 2.6 \text{ g DM/d}$ for a mean feed intake of $113.7 \text{ g DM/d}$. During the control period, daily hard faeces excretion was not significantly different ($36.4 \pm 3.0 \text{ g DM/d}$, for a feed intake of $110.8 \text{ g DM/d}$). The DM digestibility did not differ during control and WSF periods (67.2 and 67.8%). If caecotrophe production is included in the DM digestibility calculation, coefficient fell by 10.5 units (mean = 57.3%). When soft faeces intake was prevented (period WSF), the mean retention time in the whole tract evolved from 23 h (Control) to 15 h (-34%) and the minimal transit time was 50% shorter. The caecal mean retention time passed from 17 h in Control to 10 h (-40%) in WSF period. Caecal retention of large particles seemed less affected (CRlp:-23%) than fine (CRfp:-48%).

Key words: rabbit, transit, caecotrophy, digestion, circadian excretion pattern.

INTRODUCTION

The nutritional benefit of caecotrophy for the rabbit was extensively studied, either for its supply in nitrogen or vitamins (KULWICH et al., 1953; BATTAGLINI, 1968),
and was reviewed by Galloin (1983). More recently, the bacterial protein contribution from soft faeces was more precisely examined (Balcells et al., 1998; Garcia et al., 2000; Gidenne and Jehl, 2001). However, the impact of caecotrophy on rate of passage of feed had been less studied, and frequently with a low number of rabbits or questionable labelling techniques (Uden and Van Soest, 1982; Piekarz, 1963). In addition, previous works on rate of passage in the rabbit, generally calculated the rate of passage only in the whole digestive tract (Sakaguchi et al., 1992, Laplace and Lebas, 1975).

Therefore the present study aimed to quantify more precisely the impact of caecotrophy on transit of particulate phase of the feed, using a precise faecal sampling combined with a radio-lanthanide labelling procedure. Moreover, procedures of modelisation of the marker kinetics will be used to estimate the retention time of particles in the caeco-colic segment.

**MATERIALS AND METHODS**

**Animals, housing and experimental protocol.**

Twelve Neo-Zealand White male rabbits were housed in a closed and ventilated breeding room (18°C ± 2°C) with 12 h light (7:00-19:00). The animals were maintained in metabolism cages from 42 to 74 d of age and received water and feed *ad libitum*. The experimental diet was formulated to meet the nutrient recommendations for growing rabbits (De Blas and Mateos, 1998). The feed ingredients and the chemical composition are reported in Table 1. The diet contained a relatively high level of digestible fibres (hemicellulose+pectins = 223 g/kg), provided by beet and citrus pulps. The live weight and feed intake were individually measured at least once a week and at the beginning and end of each transit measurement period.

Animals were firstly adapted to the diet and cages for two weeks (42-55 d of age), and they were submitted to a 24-h period of adaptation to the collar (52 to 53
d), but without transit measurement. Then, the rate of passage was measured on the animals which were allowed to practice caecotrophy (Control period) during a 4-d period (from 56 to 60 d of age). Finally, rate of passage measurements were performed.

