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Reprod. Nutr. Dev. 39 (1999) 223-230

Original article

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(Received 26 August 1998; accepted 27 January 1999)

Abstract — Thirty-two SPF New Zealand White rabbits of both sexes were used in this experiment. They were sequentially slaughtered at 22, 29, 35 and 42 days of age. The rabbits were only milked by their mother and by foster mothers (no access to solid food) throughout the experiment. They exhibited a caecal fermentation pattern that turned towards a proteolytic metabolic activity (high levels in NH_3 and in branched-chain fatty acids and valeric acid: 17 mmol·L^{-1} and 1 mmol·L^{-1}, on average, respectively). The absence of caecal 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^{-1}) and high NH_3 concentrations (16.5 mmol·L^{-1}) explained the high pH value (6.8 on average). This could be considered as a pathological value in weaned rabbits, but in our case no clinical signs of diarrhoea were 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 dependent on ontogenic factors rather than on the composition of the caecal media (pH, etc.). © Inra/Elsevier, Paris.

rabbit / milky diet / caecal microflora / caecal fermentation / pH

Résumé — Étude de la microflore et de l'activité fermentaire cæcale chez le lapereau nourri exclusivement avec un régime lacté. Trente-deux lapins Néo-Zélandais, SPF ont été utilisés et abattus à 22, 29, 35 et 42 j d'âge. Le maintien d'une alimentation uniquement lactée jusqu'à 42 j, fournie par leur mère et par une femelle nourricière, a conduit à un profil fermentaire orienté vers une activité métabolique protéolytique (teneur élevée en NH_3 et en AGV mineurs : respectivement 17 mmol·L^{-1} et 1 mmol·L^{-1} de contenu cæcal, en moyenne). L'absence de flore cellulolytique et la

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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. À partir du 29e jour, les teneurs faibles en AGVt (12,5 mmol·L^{-1}) et fortes en NH_3 (16,5 mmol·L^{-1}) expliquent une valeur élevée et stable du pH, à 6,8 en moyenne. Ceci peut est considéré 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 (de 10^7 à 10^3 bact·g^{-1} en moyenne, entre 22 et 42 j), a été similaire à celle qui est classiquement décrite. La corrélation positive entre le pH cæcal et les *Escherichia coli* mentionnées par certains auteurs n'est par conséquent pas retrouvée ici. De même, nos résultats n'indiquent pas de corrélation significative entre le niveau de colibacilles dans le cæcum et la teneur en NH_3 cæcal, ou avec la présence d'une flore cellulolytique mesurable. L'évolution de la population colibacillaire semble donc plutôt dépendante de facteurs ontogéniques et non pas de facteurs dépendant de l'alimentation solide.

lapin / alimentation lactée / microflore cæcale / fermentation cæcale / pH

1. INTRODUCTION

Intestinal pathology is the main cause of mortality in the growing rabbit and reaches levels of 12 % in France [12]. It could be hypothesized that the caecal microbial activity level (CMA) plays an important role in the development of enteritis after weaning [6]. Few studies, however, have been carried out on the flora colonization of the digestive tract of rabbits between birth and weaning [9, 21]. Only one study has focused on caecal fermentation and its relationship to microflora in SPF (specified pathogen free) rabbits during the weaning period [18]. In standard breeding conditions, before weaning (28–35 days of age), young rabbits are exclusively suckled by their mothers until 18–20 days and then they begin to consume solid pelleted food. Therefore, it was of interest to establish the respective effects of milk versus dry feed intake on the establishment of CMA in the pre-weaned rabbit, and thus to evaluate the impact of ontogenic factors. For this purpose, we have used an experimental model based on rabbits fed exclusively with milk and therefore excluding the influence of solid feed intake on CMA. The aim of our work was to describe the effect of extending the milk-based diet period on the development of caecal microflora (colibacilli, anaerobic total flora, cellulolytic and amylolytic flora) and of the fermentation pattern. The results are compared to those obtained in the same type of rabbit fed with solid feed, described previously by Padilha et al. [18].

2. MATERIALS AND METHODS

2.1. Animals and housing

New Zealand White rabbits (Inra strain A 1077) of both sexes, housed in wire cages with 14 h of light (from 06h00 to 20h00) 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 [5].

