CAECAL MICROFLORA AND FERMENTATION PATTERN IN EXCLUSIVELY MILK-FED YOUNG RABBITS

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Abstract - Thirty two 22- to 42-day-old SPF New Zealand White rabbits of both sexes were used and sequentially slaughtered, 8 per day, at 22, 29, 35 and 42 days of age. Data were treated by variance analysis. Rabbits given only a milky diet up to 42 days of age exhibited a caecal fermentation pattern turned towards a proteolytic metabolic activity (high levels in NH3 and in branched-chain fatty acids and valeric acid: 17 mmol/l and 1 mmol/l, on average, respectively). The absence of cellulolytic microflora and the low concentration of the total volatile fatty acids (tVFA) could be explained by the lack of substrate that would have been brought by a solid feed. From day 29 onwards, the low tVFA (12.5 mmol/l) and high NH3 concentrations (16.5 mmol/l) explained the high pH value (6.8 on average). This may be considered as a pathological value for weaned rabbit but in our case no clinical sign of diarrhoea was observed. The evolution of the colibacilli flora according to age was similar to that usually described and thus was not correlated with pH, tVFA or cellulolytic flora. The evolution of the colibacilli population (from 10^7 bact/g on day 22 to 10^3 on day 42) seemed dependant on ontogenic factors rather than on nutritional ones.

INTRODUCTION

Intestinal pathology is the main cause of mortality in the growing rabbit reaching 12% in France (KOEHL, 1995). It could be hypothesised that the caecal microbial activity (CMA) play an important role in the development of enteritis after weaning. Little is known about the caecal fermentation related to the microflora in the young rabbit around the weaning period, except a recent study of PADILHA *et al.* (1995) performed on SPF (specified pathogen free) rabbits given a commercial diet. However, it is of interest to elicit the effect of milking from dry feed intake on the establishment of CMA in the pre-weaned rabbit. Thus our work aimed to described the incidence of an extended exclusive milking on the development of the caecal microflora (colibacilli, anaerobic total flora, cellulolytic and amylolytic flora) and of fermentation pattern.

MATERIAL AND METHODS

Animals and housing

New Zealand White rabbit (INRA strain A 1077) of both sexes, housed in wire cages under 14 hours of light (from 6 a.m. to 8 p.m.) and at a room temperature of 18 ± 0.5 °C, were used in this study. They came from the SPF breeding colony of the Station de Pathologie Aviaire et de Parasitologie, INRA, Tours, France (COUDERT *et al.*, 1988).

Four litters of 8 rabbits each were used. From the birth to the 29th day of age, the animals were milked once a day (8h30) by their own mothers. In order to prevent an insufficient production of milk which occurs at the end of lactation (LEBAS, 1972), supplementary lactating does were used from 22nd day onwards. The second doe was at the same stage of lactation (itslitter was weaned at day 22) as the natural mother and was used as a foster-mother (suckling of the youngs at 16h30). Between days 29 and 36, the natural mother practically dried up, was replaced by a third doe (8th day of lactation and litter discarded). The young rabbits continued to be suckled twice a day (at 8h30 with the third doe and at 16h30 with the second one till the 42nd day of age. To maintain an optimum milk production, the suckling does were not pregnant during this time.

The diet composition received by the does and the milk composition were the same as those previously mentioned by PADILHA *et al.* (1995), *i.e*: 13.5% starch, 17.6% crude protein and 21.5% ADF for the pelleted feed, and 33% and 43% crude protein for the milk, determined at 22 and 29 days of lactation, respectively. Water was available *ad libitum*.

Measured parameters

Two rabbits per litter were slaughtered at 22, 29, 36 and 42 days of age.
All the measures (zootechnical, biochemical and microbiological) and the methods used were those described by PADILHA *et al.* (1995). Briefly, VFA determination was assessed by gas chromatography (SPILLER *et al.*, 1980) and that of NH_3 by colorimetry (VERDOW *et al.*, 1977). Colibacilli flora was enumerated on Drigalski agarose and anaerobic flora were analysed under strictly anaerobic conditions, in « roll tubes », according to the method of Hungate (1966).

Statistical analysis

Data were treated by variance analysis with comparison of means by Tukey's test using the SYSTAT program (version 5.04, 1994).

RESULTS AND DISCUSSION

Zootechnical traits

The live weight increased by 36% ($P<0.05$) between days 22 and 29 (Table 1), but this is markedly less than that observed in standard conditions (+67%) (LEBAS, 1972; PADILHA *et al.*, 1995) when rabbits are milked and also have a dry feed *ad libitum*. Consequently, a slight (not significant) increase in daily weight gain (DWG) was observed (+9%), compared to an increase of 27% for standard conditions (same period in the work of PADILHA *et al.*, 1995).