Table 1: Ingredients and chemical composition of the experimental diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(%)</th>
<th>Chemical composition and nutritive value</th>
<th>g/kg (air dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>19.00</td>
<td>Dry matter</td>
<td>874</td>
</tr>
<tr>
<td>Wheat meal</td>
<td>8.00</td>
<td>Ash</td>
<td>71</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>10.50</td>
<td>Crude Fat</td>
<td>20</td>
</tr>
<tr>
<td>Lucerne meal</td>
<td>19.60</td>
<td>Crude protein (Nx6.25)</td>
<td>161</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>6.00</td>
<td>Gross energy (MJ/kg)</td>
<td>15.95</td>
</tr>
<tr>
<td>Citrus pulp</td>
<td>10.00</td>
<td>Starch</td>
<td>118</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>17.00</td>
<td>Crude fibre</td>
<td>155</td>
</tr>
<tr>
<td>Soya bean hulls</td>
<td>1.85</td>
<td>NDF$^2$</td>
<td>320</td>
</tr>
<tr>
<td>Grape seed meal</td>
<td>2.00</td>
<td>Lignocellulose, ADF$^2$</td>
<td>191</td>
</tr>
<tr>
<td>Beet molasses</td>
<td>4.50</td>
<td>Lignins (ADL)$^2$</td>
<td>52</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.25</td>
<td>Hemicellulose (NDF-ADF)</td>
<td>129</td>
</tr>
<tr>
<td>Salt</td>
<td>0.60</td>
<td>Cellulose (ADF-ADL)</td>
<td>138</td>
</tr>
<tr>
<td>Vitamin premix$^1$</td>
<td>0.50</td>
<td>Pectins$^3$</td>
<td>94</td>
</tr>
<tr>
<td>L Lysine</td>
<td>0.05</td>
<td>NNCC$^4$</td>
<td>322</td>
</tr>
<tr>
<td>DL methionine</td>
<td>0.15</td>
<td>Digestible protein$^5$ (g/kg)</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digestible energy$^5$ (MJ/kg)</td>
<td>9.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DP/DE (g/MJ)</td>
<td>11.38</td>
</tr>
</tbody>
</table>

$^1$containing vitamins: A, 1500000 UI/kg premix, D3, 200000 UI/kg, E 3000 mg/kg, B, 200 mg/kg; oligo-elements: copper 4 g/kg, iron 8 g/kg, zinc 20 g/kg, manganese 4 g/kg, without antibiotic or coccidiostatic.

$^2$according to the sequential procedure of Van Soest et al. (1991); NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin.

$^3$Water insoluble pectins calculated according to Gidenne (2003).

$^4$Non nitrogenous cellular content = Organic matter - NDF - Crude protein.

$^5$Nutritive value measured in vivo on 10 rabbits according to European referenced procedure (Perez et al., 1995).
on the same rabbits but wearing a plastic collar (without soft faeces intake = WSF periods, soft faeces intake not allowed), for two periods of three days (63-66 d and 70-73 d of age, i.e. WSF1 and WSF2 periods), in order to estimate the effect of the period on transit measurements. When soft faeces intake was prevented, a three day period of faeces collection was sufficient, as no more marker was excreted over 72-h delay after dosing. A flat shape collar was used to prevent soft faeces intake, as described by GIDENNE and Lapanouse (2000).

**Rate of passage measurements**

Measurements of digestive transit of the solid phase of the digesta were carried out by analysing the kinetics of faecal excretion of a fibre particles labelled with $^{141}$Ce. A dose of labelled particle (100 mg of NDF residue of the diet, 2 kBq/dose) was orally administrated using a modified plastic syringe (1 ml), between 11:00 and 11:15. This time was chosen to obtained the lowest variations in retention time, in agreement with GIDENNE and Lapanouse (1997). It allowed also the largest delay between the end of caecotrophy and the next period of feed intake, therefore giving more time to animals to recover after a possible stress associated with marker dosing. Faecal excretion was then hourly fractionated over 4 days (96 samples) for the Control period, and over 3 days for the WSF periods (72 samples), using an automatic faecal sampler (API, Castanet, France). For each sample, the type of faeces (hard or soft) was separated and weighed (Figure 1). Intermediate faeces (aspect between hard and soft) were recorded as hard faeces, in agreement with JILGE (1974) who observed they were not consumed by the rabbit. The quantity of marker excreted in each faecal sample was then analysed by gamma spectrometry (Packard Instrument, Model 5530, Downersgrove, IL, USA), and expressed in counts per minute (cpm) to take into account the counting yield of the apparatus.