Four litters of eight rabbits each were used. From birth to day 29, the animals were fed milk once a day (08h30) by their own mothers. In order to prevent an insufficient production of milk, a phenomenon which occurs at the end of lactation [13], supplementary lactating does were used from the 22nd day onwards. A second doe, which was at the same stage of lactation (its litter was weaned at day 22) as the natural mother, was used as a foster-mother (suckling young at 16h30). Between days 29 and 36, the natural mother became practically dried up. She was replaced by a third doe (8th day of lactation and litter discarded). The young rabbits continued to be suckled twice a day (at 08h30 with the third doe and at 16h30 with the second one) until day 42. To maintain an optimum milk production, the suckling does were not pregnant during this time.

The diet of the does and their milk composition were the same as those previously mentioned by Padilha et al. [18], i.e. 13.5 % starch, 17.6 % crude protein and 21.5 % ADF (acid detergent fibre) for the pelleted feed, and 33 and 43 % crude protein for the milk, determined according to the method described by Amariglio [1], at 22 and 29 days of lactation, respectively. Water was available ad libitum.

2.2. Measured parameters

Two rabbits per litter were slaughtered at 22, 29, 36 and 42 days of age. All the measurements (zootechnical, biochemical and microbiological) and the methods used were those described by Padilha et al. [18]. Briefly, the young rabbits were weighed on the day of slaughtering and also 24 and 48 h beforehand in order to calculate the daily weight gain. Milk consumption was determined by weighing animals before and after suckling. VFA (volatile fatty acids) determination was assessed by gas chromatography [23] and that of NH_3 by colorimetry [25]. 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 [11].

2.3. Statistical analysis

The data were treated by variance analysis with mean comparisons by the Tukey test using the SYSTAT statistical software package [24].

3. RESULTS

3.1. Zootechnical traits

The live weight increased by 36 % ($P < 0.05$) between days 22 and 29 (*table I*). Consequently, a slight (not significant) increase (+9 %) in daily weight gain (DWG) was observed.

Milk consumption reached 33 $g \cdot d^{-1}$ on day 22 and decreased by 50 % on day 29 in spite of the foster mother (*table I*). In fact it was observed on day 29, that at the second suckling at 16h30 the rabbits consumed about one third of the daily milk consumption, without increasing their global milk intake. This implied that the milk consumption at 08h30 was reduced. On days 36 and 42, the consumption of milk was not

Table I. 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 milk only.

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 ($g \cdot d^{-1}$)*	15.7^{a}	(0.88)	22.5^{a}	(3.07)	-0.9^{b}	(2.06)	6.2^{b}	(3.38)
MI ($g \cdot d^{-1}$)	32.8^{a}	(2.75)	16.8^{b}	(4.26)	8.5^{b}	(4.76)	$18.4^{a,b}$	(3.72)
Caecal weight traits								
CW (g)	3.3^{a}	(0.24)	6.9^{b}	(0.42)	$8.5^{b,c}$	(0.41)	9.3^{c}	(0.53)
CC (g)	4.2^{a}	(0.60)	18.0^{b}	(1.16)	$21.9^{b,c}$	(2.89)	27.7^{c}	(3.40)
CW/BW (%)	0.86^{a}	(0.08)	1.29^{b}	(0.08)	$1.63^{b,c}$	(0.09)	$1.70^{b,c}$	(0.17)
CDM (%)	21.7^{a}	(1.41)	$26.2^{a,b}$	(0.70)	$27.2^{a,b}$	(1.76)	31.2^{b}	(2.78)

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 ($P < 0.05$).
* Rabbits were weighed 24 and 48 h before, and on the slaughter day to calculate the daily weight gain.

significantly different from that recorded on day 29. On day 36 the milk intake was particularly low (mean = 9 g per animal). This could be attributed either to a bad milking ability of the third doe or to behavioural problems between the young and this doe. As a result, a significant decrease in the DWG from 22.5 to –0.9 $g \cdot d^{-1}$ was noted between days 29 and 36.

3.2. Caecal weight traits

All parameters significantly increased ($P < 0.05$) between days 22 and 42 (*table I*). Between days 29 and 42, a significant increase of the caecal content and of the caecal wall was observed.

3.3. Caecal fermentation pattern

The concentration of NH_3 significantly increased ($P < 0.05$) between days 22 and 29 and then remained constant up to day 42 (16.5 $mmol \cdot L^{-1}$ on average) (*table II*).

The total caecal VFA concentration (tVFA) remained at a very low level 12.5 $mmol \cdot L^{-1}$ throughout the experiment (*table II*). A 35 % lower tVFA concentration was observed on day 36 compared to that of day 22. This could be explained by a significant reduction of the acetate concentration by 50 % ($P < 0.05$) between days 22 and 36, while the propionate and butyrate concentrations remained steady. The lower fermentation activity observed at 36 days of age could be related to the very low milk intake during this period. The concentrations of the branched-chain fatty acids (isobutyric and isovaleric acid) and valeric acid (mVFA) were not significantly different according to age (1 $mmol \cdot L^{-1}$ on average). The caecal pH remained steady and close to neutrality (mean 6.81) throughout the experiment.