Table 1 : Evolution of zootechnical traits (body weight (BW), daily weight gain (DWG) and milk intake (MI)) and caecal weight traits (weight of the caecal wall (CW), caecal content (CC), percentage of the weight of the caecal wall compared to the body weight (CW/BW) and caecal dry matter (CDM)) in young rabbits given only milk.
(Data are mean (n=8) ± standard errors of the mean (SEM) in parentheses. Mean values in a same row with common superscripts were not significantly different (P<0.05)

Trait	*Age (d)*							
	22		*29*		*36*		*42*	
Zootechnical traits								
BW (g)	397^{a}	(12.1)	541^{b}	(21.5)	530^{b}	(33.4)	563^{b}	(31.3)
DWG (g/d)	15.7^{a}	(0.88)	22.5^{a}	(3.07)	-0.9^{b}	(2.06)	6.2^{b}	(3.38)
MI	32.8^{a}	(2.75)	16.8^{b}	(4.26)	8.5^{b}	(4.76)	18.4^{ab}	(3.72)
Caecal weight traits								
CW (g)	3.3^{a}	(0.24)	6.9^{b}	(0.42)	8.5^{bc}	(0.41)	9.3^{c}	(0.53)
CC (g)	4.2^{a}	(0.60)	18.0^{b}	(1.16)	21.9^{bc}	(2.89)	27.7^{c}	(3.40)
CW/BW (%)	0.86^{a}	(0.08)	1.29^{b}	(0.08)	1.63^{bc}	(0.09)	1.70^{bc}	(0.17)
CDM (%)	21.7^{a}	(1.41)	26.2^{ab}	(0.70)	27.2^{ab}	(1.76)	31.2^{b}	(2.78)

Milk consumption on days 22 and 29 (Table 1) was close to the results of PADILHA *et al.*, 1995, including a reduction by about 50% ($P<0.05$) of milk intake between the 22nd and 29th days. Milking from a supplementary doe did not increase the amount of milk consumed by the youngs. In fact, it could be observed on day 29 (one week after the establishment of the second suckling at 16h30), that the rabbits ingested at 16h30 about one third of the daily milk consumption without an increase of global milk intake, meaning that milk consumption at 8h30 was reduced.
On days 36 and 42, the consumption of milk did not vary significantly compared to that recorded on day 29. On day 36 the milk intake was particularly low (mean = 9g per animal). This could be attributed to the third doe, badly accepted by the females themselves and/or by the youngs. As a result, a significant decrease of the DWG from 22.5 to -0.9g/d was noted between days 22 and 29.

Caecal weight traits

All parameters significantly increased ($P<0.05$) between day 22 and day 42 (Table 1).
Between days 29 and 42, a significant increase of the caecal content and of the caecal wall was observed but on day 42 the mean weight of the empty caecum represented only 55% of that measured in standard conditions (PADILHA *et al.*, 1995). Nevertheless the relative weight (CW/BW %) of the caecal wall was superior (1.7 %) to that observed in weaned animals (1.5% on average) (LEBAS and LAPLACE, 1972;PADILHA *et al.*, 1995). On day 42, the caecal content weight showed a two fold reduction compared to weaned rabbits (PADILHA *et al.*, 1995). On the contrary, the caecal dry matter level was 6 points higher, corresponding to a pasty and compact content. This suggests an accumulation of milky matter and a stasis in the caecum. Although it was not controlled, the lack of solid feed may have disturbed the caecotrophy or even prevented the installation of the caecotrophy, thus impairing the normal stemming of liquids towards the caecum as described previously (BJÖRNHAG, 1972; HÖRNICKE and BJÖRNHAG, 1980; EHRLEIN *et al.*, 1983). It could be also hypothesised that the drying of the caecal content was related to a change in the intestinal transit. The amount of substrate entering the caecum could be low and thus may induce an increase of the retention time in the caecum associated to a reduction of the colic motility and then a reduction of the turnover rate of the caecal content.