Before labelling, fibre particles were extracted with neutral detergent solution and Termamyl 120L (thermostable amylase, Novo A/S Copenhagen) for 1 h at 100°C, then rinsed and filtered on a 0.05 mm sieve. Particles were then labelled using a competitive binding technique (ELLIS and BEEVER, 1984). First, particles were maintained in suspension by magnetic stirring for 24 h in 100 ml of an acid solution
(pH between 2.1 and 2.4, to simulate the acid conditions of the rabbit stomach), containing CeCl$_3$ and citric acid (competitive ligand) in half molar proportions. Labelled particles were then rinsed with tap water.

The mean retention time (MRT) of solid phase of digesta was calculated according to the general formula $MRT = \sum M_i T_i$, where $T_i$ represented time elapsed between $T_0$ (administration of the marker) and the $i^{th}$ collect, and $M_i$ the mass of marker excreted between $T_{i-1}$ and $T_i$. The minimum transit time (TTm) was the delay time (average time between two collects) for first appearance of the marker in the faeces, which is equivalent to the passage time in the small intestine and the distal colon of the rabbit (GIDENNE, 1994).

To estimate retention time in mixing compartments (mainly caecum) and the impact of the caecotrophy, we analysed more particularly the kinetics of Ce concentration in the faeces (Figures 2 and 3). It is generally divided in two phases: a short ascending part, associated with retention time in the first mixing compartment (i.e. the stomach), and a longer descending part, corresponding to marker retention in the second mixing compartment (i.e. mainly the caecum) (GIDENNE, 1994). The caecotrophy, associated with soft faeces intake, causes the decrease of the faecal concentration ($C_t$) of the marker of the solid phase to inflect, because of a recycling of labelled particles through soft faeces consumption.

A single adjustment of the decreasing part of the kinetic was firstly performed using a non-linear regression according to an exponential ($C_t = C_0 \times \exp^{-kt}$), where the inverse of the constant of time “$k$” represented the mean retention time in the caecum (figure 2). We also carried out a double adjustment of the decreasing part of the kinetic, before and after the first phase of caecotrophy (Figure 3), using a non-linear regression according to an exponential ($C_t = C_0 \times \exp^{kt}$). We defined two indices, $CRlp$ and $CRfp$, as the inverse of the two constants of time of the 2 equations, each one representing a residence time in the “caecum-proximal colon” compartment. The caecal retention time of large particles “$CRlp$” would correspond to particles larger than 0.3 mm and quickly excreted in hard faeces (BJÖRNHAG,
The caecal retention of fine particles “CRfp” would correspond to particles (<0.3 mm) driven back to caecum by the proximal colon during the period of hard faeces excretion, and which are thus potentially incorporated in caecotrophes and consumed a second time (Pickard and Stevens, 1972; Björnhag, 1981).

Chemical analyses

Dry matter was determined in feed and faeces by heating 24 h at 103°C. Ash and fibre fractions were determined in feeds according to EGRAN (2001). Nitrogen was determined by DUMAS combustion method using Leco apparatus (model FP-428, Leco Corp., St Joseph, MI, USA) and converted to crude protein using the factor 6.25.

Statistical analyses

Results were subjected to analysis of variance according to the general linear model (GLM, SAS OnlineDoc. release 8.01 for SunOs, SAS Institute Inc., Cary, NC USA). First, the effect of the period of measurement was analysed (Table 2). As no effect of the period was detected, a bi-factorial model was used including the effect of the treatment (control vs WSF) and the effect of the animal, to take into account the fact that the measurements were repeated on the same animals. Rabbits wearing a collar and having a decrease of intake exceeding 20% of the control period were excluded from statistical analysis.

RESULTS

Faecal excretion pattern and kinetics of marker

The excretion pattern of hard and soft faeces was hourly quantified during three complete 24-h cycle on rabbits prevented from caecotrophe consumption (Figure 1). Hard faeces excretion averaged 35.3 ± 3.2 g DM/d, while caecotrophe excretion was on average 10.7 ± 2.6 g DM/d for a mean feed intake of 113.7 g DM/d. During the control period, daily hard faeces excretion was not significantly different (36.4
± 3.0 g DM/d, for a feed intake of 110.8 g DM/d). Accordingly, the dry matter digestibility did not differ during control and WSF periods but seemed more variable (67.2 ± 1.6 and 67.8 ± 5.4%, respectively). If caecotrophe production is included in the DM digestibility calculation then coefficient fell by 10.5 units (57.3% on average). Furthermore within WSF periods, caecotrophe excretion was relatively variable compared to hard faeces. For instance, daily caecotrophe production could vary from 10 to 26 g DM.