3.4. Microbiological traits

The anaerobic cellulolytic microflora were absent or at an inferior level than the threshold values for our method (*table III*). 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 the animals.

The level of colibacilli flora decreased sharply ($P < 0.05$) from 10^7 to 10^4 bacteria per g of caecal contents between days 22 and 29 (*table III*), and then decreased more slightly to a level of 10^3–10^2 on average, on day 42.

Table II. Evolution of the caecal pH and fermentary traits (ammonia (NH_3), acetate (C_2), propionate (C_3), butyrate (C_4), minor volatile fatty acid (mVFA, i.e. branched-chain fatty acid and valeric acid) and total volatile fatty acids (tVFA)) in young rabbits given milk only.

	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 ($mmol \cdot L^{-1}$)	10.3^a	(0.72)	16.0^b	(1.73)	16.7^b	(1.15)	16.9^b	(1.63)
C_2 ($mmol \cdot L^{-1}$)	11.0^a	(1.99)	8.92^{a,b}	(1.44)	5.92^b	(0.56)	7.36^{a,b}	(0.93)
C_3 ($mmol \cdot L^{-1}$)	2.45^a	(0.41)	3.14^a	(0.42)	2.04^a	(0.32)	2.46^a	(0.29)
C_4 ($mmol \cdot L^{-1}$)	0.75^a	(0.12)	0.86^a	(0.18)	0.43^a	(0.10)	0.64^a	(0.11)
mVFA ($mmol \cdot L^{-1}$)	0.96^a	(0.14)	1.16^a	(0.13)	0.78^a	(0.16)	0.96^a	(0.20)
tVFA ($mmol \cdot L^{-1}$)	15.1^a	(2.46)	14.1^a	(1.87)	9.16^a	(0.97)	11.4^a	(1.26)

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 ($P < 0.05$).

4. DISCUSSION

The respective impact of factors linked to the intake and to ontogenic factors was evaluated for the caecal microbial activity of the rabbit around the weaning period. An original experimental model, based on exclusively milk-fed rabbits, was thus compared to rabbits fed milk and solid feed (standard model [18]), in order to discriminate the effects of dry feeding by difference.

Although the live weight increased by 36 % (between 22 and 29 days of age) in strictly milk-fed rabbits, this was much less than that observed in standard conditions (+67 %) [13, 18], when rabbits are suckled and also have an ad libitum access to dry feed. As a consequence, the daily weight gain increased slightly (+9 %), compared to an increase of 27 % for the standard model.

Despite the increase in the caecal content between 29 and 42 days, the mean weight of the empty caecum on day 42 represented only 55 % of that measured in the standard model. Nevertheless, the relative weight (CW/BW %) of the caecal wall was superior (1.7 %) to that observed in weaned animals (1.5 % on average) [14, 18]. On day 42, the weight of the caecal contents showed a two-fold reduction compared to that of the weaned rabbits in the standard model. In contrast, the caecal dry matter level was six points higher, corresponding to a pasty and compact content. This suggests an accumulation of milky matter and a stasis in the caecum. Although this was not verified, 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 previously described [3, 10]. It may also be hypothesized that the drying of the caecal contents was related to a change in the intestinal transit. The amount of substrate entering the caecum could be low and therefore may induce an increase in the retention time in the caecum associated with a reduction in colic motility and then a reduction in the turnover rate of the caecal contents.

With respect to the caecal fermentation pattern, the ammonia level was similar to that reported by Piattoni et al. [19], but was two times higher than that found in the standard model [18]. Inversely, the total VFA concentration was lower than that mentioned by Padilha et al. [18] (30–35 $mmol{\cdot}L^{-1}$) using SPF (but weaned) rabbits, or that observed in conventional weaned rabbits (50–80 $mmol{\cdot}L^{-1}$) [2, 16, 19, 20]. The minor VFA level reached 1 $mmol{\cdot}L^{-1}$, which was 60 % higher than previous data observed in 42-day-old SPF rabbits (standard model). Thus,

Table III. Evolution of the caecal colibacilli flora (Coli), total anaerobic microflora (tMF), amyloytic microflora (Amyl) and cellulolytic microflora (Cell) (in log10 of bacteria per g of caecal content) in young rabbits given milk only.