Caecal fermentation pattern

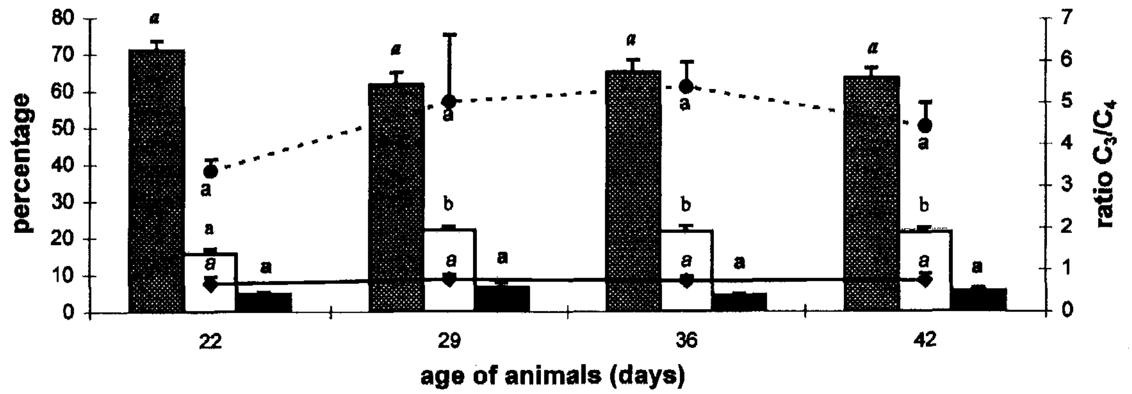
The concentration of NH3 significantly increased ($P<0.05$) between days 22 and 29 and then remain steady up to day 42 (16.5 mmol/l on average) (Table 2). Ammonia level was similar to that reported by PIATTONI *et al.* (1995) but was two times higher than that found by PADILHA *et al.* (1995).

Table 2 : Evolution of the caecal pH and fermentary traits (ammonia (NH3), acetate (C2), propionate (C3), butyrate (C4), minor volatile fatty acid (mVFA, i.e. branched-chain fatty acid and valeric acid) and total volatile fatty acids (tVFA)) in young rabbits given only milk. *(Data are mean (n=8) ± standard errors of the mean (SEM) in parentheses. Mean values in a same row with common superscripts were not significantly different (P<0.05).*

	Age (d)							
	22		*29*		*36*		*42*	
pH	6.93^{a}	(0.12)	6.74^{a}	(0.07)	6.68^{a}	(0.05)	6.90^{a}	(0.03)
NH_3 (mmol/l)	10.3^{a}	(0.72)	16.0^{b}	(1.73	16.7^{b}	(1.15)	16.9^{b}	(1.63)
C_2 (mmol/l)	11.0^{a}	(1.99)	8.92^{ab}	(1.44)	5.92^{b}	(0.56)	7.36^{ab}	(0.93)
C_3 (mmol/l)	2.45^{a}	(0.41)	3.14^{a}	(0.42)	2.04^{a}	(0.32)	2.46^{a}	(0.29)
C_4 (mmol/l)	0.75^{a}	(0.12)	0.86^{a}	(0.18)	0.43^{a}	(0.10)	0.64^{a}	(0.11)
mVFA (mmol/l)	0.96^{a}	(0.14)	1.16^{a}	(0.13)	0.78^{a}	(0.16)	0.96^{a}	(0.20)
tVFA (mmol/l)	15.1^{a}	(2.46)	14.1^{a}	(1.87)	9.16^{a}	(0.97)	11.4^{a}	(1.26)

The total VFA concentration (tVFA) remained at a very low level 12.5 mmol/l throughout the experiment (Table 2). This value was smaller than those mentionned by PADILHA *et al.* (1995) (30-35 mmol/l) using SPF (but weaned) rabbits or those observed in conventional weaned rabbits (50-80 mmol/l) (PROHAZSKA, 1980; MORISSE *et al.*, 1990; BELLIER *et al.*, 1995, PIATTONI *et al.*, 1995). Nevertheless it could be noted a 35% lower tVFA concentration on day 36 compared to that of day 22. This could be explained by a significant reduction by 50% of the acetate concentration ($p<0.05$) between days 22 and 36, while propionate and butyrate concentration remained steady. The lower fermantative activity observed at 36 days of age, could be of course related to the very low milk intake at this period. The concentrations of the branched-chain fatty acids (isobutyric and isovaleric acid) and valeric acid did (mVFA) were not affected by sampling age (1 mmol/l on average). This value is 60% higher than previous data noticed in 42-day-old SPF rabbits (PADILHA *et al.*, 1995).The fermentary pattern was strongly different from that observed by PADILHA *et al.* (1995) as only slight changes were recorded between 29 and 42 days (figure 1). The molar proportion of acetate did not exceed 65% of the tVFA, instead of 78 to 85% in normaly fed rabbits. That of butyrate was lower than 6%, while it is usually close to 8 to 10% (BELLIER *et al.*, 1995). The molar proportion of propionate increased significantly between 22 and 29 days and reached then 22%, while in standard conditions the propionate molar proportion does not exceed 10%. In terms of molar proportion the sum of branched-chain fatty acids and valeric acid was 4 to 5 times higher than that observed in normally fed rabbits.

