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CAECAL CANNULATION IN FIVE WEEK OLD RABBIT. AN IN-VIVO STUDY OF THE CIRCADIAN VARIATIONS OF THE FERMENTATION PATTERN.

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ABSTRACT

A glass cannula and surgical procedure suitable for caecal cannulation of five week old rabbit was described. The main advantages of this cannula were resistance against rabbit damages, absence of caecal liquid leakage, simple surgical procedure, easier digesta outflow during collection, good tolerance by the animals for several months.

Caecal digesta collection were performed throughout a 24 hours period during 6 consecutive days (2 collections/day) in 6 week old rabbits receiving ad libitum a standard diet. Low level of VFA were measured during the light period (meanly 60 mM/L between 8.00 and 16.00h) which correspond to the caecotrophy phase (≈8.00 to 12.00h) and to the low feed intake period. At the same time the level of butyrate decreased, while level of propionate or those of ammonia remain steady throughout 24 h. Compare to adult rabbit, the caecal fermentation pattern in the post-weaned rabbit differed essentially by slighter VFA variations during a 24 hour cycle and by a lower level of butyrate and a higher level of acetate.

INTRODUCTION

In rabbit the fiber degrading bacterial activity take place mainly in the caecum (40% of the total volume of the digestive tract), and absorption of the end-products of fermentation (volatile fatty acids, ammonia..) from this organ has been reported (BARCROFT et al 1944, COOLS and JEUNIAUX 1961). Changes in the normal fermentation pattern were observed on subject affected by enteritis, especially in the young rabbit during the post-weaning phase. However, few studies have described the caecal fermentation in the young rabbit (MORISSE et al 1985). In addition, the caecal fermentation has been generally studied using material taken from the animal after slaughter, and extrapolated to the situation in-vivo. Thus, we have recently developped an improved technique of caecal cannulation in

adult rabbit using a glass cannula design, in order to obtain caecal material with a minimal disturbance in the normal fonctionning of the digestive tract (GIDENNE and BELLIER, 1992). However, differences in fermentation pattern were found between adult and post-weaned rabbit, then it is necessary to adapt a cannula to the young rabbit in order to perform in vivo studies of the bacterial activity at this particular physiological stage.

This paper describes a technique for caecal cannulation in the five week old rabbit, which allowed repeated sample collection. In order to check the effect of the collection time, we also investigate in vivo circadian variations of the fermentation pattern, because of the incidence of caecotrophy on the metabolism of the rabbit hindgut (VERNAY et al 1984, VERNAY 1987, VERNAY 1989).

MATERIALS AND METHODS

ANIMAL AND FEEDING.

A group of seven New Zealand White rabbits was constituted at weaning in order to perform cannulation of the caecum at 37 days old (mean live-weight 816 ± 134 g).

Animals were kept during all the experiment in metabolism cages and submitted to a 12h light (7.00 to 19.00h) 12h dark schedule. An experimental diet (table 1) was provided ad libitum throughout the experiment.

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

Ingredients (%)		Chemical composition (% dry matter)	
Wheat	37.4	Organic matter	90.8
Dehydrated lucerne meal	32.0	Crude protein	18.5
Wheat bran	10.2	Crude fiber	12.3
Wheat straw	6.1	Neutral detergent fiber	32.6
Soya bean meal	11.2		
Minerals and vitamins	3.1		

CANNULA DESIGN.

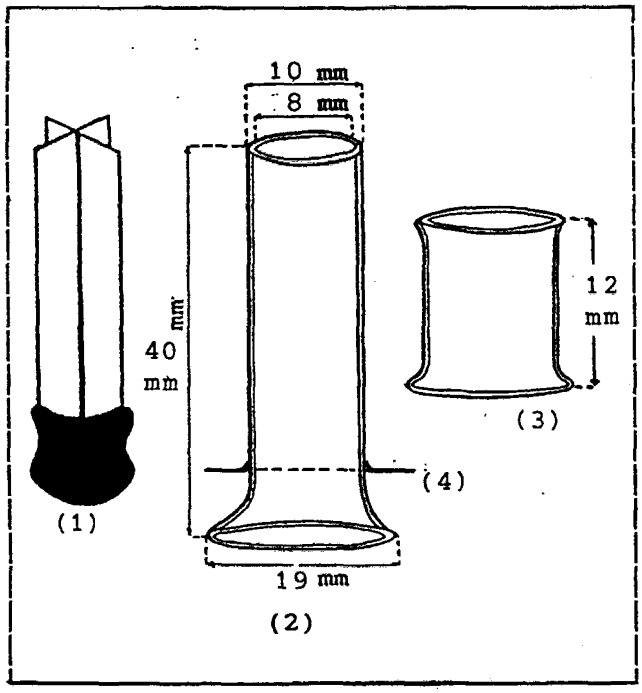
Previous work concerning ileal cannulation (GIDENNE et al.,1988) and caecal cannulation (GIDENNE and BELLIER 1992) in adult rabbit have demonstrated the advantage of glass cannula (Pyrex) compare to plastic or Teflon one: higher resistance against accidental damage by the rabbit, easier caecal digesta outflow during sampling and simplification of the surgical procedure.

