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# CAECAI MICROBIAL ACTIVITY OF THE YOUNG RABBIT : INCIDENCE OF A FIBRE DEFICIENCY AND OF FEED INTAKE LEVEL.

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## ABSTRACT

Caecal fermentative activity (CFA) and bacterial fibrolytic activity (BFA) was described before and after weaning (28d). Rabbits (6 litters of eight per diet) were fed a low fibre diet (P13, 9.2% ADF), or a standard fibre diet (16.5% ADF) containing a normal (P31) or excessive methionine level (P31M), to estimate the global fibre effect (P13 vs P31) or the fibre effect itself without change in intake level (P13 vs P31M). Feed intake (from 28 to 42d) was 13% lower for P31M than for P31 group, but was similar to P13 group. Between 21d and weaning, VFA level as well BFA doubled and then slightly increase until 42 days of age. A sharp reduction for propionate and inversely an increase in butyrate molar proportions were observed with age. Until weaning, VFA level were similar among the three diets, although it tended to be lower for P13. Then VFA level of P31 group increased slightly, while for P13 it tended to decrease (resp. 72 vs 40 mM at 42d.,  $P < 0.05$ ). Bacterial cellulolytic and xylanasic activity were similar among diets. After weaning, pectinasic activity was 10% higher in P31 than in P13 group. CFA of P31M group was intermediate between P31 and P13, suggesting that fibre effect partly depended of the intake level.

## INTRODUCTION

The main hypothesis currently acknowledged to explain the origin of non-specific enteritis (e.g. with low fibre diets) in the growing rabbit is an unbalance caecal flora activity, itself resulting of changes in bacterial population (GIDENNE, 1997). It is therefore essential to develop methodological approach for studying microbial activity, and particularly in the young rabbit (around weaning). Two methods were combined here to describe the microbial activity, before and after weaning : an analysis of fermentation products and of fibrolytic activity of caecal flora. On the other hand, incidence of non-specific enteritis is increased with low dietary fibre supply (LEBAS *et al.*, 1998). However, as rabbits are fed ad libitum, reducing the fibre supply also reduce the voluntary feed intake, since animals adjust their feed intake to the digestible energy level of the feed. Consequently, we aimed to evaluate the respective impact of the effect of fibre itself from that of intake level. A standard (P31, ADF=16.5%) and a fibre deficient level (P13, ADF=9.2%) diet were compared to estimate the global fibre effect (effect of intake and fibre itself). To modulate the voluntary feed intake we used the procedure of COLIN *et al.* (1973), consisting in an enrichment of methionine in "standard" diet (P31M) to obtain a reduction of the intake without change in fibre level.

## MATERIAL AND METHODS

### *Experimental design*

The dietary model (table1) combined two aims : a 40% reduction of the fibre level (NDF or ADF) without changes in the fibre quality (P31 vs P13), and a sharp increase in methionine level: 0.38 and 1.4% resp. for P31 and P31M. Water and feeds (pellets of 3.5 mm) were given ad libitum. Eighteen litters were equalised at birth to eight pups and were randomly attributed to each diet. From 18d of age until weaning (28d), pups were caged separately from their mother to control the milking and mainly to fed pups with the experimental diets, while the mothers were fed a commercial diet. To perform soft faeces collections before weaning, we must managed sufficient

space for animals equipped with a collar. Therefore at 21d of age, each litter was separated in two groups (5 and 3 pups) and caged separately (for milking the two groups of pups are simultaneously introduced in the female cage, at 9:00, for  $\approx$  5 minutes). Pups caged in group of three were used for soft faeces collection, while those caged in group of five were used for caecal content sampling. Rabbits were then caged individually from weaning until 44d. of age.

*Caecal content and soft faeces collection to evaluate microbial activity of the caecal flora.*

Caecal contents were obtained at 21, 28 and 42 days of age, after slaughtering by cervical dislocation, at the end of the caecotrophy period (about 11:00 h). Volatile fatty acids (VFA) were analysed on caecal digesta samples by gas liquid chromatography, on a semi capillary column.