Figure 1 : Evolution of the molar proportion of the different volatil fatty acids (acetic (■), propionic (□), butyric (■) and minor volatil fatty acids (mVFA) (_◆_)) and of the ratio C3/C4 (-●-) in the caecum of young rabbits given only milk. *(Data are mean (n=8) ± standard errors of the mean (SEM) in parentheses. Mean values with common superscripts were not significantly different (P<0.05)).*



No clinical sign of diarrhoea was observed here, in spite of decreased weight gain by the animals and although their fermentation pattern (including C3/C4 ratio up to 3.5) was close to that observed in sick rabbits (MORISSE *et al.*, 1985; GIDENNE and JEHL, 1994). These traits seemed relevant to a proteolytic metabolic activity. The high levels in NH3, propionate and branched-chain fatty acids and valeric acid could be the result of fermentative process of proteic residues reaching the caecum (GOODLAD and MATHERS, 1990; MORTENSEN *et al.*, 1990).

Microbiological traits

The anaerobic cellulolytic microflora was absent or at least inferior to the threshold of our method (Table 3). Total and amylolytic anaerobic flora were at a high but normal level (10^{10}-10^{11} bacteria per gram of caecal content), without any noticeable variation according to the age of animals. These results are similar to those previously obtained in rabbit given solid food (PADILHA *et al.*, 1995).
The colibacilli flora significantly fell from 10^7 to 10^4 bacteria/g of caecal content (p<0.05) between day 22 and day 29 (Table 3). This reduction tended to continue down to 10^3-10^2 on average, on day 42, as previously reported in healthy weaned rabbits (SMITH, 1965; MORISSE *et al.*, 1985; LICOIS *et al.*, 1992). Therefore, the colibacilli flora is the only parameter that shows a normal development, which should be independent of the milky diet imposed on the animals. This should mean that the colibacilli flora would rather vary according to the age of animals than with regard to alimentary conditions.

Table 3. Evolution of the caecal colibacilli flora (Coli), total anaerobic microflora (tMF), amyloytic microflora (Amyl) and cellulolytic microflora (Cell) (in log10 of bacteria/g of caecal content) in young rabbits given only milk. *(Data are mean (n=8) ± standard errors of the mean (SEM) in parentheses. Mean values in a same row with common superscripts were not significantly different (P<0.05). *ND: non detectable flora*

	Age (d)							
	22		*29*		*36*		*42*	
Coli	7.30^a	(0.80)	4.56^b	(0.68)	3.79^b	(0.70)	3.46^b	(0.39)
tMF	10.81^a	(0.06)	10.74^a	(0.02)	10.64^a	(0.06)	10.58^a	(0.08)
Amyl	10.41^a	(0.12)	9.92^a	(0.09)	10.31^a	(0.03)	10.08^a	(0.07)
Cell	ND*		ND		ND		ND	

In conclusion, this study clearly showed that there was no relationship between the level of the colibacilli flora and the pH or the tVFA concentration, while MORISSE *et al.* (1985), in an *in vivo* study and PROHAZSKA (1980), in an *in vitro* study mentionned a correlation between these parameters.
An extended milking reproduced a microbial activity close to that found in case of diarrhoea but without any symptom of digestive troubles. Our results also demonstrated that the intake of solid feed is the major factor responsible of the establishment of the fibrolytic flora and of the caecal fermentation pattern, without relation with *E.coli* flora. It seemed thus of interest to stimulate as soon as possible the feed intake of the young rabbit before the weaning, in order to promote the establishment of the symbiotic flora.

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Etude de la microflore et de l'activité fermentaire caecale chez le lapereau nourri exclusivement avec un régime lacté - Trente deux lapins Néo Zélandais, SPF ont été utilisés et abattus à raison de 8 animaux par jour à 22, 29, 35 et 42 jours d'âge. Le maintien d'une alimentation uniquement lactée jusqu'à 42 jours a conduit à un profil fermentaire orienté vers une activité métabolique protéolytique (teneur élevée en NH3 et en AGV mineurs : respectivement 17 mmol/l et 1 mmol/l de contenu caecal, en moyenne). L'absence de flore cellulolytique et la concentration très faible en acides gras volatils totaux (tAGV) s'expliquent par l'absence de substrat qui aurait pu être apporté par l'aliment solide. A partirdu 29ème jour, les teneurs faibles en AGVt (12.5 mmol/l) et fortes en NH_3 (16.5 mmol/l) expliquent une valeur élevée et stable du pH, à 6,8 en moyenne. Ceci peut est considérée comme une valeur pathologique chez le lapin sevré mais dans notre cas aucun signe clinique de diarrhée n'a été observé. L'évolution de la flore colibacillaire en fonction de l'âge (de10^7 à 10^3 bact/g en moyenne, entre 22 et 42 jours), a été similaire à celle qui est classiquement décrite. La corrélation positive entre le pH cæcal et les *E.coli* mentionnées par certains auteurs n'est par conséquent pas retrouvée ici. L'évolution de la population colibacillaire semble liée à l'âge et non directement dépendante de l'alimentation solide. Cette expérience démontre donc que le niveau de colibacilles dans le cæcum n'est pas systématiquement lié au pH, ni à la teneur en NH_3, ni à la présence d'une flore cellulolytique mesurable.