	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	

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.

the fermentation pattern differed greatly from what we observed previously in standard conditions with the same type of rabbits (SPF), 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, whereas it was between 78 and 85 % in normally fed rabbits. The butyrate molar proportion was inferior to 6 % (compared to 8–10 % in the standard model [2]), whereas that of propionate increased significantly between days 22 and 29 and then reached 22 % (in the standard model it remained under 10 %). The molar proportion of the sum of branched-chain fatty acids and valeric acid was four to five times higher than that observed in normally fed rabbits.

No clinical signs of diarrhoea were observed throughout the experiment despite a decrease in weight gain and a fermentation pattern (including a C3/C4 ratio of up to 3.5) typical of that observed in sick rabbits [7, 16]. These traits seemed relevant to a proteolytic metabolic activity. The high levels of NH_3, propionate and branched-chain fatty acids and valeric acid could be a result of the fermentation of the protein residues reaching the caecum [8, 17].

The development of anaerobic flora with age is similar to the pattern depicted in the standard model [18]. The decrease in the level of colibacilli flora between 22 and 29 days of age was also in agreement with the results previously reported in healthy weaned rabbits [15, 16, 22]. Inversely, the cellulolytic flora remained at a lower level while it could have reached 10^6 bacteria per g at 42 days of age [4]. Colibacilli flora is the only parameter that shows a normal development, independent of the feeding (milk only or milk + solid feed) imposed on the animals. Thus, in contrast to cellulolytic flora, colibacilli flora levels would be mainly dependant on ontogenic factors.

Our study clearly demonstrated that there is no relationship between the colibacilli flora level and chemical characteristics of the caecal media (DM, pH, VFA, NH_3), although a relationship between pH and *E. coli* has been suggested in vitro [20] and in vivo [16]. An extended suckling period reproduced a microbial activity similar to that found in cases of diarrhoea, but without any symptoms of digestive troubles. Previous studies [6, 16] demonstrated that caecal pH close to neutrality (pH 6.8–7.0) were associated with digestive troubles. But, the

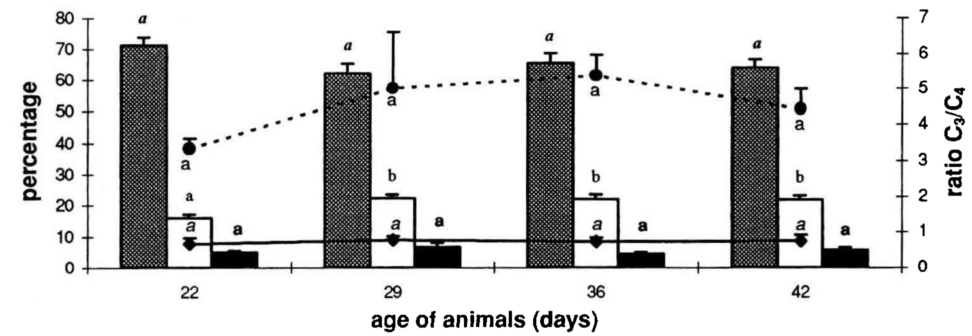

Figure 1. Evolution of the molar proportion of the different volatile fatty acids: (acetic (▩), propionic (□), butyric (■) and minor volatile fatty acids (mVFA) (–◆–)) and of the ratio C_3/C_4 (- - ● - -) in the caecum of young rabbits given milk only. Data are mean (n = 8) ± standard errors of the mean (SEM) in parentheses. Mean values in a same row with common superscripts were not significantly different ($P < 0.05$).

pH level here was relatively high (6.81) and was not connected with any clinical signs of disease, thus suggesting that caecal pH itself is not a determinant factor leading to digestive disorders.

In conclusion, the intake of solid feed is the major factor responsible for the establishment of the fibrolytic flora and the caecal fermentation pattern, without a major role of the *E. coli* flora. Therefore, it seems of interest to promote an early intake of solid feed before the weaning of the young rabbits, in order to promote the establishment of symbiotic flora.

ACKNOWLEDGEMENTS

We thank P. Coudert, G. Fonty and F. Lebas for their hepful discussions and critical comments regarding this work. We are also grateful to M. Boivin, Y. Breuzin and A. Francineau, (station de pathologie aviaire et de parasitologie, Inra Tours), J. Gomez (station de recherches avicoles, Inra Tours), Lapanouse and M. Segura (station de recherches cunicoles, Inra Toulouse) for their skilful assistance. The authors also wish to thank the National Council for Scientific and Technological Development (Brazil), for its support.

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