The caecotrophy was centred between 10:00 and 16:00, when there was no hard faeces excretion in any of the rabbits (Figure 1). The duration of the caecotrophy was meanly 9 h with little inter-individual variability (± 0.5 h), while the starting time could range from 6:00 to 8:00 a.m. Nevertheless, on 7 of 39 occasions (number of 24-h cycle controlled for the two WSF periods), another short caecotrophy period occurred between 1:00 and 4:00, characterised by a low quantity of excreted caecotrophes (1 to 3 g DM). In one animal, this double caecotrophy period was not transitory, as it was repeated 3 times over the three day observation period.

In certain rabbits, we observed a stabilisation or even a short increase in marker concentration just after the caecotrophy period, because of recycling of marker in digestive system associated with the soft faeces intake (Figures 2 and 3). As marker

![Figure 1: Circadian faecal excretion pattern in 7 weeks old rabbits, not allowed to consume their caecotrophes.](image-url)
dosing was performed during the caecotrophy period (11:00, see Figure 1), the ascending phase of the kinetic was generally lacking in the control period and appeared more clearly (although very short) in the WSF period (Figure 4).

**Figure 2**: Kinetics of faecal marker concentration versus time, in rabbits allowed to practice caecotrophy: observed values and single non-linear adjustment according to a one compartment model.

**Figure 3**: Kinetics of faecal marker concentration versus time in rabbits allowed to practice caecotrophy: observed values and double non-linear adjustment according to one compartment models.
CAECOTROPHY AND TRANSIT IN THE RABBIT

Rate of passage parameters and impact of caecotrophes intake

The effect of the period of measurement was analysed on the same five rabbits having successful transit measurements for the two consecutive WSF periods. Whatever the criteria of transit (MRT, TTm, CRlp,…) the effect of the period was

<table>
<thead>
<tr>
<th>Table 2: Effect of the period of measurements on transit of rabbits not allowed to consume their caecotrophes (WSF periods).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period WSF1 (n=5)</td>
</tr>
<tr>
<td>Live weight¹, g</td>
</tr>
<tr>
<td>Feed intake¹, g/d/kg LW</td>
</tr>
<tr>
<td>MRT, h</td>
</tr>
<tr>
<td>Caecal retention time, h</td>
</tr>
<tr>
<td>CRlp, h</td>
</tr>
<tr>
<td>CRfp, h</td>
</tr>
</tbody>
</table>

¹mean values during rate of passage measurements (4 days).

Figure 4: Example of faecal marker concentration versus time, in rabbit not allowed to consume its soft faeces (WSF periods).
not significant (Table 2), while the effect of the animal was always significant. Among the two periods, the feed intake remained similar and animals gained almost 200 g of weight, thus indicating that prevention of caecotrophes intake by wearing a collar for 3 days did not seem very stressful for these animals. No significant interactions were detected among the effect of the treatment and the effect of the animal, therefore results were presented only according to the effect of the treatment.

As growing rabbits were used, their live weight increased with age, even when animals were wearing a collar (period WSF). Consequently, rabbit weights were 200 g higher in WSF than in the control period (Table 3). However, the relative feed intake (g/kg of LW) was significantly reduced by 18% during the WSF period. This was probably the consequence of a moderate stress associated with collar wearing, and with the prevention of soft faeces intake. The effect of the intake variation among periods on transit criteria was taken into account in the statistical model, as it was integrated in the “animal” factor.