The design of the cannula is similar to that employed for adult, except a reduction in size for the adaptation to five week old rabbit (fig 1).

SURGICAL PROCEDURE.

A special attention was taken for the anesthesia of the young rabbit, we used a coktail of xylazine and ketamine administrated via the marginal ear vain. Under full aseptic conditions, a laparotomy was realized for approximatevely 4 cm. First, a purse string suture was placed between the 4th and the 5th caecum whorl. After incision of the double serosa wall of the caecum, the base of the cannula was inserted, then the purse string suture was tightened and four single sutures between the outer ruff and the caecal wall were placed to maintain the cannula. Then the tubular part was brouht out through a stab incision on the right side of the abdomen, and the external protection piece (fig 1) was fitted on the cannula to avoid excessive movement and to improve the resistance of the tubular part. During surgery any obvious seepage of caecal material was carefully removed. Finally the abdominal muscle and skin incisions were closed separately after flooding the peritoneal cavity with a sulfamid solution. A further antibiotic prophylaxis was given on the day of surgery and for 2 days therafter.

Figure 1. Design of the caecal cannula for 5 week old rabbit.



(1). Plastic plug.

(2) Cannula shaped from a glass (Pyrex) tube.

(3) External piece (Pyrex) of protection fitted on the cannula.

(4) Outer ruff (silicon rubber subdermal material 0.5mm thick) pasted on the cannula

SAMPLE COLLECTION

Caecal material was collected via the cannula only seven days post-operatively. Rabbits were placed in a special hammock with an opening to admit the cannula. The caecal contents drained under gravity in PVC tubes containing a phosphoric acid solution and they were stored immediately at -18°c. A series of twelve collections (2 collections/day) was made for 6 consecutive days throughout a period of 24 hours (one collect each two hours). Total duration of a collection did not exceed 15 mn, the quantity of caecal material collected was about 5 gram fresh matter (less than 10% of the total caecal content).

ANALYSES

Dry matter was determined on caecal sample by heating at 105°c for 24 h, and ash was determined after incineration at 550°c for 5 h. The pH was taken immediately after the collection with a glass electrode pH meter. Volatile Fatty Acids (VFA) were measured by gas chromatography according to the method of JOUANY (1982) adapted to a semi-capillary column, and ammonia (NH_3) concentrations by the technique of WEATHERBURN (1967).

STATISTICS

Analysis of variance was conducted with GLM procedure of Statistical Analysis System (1985), with a two-way model : collection time effect and individual effect.

RESULTS AND DISCUSSION

POST-SURGICAL RECOVERY.

The cannula was installed in six rabbits weighing 816 ± 134 g at 37 d of age. The post-surgical live-weight loss was very low since all animals (except one) have returned their initial intake and have increased their weight by 64 ± 27g (n=5) four days after surgery. The rabbits showed no lesions around the cannula, and any digesta leakage was observed. It was possible to perform sample collection by only seven days post surgery, mean live-weight was then 965 ± 143 and daily waight gain (since surgery) was 21.3 g/d. In comparison with adult rabbit, post-surgical recovery time reach 10 d if using a glass cannula (GIDENNE and BELLIER 1992) and 30 d if using PVC cannula (CARMAN and WAYNFORTH 1984).

Repetitive digesta sampling were realised (two collections/day) during six consecutive days and daily feed consumption was 78.5 ± 12.8 g. Cannula remained functionnal for at least 3 months.

Table 2. Mean composition of caecal content collected in-vivo on five week old rabbits.