The enzymatic fibrolytic activity of caecal bacteria (BFA) was determined on soft faeces since it

**Table 1: Ingredients and composition of experimental diets.**

Diets	P13	P31	P31M
Dehydrated Beet pulp	13.0	31.0	30.9
Dehydrated alfalfa	4.0	9.5	9.4
Wheat straw	4.0	9.5	9.4
Soya bean meal	18.0	17.2	17.1
Wheat	57.6	30.0	29.8
DL Methionine (99%)	0.1	0.2	1.2
Vitamins and minerals	3.3	2.6	2.2
<i>Chemical composition (% air dry basis)</i>			
Starch	37.4	19.6	17.6
Crude Protein (Nx6,25)	18.5	16.2	16.6
Methionine <sup>1</sup>	0.32	0.38	1.4
NDF	19.4	31.1	29.6
Lignocellulose (ADF)	9.2	16.5	15.8
Lignins (ADL)	1.8	2.4	2.3
Pectins (water insoluble) <sup>1</sup>	5.0	9.2	9.2

<sup>1</sup>: calculated values from tables of ingredients.

give a similar result compared to sample of caecal content (JEHL *et al.*, 1996). Measurements were performed at 25, 34 and 44d of age. A light plastic collar (50g, GIDENNE and LEBAS, 1987) was placed (from 17:00 until 12:00h) on each animal to prevent caecotrophy and thus allowed to collect soft faeces. Immediately after excretion a sample of soft faeces was collected and treated for bacterial enzyme extraction as described JEHL *et al.* (1996). Cellulolytic, xylanolytic and

pectinolytic activities were assayed respectively on carboxymethylcellulose (CMC), wood xylan or citrus pectin substrates, by measuring the amount of reducing sugars released, after incubation of 0.1ml of enzyme preparation and 1ml of substrate for 60' at 39°C.

*Statistical analysis*

Data were first statistically treated, using SAS software package, according to a two factorial design : effect of age - effect of diet and interaction. However, for most of the criteria, significant interaction occurred between the effect of age and the effect of the diet. Results were thus further subjected to two types of statistical analyses: within the same age (among the three diets) and within the same diet (among the age), and are presented in figure 1 to 4.

**RESULTS AND DISCUSSION**

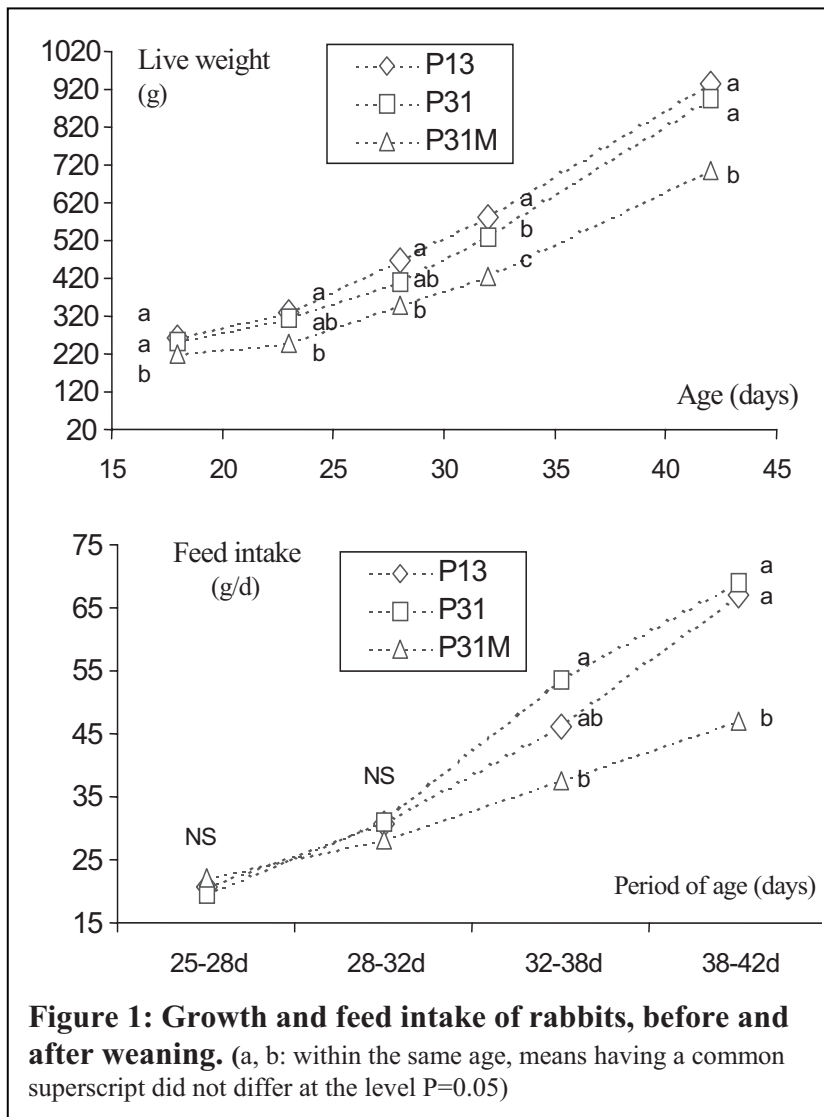
*Feed intake regulation and growth*

A 40% reduction of the ADF level (P31 vs P13) did not significantly affect zootechnical performances (fig. 1), as size of rabbits groups was here relatively low. However we noticed, before weaning a significantly higher ( $P < 0.01$ ) daily weight gain (DWG) for rabbits fed P13 compared to P31 or P31M (resp. DWG = 26.9a , 19.0b, 19.7b g/d on period 23-28d.of age, figure