Table 3: Rate of passage measurements of solid phase for Control and caecotrophy prevented rabbits (WSF).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>WSF (n=9)</th>
<th>RMSE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight1, g</td>
<td>1910</td>
<td>2140</td>
<td>100</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Feed intake1, g/d/kg LW</td>
<td>64.5</td>
<td>52.3</td>
<td>5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean retention time, h</td>
<td>23.1</td>
<td>15.2</td>
<td>1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimal transit time2, h</td>
<td>5.7</td>
<td>2.9</td>
<td>0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caecal retention time, h</td>
<td>16.9</td>
<td>10.2</td>
<td>2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRlp3, h</td>
<td>12.4</td>
<td>9.5</td>
<td>2.3</td>
<td>0.035</td>
</tr>
<tr>
<td>CRfp3, h</td>
<td>23.9</td>
<td>12.4</td>
<td>2.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

RMSE: Root error mean square.
Values are least square means obtained from the bifactorial variance analysis (effect of treatment and effect of animal).
1Mean live weight and feed intake during rate of passage measurements (4 days).
2Minimal transit time: first appearance of marker in faeces.
3CRlp, CRfp: mean retention time in the caecum, for large particles (>0.3 mm) and fine particles (<0.3 mm) respectively.
The mean retention time (MRT) in the whole tract evolved from 23 h in control to 15 h (-34%) in the WSF period, while their minimal transit time was 50% shorter (Table 3). Similarly, in the caecum the mean retention time passed from 17 to 10 h (-40%) for rabbits not allowed to perform caecotrophy. Caecal retention of large particles was meanly of 12 h and was 9 h shorter than fine particles, during the control period. In contrast, CRlp was only 3 h shorter than CRfp when soft faeces intake was not allowed. Transit of large particles was thus significantly less affected (CRlp: -23%) than fine (CRfp: -48%) during the WSF period.

**DISCUSSION**

The mean caecotrophe production, using the “collar” method over a three day period, averaged 10% of the feed intake (DM basis). Literature reported slightly higher values of about 12-13%, but obtained with rabbits wearing the collar only 24 h (PROTO, 1968; FEKETE and BOKORI, 1985; GIDENNE and LEBAS, 1987). We effectively registered a decrease of caecotrophe excretion from day 1 to day 2 with collar (on average -20%) due to an adaptation delay of animals. For instance, GIDENNE and PONCET (1985) estimated with a “feed labelling” procedure (without collar) that caecotrophe production reached 15% of the intake. However, caecotrophy itself seemed slightly affected, as the duration of the caecotrophy period and the frequency of the double caecotrophy were similar to that reported by JILGE (1974). Moreover, the caecotrophy impact on DM digestibility was similar to previous studies (FRAGA and DE BLAS, 1977; LUICK et al., 1992).

The caecotrophy affected the marker kinetics in the faeces. The small increase of marker concentration during the descending phase was generally noticed even in collared rabbits. In fact, wearing a collar only prevented the rabbits from consuming their soft faeces, but the caecotrophy itself was not stopped (soft faeces production still continued). For instance, antiperistaltic movement in the proximal colon occurred during hard faeces production and thus fine labelled particles were moved back to the caecum. This led to an increase of the marker concentration in caecal digesta
that further constituted the soft faeces. We thus found that caecotrophy increased the caecal retention of fine particles more than for large particles. This was consistent with Jilge (1982) who reported a higher proportion of fine particles in soft than in hard faeces. With respect to the whole tract, the caecotrophy increased the retention time by 8 hours. A similar impact was also reported by Luick et al. (1992), using chromium mordanted particles and more fibrous diets. In contrast, a more restricted impact (+2 to +4 h) of caecotrophy on transit was reported by Fraga et al. (1984), but using particles coloured with fuchsin.

In conclusion, our study confirmed the key role of caecotrophy in the control of transit in the rabbit. Prevention of soft faeces intake led to a one third decrease of the digesta retention time in the alimentary tract of the rabbit, with a particular impact on fine particles.

REFERENCES


Perez J.M., Lebas F., Gidenne T., Maertens L., Xiccato G., Parigi-Bini R., Dalle