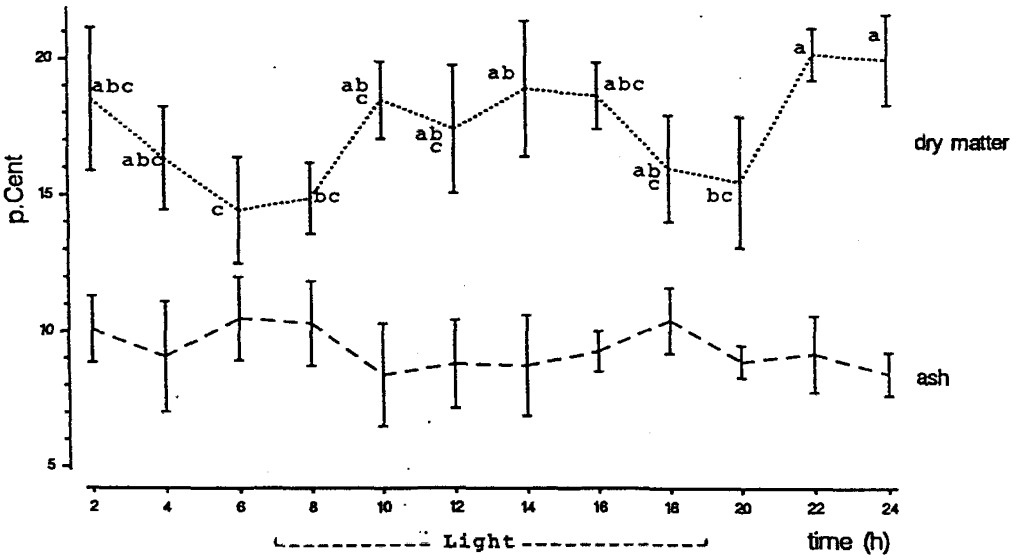
	DM (%)	Ash	pH	NH3 (mM)	VFA (mM)	C2 (%)	C3 (%)	C4 (%)	Cm (%)
General mean	17.6	9.3	6.0	7.6	65.4	83.2	5.4	9.9	1.4
S.E.M	1.9	1.2	0.2	2.2	8.2	2.6	1.2	2.1	0.5
Effect of:									
Sampling time	0.001	0.11	0.001	0.058	0.001	0.28	0.003	0.37	0.65
Individual	0.18	0.018	0.023	0.017	0.018	0.001	0.001	0.001	0.72

S.E.M: Residual standard error of the mean.
C2: acetate; C3: propionate; C4: butyrate;
Cm: valerate + isobutyrate + isovalerate.

CIRCADIAN VARIATIONS OF THE CAECAL FERMENTATION PATTERN IN THE SIX WEEK OLD RABBIT.

Contrary to the ash level, the dry matter level of caecal content changed significantly with time (table 2 and fig.2). During the light period dry matter level was high and it fall from 19% to 15% between 22.00 and 6.00h before the caecotrophy phase (6.00 to 12.00h); it correspond also to a decrease of the caecal volume before caecotrophy as showned by LENG and HÖRNICKE (1976). No such variations in caecal DM level were found in adult rabbit by LENG and HÖRNICKE (1975) or in growing rabbit by GIDENNE and LEBAS (1984).

Figure 2. Circadian changes of dry matter and ash content of the caecal digesta.