1). Reversely after weaning, DWG was similar for P13 compared to P31 (mean DWG = 33.8 g/d on period 28-42d.), while DWG of rabbits fed P31M was reduced by 23% (DWG on 28-42d. = 26.0 g/d, figure 1). Feed intake of P31M group is significantly reduced after weaning. As previously observed by Colin *et al.* (1973) a two weeks delay is necessary to observe a reduction of a feed intake due to an excessive methionine. Expressed according to the live-weight, the feed intake was 13% lower for P31M than for P31 group (resp. 72.8 vs 84.2 g/kg LW), and did not differ from that of P13 group (71.0 g/kg LW). Thus, as expected, differences in microbial activity between P13 and P31M originated from a fibre effect itself (without effect of intake level).

*Microbial activity : methodology and effect of age.*

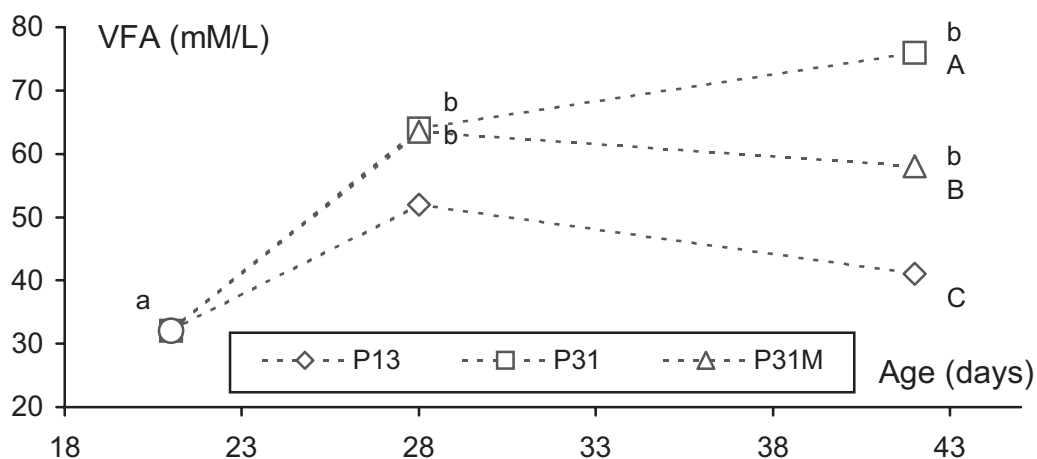
Two approaches were combined to describe the caecal microbial activity : an analysis of the caecal substrate (pH and VFA measurement) that is a consequence of the caecal fermentative



**Figure 1: Growth and feed intake of rabbits, before and after weaning.** (a, b: within the same age, means having a common superscript did not differ at the level P=0.05)

