Original article

Relationships between microflora and caecal fermentation in rabbits before and after weaning

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Summary — Some microbiological and biochemical parameters of caecal content were studied in 15- to 49-d-old rabbits that were slaughtered sequentially. The ammonia level did not differ before weaning (11.5 mmol/L on average) ($P = 0.41$) or after weaning (7.4 mmol/L on average) ($P = 0.19$) but decreased by 40% ($P < 0.001$) between days 29 and 32. The level of pH decreased linearly ($P < 0.001$) throughout the period studied. The *Escherichia coli* counts decreased up to weaning ($P < 0.001$) and was then not significantly affected by age ($P = 0.12$). The total volatile fatty acid (VFA) concentration increased between days 15 (8.2 mmol/L) and 25 (33.9 mmol/L) ($P < 0.05$) and then levelled off below 40 mmol/L. Molar proportions in propionate and in branched-chain fatty acid (BCFA) and valeric acid were high at day 15 but decreased when the animals began to eat solid feed. The C3/C4 ratio reversed at weaning (3.8 on day 15 and 0.5 on day 49) whereas the acetic acid proportion was not affected by age ($P = 0.19$). High counts of anaerobic microflora were found between 15 and 22 d of age (10^{11} bacteria/g of caecal content, on average) and did not change significantly according to the age (at about 10^{10} bacteria/g), from day 29 until the end of the experiment ($P = 0.29$). Amylolytic flora had a similar evolution at a slightly lower level. In contrast, under our breeding conditions cellulolytic microflora slowly colonized the caecum and remained at a low level. The discriminant analysis revealed relationships between ages, intestinal microflora and fermentation parameter; the colibacilli flora was associated with mother-fed animals and amylolytic flora which was linked to BCFA and valeric acid, while the cellulolytic flora was associated with animals older than 4 weeks and linked to the production of C2, C3, C4 and ammonia.

rabbit / caecal microflora / caecal fermentation / volatile fatty acid / ammonia / pH

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Résumé — Évolution de la microflore et de l'activité fermentaire cæcale, chez le lapereau pendant la période péri-sevrage. *Basée sur une méthode d'abattages séquentiels, l'évolution de certains paramètres microbiologiques et biochimiques cæcaux a été analysée chez des lapereaux âgés de 15 à 49 j. La teneur en ammoniaque est stable avant (11,5 mmol/L en moyenne) (P = 0,41) ou après le sevrage (7,4 mmol/L, en moyenne) (P = 0,19), mais chute de 40% (P < 0,001) entre le 29e et le 32e jour d'âge. Alors que la flore colibacillaire diminue jusqu'au sevrage (P < 0,001) pour se stabiliser ensuite (P = 0,12), la valeur du pH décroit linéairement (P < 0,001) tout au long de l'expérimentation. En ce qui concerne les acides gras volatils (AGV), on note une élévation (P < 0,05) des AGV totaux entre 15 et 22 j (de 8,2 à 33,9 mmol/L) puis une stabilisation par la suite à un niveau peu élevé (< 40 mmol/L). Les proportions molaires en propionate et en acides gras ramifiés (AGR) plus acide valérique sont élevées à 15 j d'âge mais diminuent dès que les animaux commencent à ingérer de l'aliment solide. Le rapport C3/C4 s'inverse au moment du sevrage (3,8 à J15 et 0,5 à J49), alors que la proportion d'acétate varie peu en fonction de l'âge (P = 0,19). Nos données sur la flore anaérobie stricte totale indiquent qu'elle s'implante sans doute précocement à un niveau élevé (10^11 bactéries/g de contenu cæcal en moyenne entre J15 et J22), puis reste stable à environ 10^10 bact/g, jusqu'à la fin de la période étudiée (P = 0,29). La flore amylolytique présente une évolution similaire mais à un niveau légèrement plus faible. En revanche, la flore anaérobie cellulolytique, dans nos conditions d'élevage, semble s'implanter très lentement et à un niveau faible. L'analyse factorielle discriminante a montré des relations entre l'âge des animaux, la microflore intestinale et les paramètres fermentaires : la flore colibacillaire est associée aux animaux allaités et à la flore amylolytique et est liée aux AGR et acide valérique alors que la flore cellulolytique est associée aux lapereaux post-sevrés et liée à la production de C2, de C3, de C4 et d'ammoniac.*

lapin / microflore cæcale / fermentations cæcales / acides gras volatils / ammoniac / pH