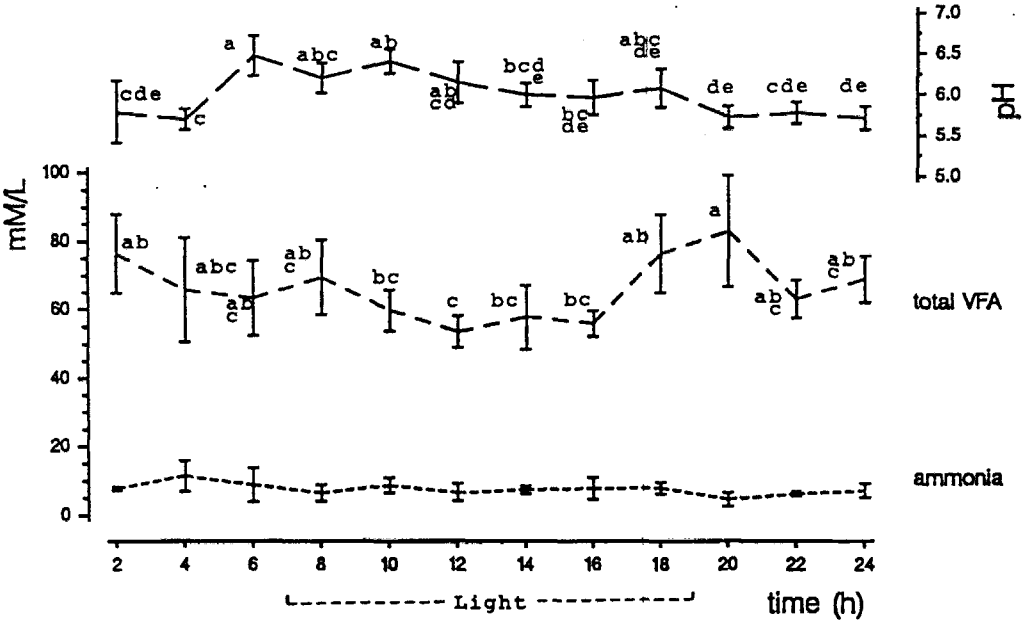


a,b: means affected with a different superscript differ significantly ($p<0.05$).

The VFA level of the caecal content were relatively low between 10.00 to 16.00h (fig.3), which correspond to caecotrophy and to a low feed intake period. Then VFA level rose from 60 to 80 mM/L between 16.00 to 20.00h. This increase in fermentation level would probably caused by two factors: increase in feed intake resulting in an increase of fermentable digesta flow in caecum, and to the antiperistaltic activity of the proximal colon inducing an enrichment in liquids and bacteria in the caecum. We noticed a decreased in VFA level between 20.00 and 22.00h, possibly corresponding to a second phase of caecotrophy as described in the young rabbit by LENG and HÖRNICKE (1975) and by LAPLACE (1978). In addition, our values indicated a lower VFA decrease during caecotrophy (-13%), than those obtained previously in adult rabbit(-20 to -30%) by PARKER and McMILLAN (1976) and by GIDENNE (1986). It could be suggested that the caecotrophy practice is not completely established in the 6 week-old rabbit, thus resulting in less circadian variations of the caecal fermentations.

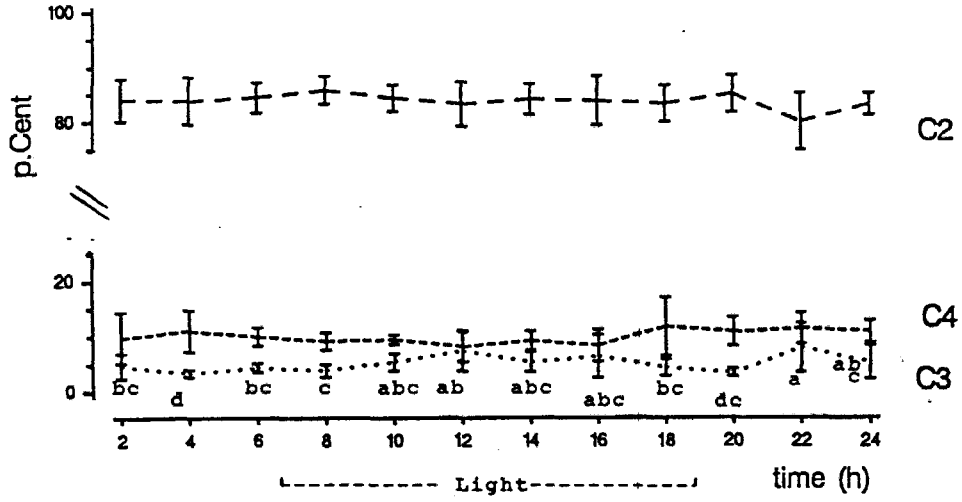
Ammonia level did not show significant circadian variations (fig 3) according to result obtained in growing rabbit (GIDENNE 1986). The pH of the caecal content increased sharply from 5.7 to 6.5 before the caecotrophy phase (4.00 to 6.00h), then it decreased slightly from 10.00 to 24.00h. Nevertheless, pH level was not correlated with ammonia or VFA level.

Figure 3. Circadian changes of pH, VFA and ammonia level in the caecum.



a,b; see figure 2.