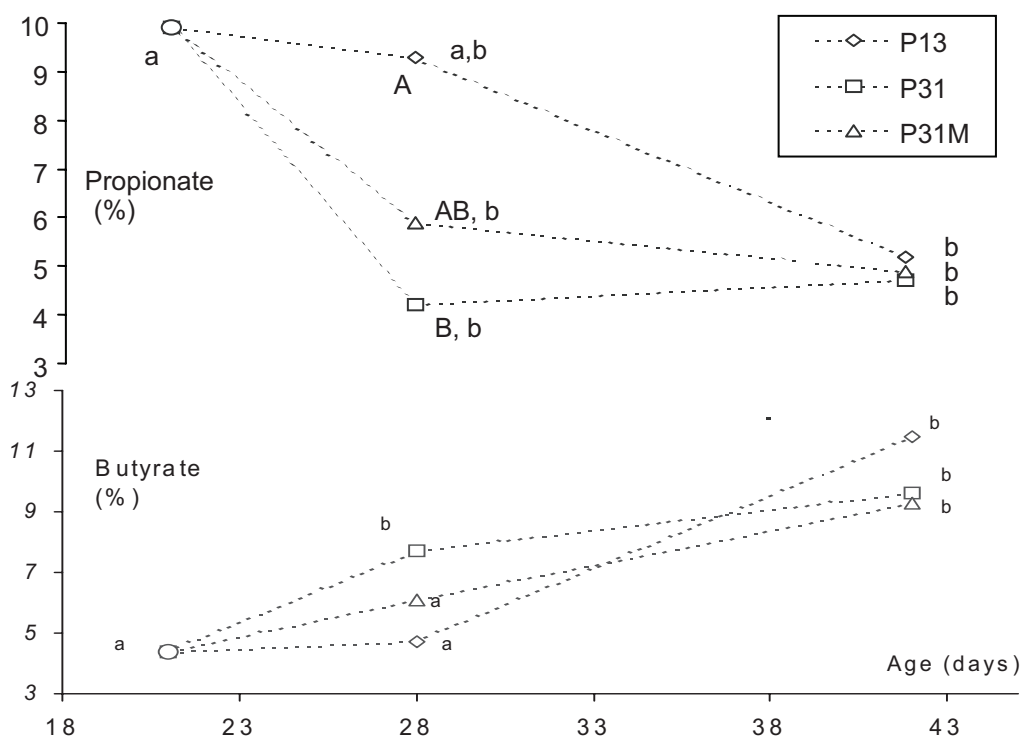
activity of bacteria (CFA), and a measurement of the bacterial fibrolytic activity that express the ability of the caecal flora to degrade a cell wall substrate. To date, these two methods were never combined to examine the microbial activity of the young rabbit, particularly before weaning.

At 21d of age, the young rabbit just begin to eat solid feed (SCAPINELLO *et al.*, 1999 ; DEBRAY *et al.*, 2000). Our results provided by the two approaches were reliable and indicated a low fermentative activity (VFA level = 30 mM/L, figure 2) associated to a low BFA (figure 4 and 5). Between 21d and weaning (28d) VFA level as well BFA doubled and only slightly increase until 42 days of age. Classical changes in molar proportions of VFA were also observed here between 21 and 28d.: sharp reduction for propionate and inversely an increase in butyrate molar proportions (figure 3), while acetate was not significantly affected. Such effects of age on CFA were already reported by PADILHA *et al.* (1995) and PIATTONI *et al.* (1995). Although not strictly comparable, because the substrates tested were different, the cellulosic activity (figure 5) appeared lower than the xylanasic one, itself half lower to pectinolytic one (JEHL *et al.*, 1996; MAROUNEK *et al.*, 1995). These results are also supported by microbiological enumeration reporting higher counts of pectinolytic and hemicellulolytic bacteria compare to cellulolytic ones (BOULAHROUF *et al.*, 1991).



**Figure 2: Total VFA level in the caecum, according to age and experimental diet.**

a, b: within the same diet, means having a common superscript did not differ at the level  $P=0.05$  (effect of age)  
 A, B: within the same age, means having a common superscript did not differ at the level  $P=0.05$  (effect of diet)



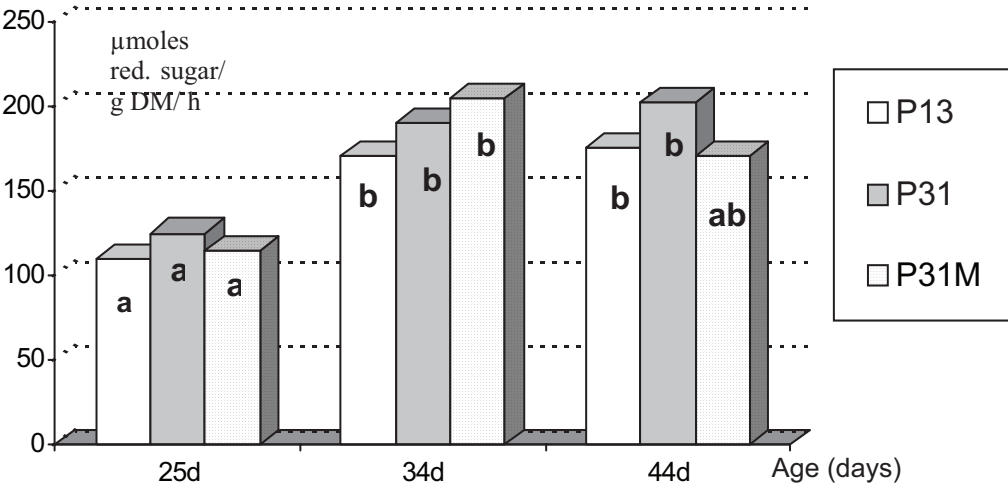
**Figure 3: Propionate and butyrate molar proportion (%) in the caecum, according to age and experimental diet.**

a, b; A, B see : figure 2.