INTRODUCTION

In the rabbit, the microbiological and biochemical compositions of the intestinal content and the balance between them, particularly in the caecum, seem to be predominant factors in the occurrence of post-weaning enteritis. Prohaszka (1980) showed that the *in vitro* concentration of volatile fatty acids (VFA) in the caecum and a low caecal pH could inhibit the proliferation of colibacilli. The extent of degradation of dietary fibre, which leads to the production of fermentation products (VFA, ammonia, *etc*) by caecal microflora, was emphasized by Cools and Jeuniaux (1961). Previous studies have reported several biochemical or bacteriological criteria and have been carried out on weaned or adult rabbits (Gidenne, 1986; Fonty and Gouet, 1989; Peeters *et al*, 1992; Bellier *et al*, 1994). The relationships between intestinal microflora and their fermentation products have however rarely been studied (Morisse *et al*, 1985).

The aim of the present work was to study the evolution of some biochemical parameters in parallel to that of the development of colibacilli flora and anaerobic microflora populations (total anaerobic flora and cellulolytic and amylolytic flora) in the caecum of young pre- and post-weaned rabbits under standard feeding conditions.

MATERIALS AND METHODS

Animals

New Zealand White rabbits (INRA strain A 1077) of both sexes were housed in wire cages under 14 h of light (from 6 am to 8 pm) and at a room temperature of $18 \pm 0.5°C$. They originated from the specified pathogen-free (SPF) breeding colony of the Station de pathologie aviaire et de parasitologie, INRA-Tours, France. They were obtained according to the method described by Coudert *et al* (1988). Briefly, the objective was to obtain animals without potentially pathogenic germs, thus prophylactic hygiene methods were

applied. These measures were based on maximum separation of young rabbits from their environment, and particularly from their mother, associated with early weaning (at 21 d). As a consequence animals were free of coccidia, oxyurids, *Pasteurella, Clostridium spiriforme* and *Escherichia coli* belonging to recognized pathogenic serogroups (O103, O15, O128, O132, O109). Eight litters of 8 rabbits were used for the experiments. One rabbit per litter was slaughtered 5 to 7 h after a meal, at 15, 22, 25, 29, 32, 36, 42 and 49 d of age. Sacrificed rabbits were replaced by rabbits from other litters raised in parallel in order to maintain a normal lactation level for each experimental litter up to weaning at 29 d.

Feeding and digestibility measurement

Animals were given *ad libitum* a pelleted commercial feed containing 17.5% crude protein and 17% crude fibre (tables I and II). To measure the feed intake of young rabbits before weaning, the access to water and food for mothers and young was separated from 12 days of age. The young were suckled once a day, at 9 am. Twenty millilitres of milk were collected on days 3, 14, 22 and 29 of lactation, from 5 does different than those used in this study but of the same age and supplemented with the same pelleted feed, in order

to determine the proportion of dry matter, crude protein, lipids and glucids provided by the milk (table III). The apparent faecal digestibility of the feed was measured in a group of 6 weanlings between 42 and 56 d of age (Colin and Lebas, 1976) after an adaptation to the feed from weaning (at 31 d).

Measured traits

Zootechnical traits

All rabbits were weighed before and after suckling on the sacrifice days in order to calculate milk consumption. Feed consumption was measured daily until weaning and subsequently on sacrifice days.

Biochemical traits

As regards the caecum, apart from the weights of the full and empty caecum, analyses concerned exclusively the contents of the caecum. Animals were slaughtered between 2 and 4 pm and the intracaecal pH was measured immediately after slaughtering (Gidenne, 1986). Dry matter content was determined by drying at 103°C for 24 h. The VFA composition of the caecal content was analysed by gas chromatography according to Spiller *et al* (1980) and the ammonia (NH_3) concentration was measured by colorimetry according to the method of Verdow *et al* (1977). NH_3

Table I. Ingredient composition of the experimental diet.