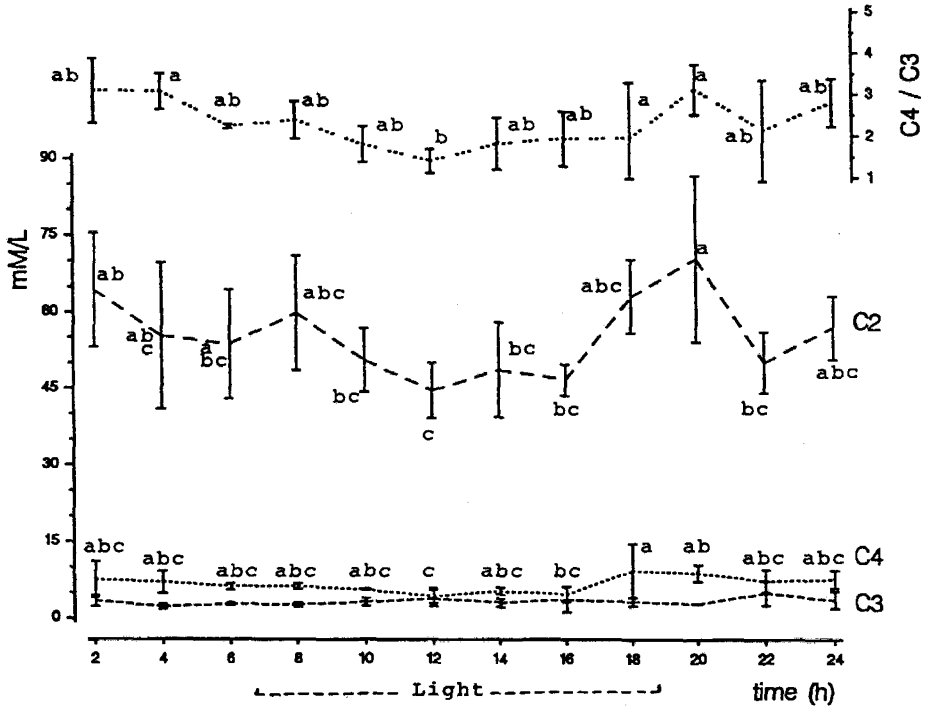
Figure 4. Circadian changes of the caecal VFA molar proportions.



a,b; see figure 2.

Any significant variation of acetate 'C2' proportions (meanly 82%) was observed according to the collection time (table 2 and fig 4), whereas the proportions of propionate 'C3'(meanly 5%) increased during the caecotrophy phase. Thus, when the level of fermentation decreased (8.00 to 12.00) the rate C4/C3 (fig.5) became lower (close to unity at 12.00h), the correlation between total VFA levels and the rate C4/C3 was highly significant ($p<0.004$, partial correlation coefficient=0.49). However in term of concentrations, C3 level did not vary significantly throughout a 24 h cycle, whereas level of butyrate 'C4' changed according to collection time (fig 5).

Figure 5. Circadian changes of the level of the different VFA and of the rate C4/C3.



C4/C3: rate of butyrate / propionate; a,b; see figure 2.

Eventually, during the low feed intake period (8.00 to 16.00) the level of the caecal fermentations decreased resulting essentially in a decrease of acetate and butyrate. This situation was also found in ad-libitum fed growing rabbit (GIDENNE 1986) and in adult rabbit on starvation (VERNAY and RAYNAUD 1975, SUSMEL and LANARI 1976). Circadian changes in C4 level could be related with his specific role as a preferential source of energy for the enterocyte (VERNAY et al 1984, VERNAY 1987).

The mean relative proportions (table 2) of the different VFA seemed to be different between adult and young rabbit. Caecal contents of 6 week old rabbits exhibited high percentage of C2 (83%) and a low one for C4 (10.0), compare to previous results obtained in adult rabbit (74 and 16% respectively for C2 and C4, PARKER and Mc MILLAN 1976) or in growing rabbit (78 and 15% resp. for C2 and C4, GIDENNE 1986).

In conclusion, the cannulation reported here was convenient to perform repetitive in vivo sampling of caecal material in a six week old rabbit. At this physiological stage the caecal flora would not already install, as the fermentation pattern were not similar to those observed in adult rabbit. This could be related also to an incomplete establishment of the caceotrophy practice in post-weaned rabbit. Further investigations are necessary to precise the regulation of caecal fermentation pattern, and more especially according to the nutritionnal status of the animal.

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