*Effect of dietary treatment on microbial activity*

Significant interaction were detected between diet and age effect for CFA, and only for pectinolytic activity ( $P<0.01$ , figure 4). Until weaning, VFA level were similar among the three diets, although it tended to be lower for P13 compared to the two others. Then, caecal VFA level

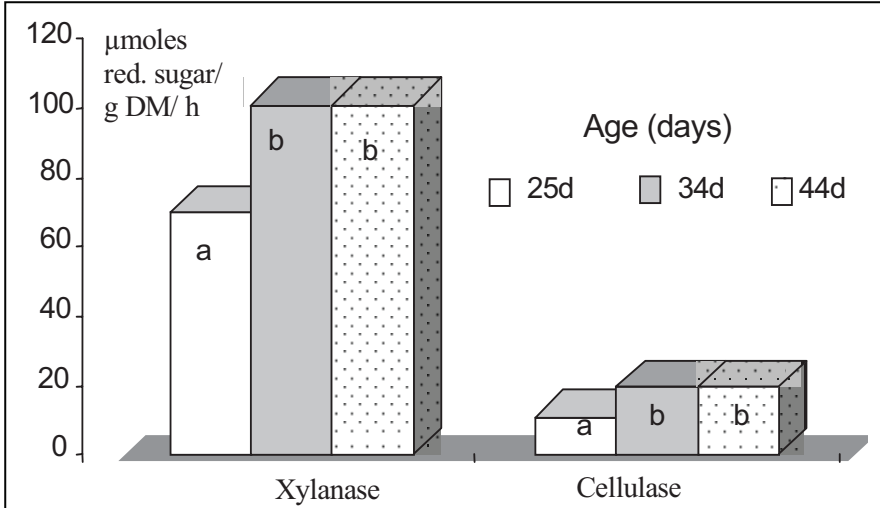
of rabbits fed P31 diets (standard fibre level) continue to increase slightly (72 mM at 42d.), while for P13 group (low fibre diet) VFA level tended to decrease (only 40 mM at 42d.). Similar reduction in CFA (at 6 weeks of age) according to a fibre level reduction were recently obtained by GIDENNE *et al.* (1999), with feeds having a lower content in digestible fibre (hemicellulose and pectins). However at ten weeks of age, the CFA did not differ among diet, suggesting an adaptation to the fibre level. Reversely, BELLIER and GIDENNE (1996) did not mentioned an effect of the fibre level reduction on CFA, but for diets having a lower level in digestible fibre than here.



**Figure 4 : Pectinase activity of bacteria according to age and diet.**

The mean effect of diet is significant (P=0.03) between P13 and P31 (means of 34 and 44d).  
 a, b: within the same diet, means (among ages) having a common superscript did not differ at the level P=0.05

The VFA molar proportions were strongly affected by dietary treatment (figure 3): at weaning, a sharp decrease in propionate and an increase in butyrate were already observed for P31 group, while fermentation pattern of fibre deficient group remain unchanged between 21 and 28d of age.



**Figure 5: Xylanase and cellulase activity of bacteria according to age of rabbits.** (a, b: means (among ages) having a common superscript did not differ at the level P=0.05)

Bacterial cellulolytic and xylanase activity were not significantly affected by dietary treatment (figure 6). In return, when analysing data of BFA (figure 4) without the group P31M, we observed a significantly (P=0.03) higher (+10%) pectinase activity for rabbits fed diet standard diet (P31) compared to

low fibre diet (resp. 197 vs 171 µmoles red. sug/g DM/h; means of 34 and 44d of age). Thus, the dietary fibre intake, even in our low fibre diet seemed not a limiting factor able to affect cellulase or xylanase activity. However, it could modulate the pectinolytic activity, which might be mainly

responsible of the variations of CFA. Besides, GIDENNE and JEHL (1999) reported a higher mortality for growing rabbits fed the same low fibre diet compared to the standard one, and this could be linked with the reduction in CMA observed here.

Rabbits fed P31M diet exhibited a fermentation pattern intermediate between the standard and low fibre diet, which was more particularly clear at weaning on butyrate and propionate molar proportions (figure 3). This highlighted the importance of the intake regulation on microbial activity and potentially on health status.

**In conclusion**, a fibre deficiency inhibited the development (quantity and quality) of the microbial activity in the young rabbit. The intermediate response of the microbial activity face to a lower feed intake, but without change in fibre level, suggested that the global effect of fibres is expressed partly (about half) through a modulation of the feed intake. Consequently, the intake regulation of the young rabbit must be more fully known in order to control microbial activity development and therefore health status.

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