Ingredients	%
Oat	5.0
Dehydrated alfalfa meal	28.5
Beet pulp	5.0
Cane molasses	4.0
Wheat straw	2.0
Wheat bran	20.0
Barley	10.3
Toasted soybean seed	5.0
Soybean meal	4.2
Sunflower meal	8.5
Remilling	5.0
Minerals	2.5

Table II. Chemical composition and nutritive value (% of dry matter) of the pelleted diet.

Dry matter	91.3%
Organic matter	91.2%
Crude protein (N x 6.25)	17.6%
Crude fibre	17.2%
Neutral detergent fibre (NDF)	40.9%
Acid detergent fibre (ADF)	21.5%
Acid detergent lignin (ADL)	6.4%
Ash	8.8%
Digestible energy (MJ/kg DM)	10.5
Digestible organic matter	56.6%
Digestible crude protein	13.5%

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Table III. Milk composition (% of dry matter).

Day of lactation	DM	CP	L	NNE
3	28.82[a] (1.27)	41.42[ab] (3.89)	29.73[ab] (4.02)	19.90[a] (4.57)
14	30.52[ab] (0.16)	37.81[ab] (0.30)	39.38[a] (1.29)	15.60[a] (1.36)
22	34.40[b] (1.21)	32.83[a] (1.90)	33.94[ab] (2.07)	27.30[a] (4.12)
29	31.47[ab] (1.15)	43.31[b] (4.27)	24.68[b] (4.37)	18.74[a] (3.12)

DM: dry matter; CP: crude protein; L: lipids; NNE: non-nitrogen extract. Values are means ($n = 5$) with the standard errors of the mean (SEM) in parentheses. Means in the same column with common superscripts did not differ significantly ($P < 0.05$).

was not determined on day 15 because of the small quantity of caecal material available at this age.

All the methods used for milk composition were those described by Amariglio (1986). For the measure of the digestibility, chemical analyses were performed on the feed and faeces according to the previously described methods (Gidenne and Jehl, 1994).

Microbiological traits

Enumerations of the different flora were carried out only at 15, 22, 29, 36, 42 and 49 d of age. Drigalski agarose (Institut Pasteur) plates were seeded with dilutions of the caecal content and incubated for 18 h at 37°C for *E coli* enumeration. Anaerobic flora were analysed under strictly anaerobic conditions in 'roll-tubes' according to Hungate's method (1966) using Caldwell and Bryant's medium 10 (M10) (1966). Enumeration of cellulolytic flora were performed in a liquid medium in which sugars were replaced by cellulose in the form of a band of Whatman No 1 filter paper (Boulahrouf *et al*, 1991). For total anaerobic and amylolytic flora, cultures were performed in solid medium. Starch replaced other sugars for the latter and colonies were evidenced with Lugol (Bryant and Burkey, 1953).

Statistical analyses

Data were treated by variance analysis with comparison of means by Tukey's test and by regression analysis using the SYSTAT program (version 5.04, 1994). As a large number of parameters was measured and totally random associations may have been revealed, we attempted to determine the correlations between the various parameters by also taking into account the age of the animals. A principal component analysis was performed to see whether homogeneous groups were formed. As the results were positive a discriminant analysis was undertaken on these groups in order to determine possible links between the different parameters according to age. Multifactorial analyses were carried out by means of the STATITCF program (version 5, 1991).

RESULTS

Zootechnical traits

The weight of the animals and the milk and feed consumption had a typical evolution (table IV). A significant reduction in milk consumption was observed between days 22 and 29 ($P < 0.01$), counterbalanced by a significant increase ($P < 0.01$) in solid feed intake. Similarly, a significant increase ($P < 0.01$) in daily weight gain (DWG) occurred from day 22 until weaning. The DWG reached 40 g/d at day 29 which was not different from the DWG observed between days 42 and 49. However, the DWG was

Table IV. Evolution of zootechnical traits (body weight (BW), daily weight gain (DWG), milk intake (MI), feed intake (FI) and feed conversion (FC)) 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 the young rabbit between 15 and 49 d of age.

Trait	Age (d)							
	15	22	25	29	32	36	42	49
Zootechnical traits								
BW (g)	294[a] (15.60)	387[ab] (12.34)	477[b] (22.54)	650[c] (22.00)	753[c] (19.09)	914[d] (26.82)	1 184[e] (28.87)	1 499[f] (26.71)
DWG (g/d)	16.9[a] (1.46)	13.5[a] (0.87)	26.50[b] (2.03)	40.75[def] (2.30)	29.03[be] (2.79)	37.30[de] (1.87)	46.44[f] (0.89)	45.18[df] (2.40)
MI (g)	28.7[a] (2.40)	31.3[a] (2.31)	26.83[ab] (2.58)	19.83[b] (1.88)	–	–	–	–
FI (g)	–	3.0[a] (0.49)	9.80[a] (0.88)	33.0[b] (3.25)	60.10[c] (1.38)	88.40[d] (2.23)	103.80[e] (1.48)	122.30[f] (1.24)
FC (%)	–	–	–	–	–	2.4[ab] (0.11)	2.24[ab] (0.06)	2.76[b] (0.16)
Caecal weight traits								
CW (g)	1.7[a] (0.12)	3.8[b] (0.18)	4.86[b] (0.25)	6.88[c] (016)	8.59[d] (0.33)	11.61[e] (0.58)	16.05[f] (0.47)	22.05[g] (0.43)
CC (g)	1.5[a] (0.19)	6.7[ab] (0.95)	12.60[bc] (1.93)	20.30[c] (1.00)	32.01[d] (1.66)	48.83[e] (2.71)	61.74[f] (2.64)	81.08[g] (3.52)
CW/BW (%)	0.6[a] (0.02)	1.0[b] (0.04)	1.02[bc] (0.04)	1.06[bc] (0.04)	1.14[c] (0.06)	1.27[dc] (0.05)	1.36[de] (0.05)	1.47[e] (0.04)
CDM (%)	12.2[a] (1.98)	21.4[bd] (0.87)	26.31[c] (1.22)	24.02[bc] (0.85)	23.35[bc] (0.58)	24.32[bc] (0.48)	24.70[cd] (0.33)	24.30[cd] (0.37)

Data are mean (*n* = 8) and standard errors of the means (SEM) in parentheses. Mean values in the same row with common superscripts were not significantly different (*P* < 0.05).

significantly lower between days 32 and 36 than those obtained later ($P < 0.05$). On day 29, the milk intake represented 6.6 g dry matter (*ie* 18% of the total dry matter ingested) and 2.4 g crude protein (31.5% of total crude protein ingested) (table III).

Caecal weight traits

There was considerable development of the caecum between 15 and 49 d of age due to the fact that the weight of the caecal wall tripled in relation to body weight, whereas the value of the caecal content was multiplied by 10 (*ie* an increase from 0.5 to 5% of body weight) (table IV). The percentage of the caecal wall weight with regard to the

body weight increased linearly according to the age, from 0.58% on day 15 to 1.47% on day 49 ($r^2 = 0.86$; $n = 64$) ($P < 0.001$). Dry matter of caecal content doubled between days 15 and 25 but did not vary significantly according to the age from day 25 with a mean of 24.5% of fresh content ($P = 0.17$).

Biochemical content

The level of NH_3 was between 10 and 12.5 mmol/L before weaning and did not differ according to the age during this period ($P = 0.41$) (fig 1). The concentration of NH_3 then fell about 40% between days 29 and 32 ($P < 0.05$). From day 32, age did not affect the level of ammonia which was between
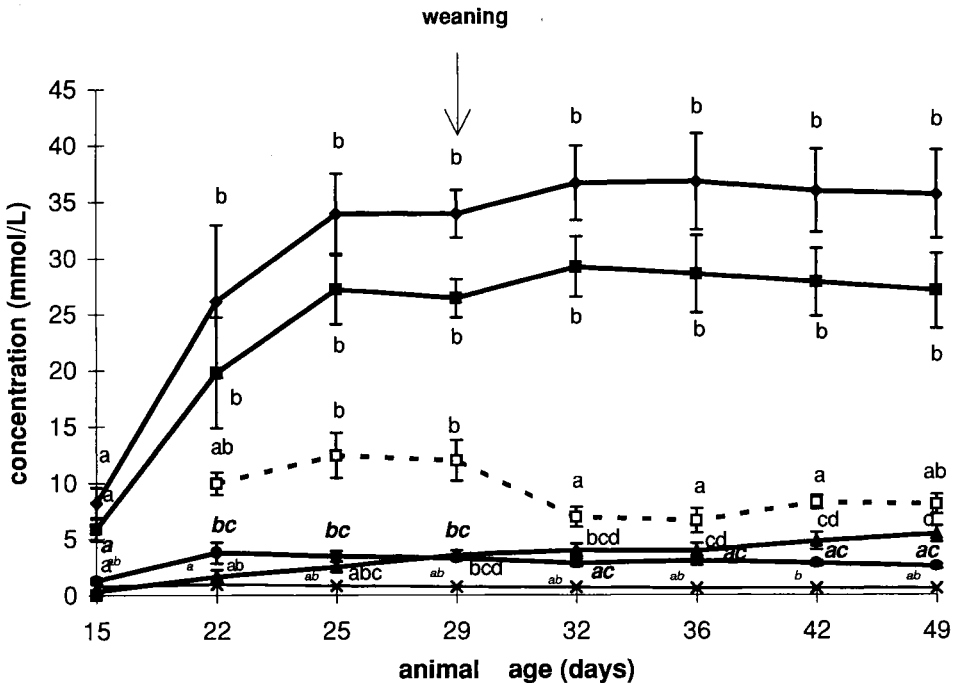

Fig 1. Evolution of the concentration of the total volatile fatty acids (◆), acetic acid (■), propionic acid (▲), butyric acid (●), branched-chain fatty acids + valeric acid (✖) and ammonia (□) of the caecal content, in the young rabbit between 15 and 49 d of age. Data are mean ± SEM (standard error of the mean). Mean values with common superscripts were not significantly different ($P < 0.05$).

6.5 and 8 mmol/L (P = 0.19). When analysed for the whole period of experiment, the NH_3 concentration was significantly correlated (P < 0.05) to the total concentration of branched-chain fatty acids (BCFA) (isovaleric and isobutyric acids) and valeric acid (r^2 = 0.32; n = 56).

A considerable increase occurred (from 8 to 34 mmol/L, P < 0.001) in the total level of VFA between 15 and 25 d of age, whereas age did not significantly affect the total VFA concentration after weaning with a mean of 36 mmol/L (P = 0.8) (fig 1). The curve of total VFA was parallel to that of acetic acid which represented on average 76% of total VFA (fig 2). Butyric acid increased significantly (P < 0.001) from 0.4 to 5.4 mmol/L (r^2 = 0.7; n = 64), ie a progression of 4 to 15% of total VFA. After a rise (P < 0.05) between day 15 (1.2 mmol/L) and day 22 (3.7 mmol/L), propionic acid decreased slightly but linearly (P < 0.05) to day 49 (2.5 mmol/L), (r^2 = 0.30; n = 56), corresponding to a reduction from 14 to 8%

of total VFA (fig 2). The total concentration of BCFA and valeric acid, which slightly increased (P = 0.31) from 0.7 to 0.9 mmol/L between days 15 and 22, decreased linearly (P < 0.05) after this time (r^2 = 0.36; n = 56).

In terms of molar proportion, while age did not affect the rate of acetic acid (P = 0.19), the molar proportion of BCFA and valeric acid decreased linearly (P < 0.001) from 11 to 1.9% between days 15 and 49 (r^2 = 0.58; n = 64). The ratio C3/C4 was at a high level (3.86) on day 15 after birth and decreased linearly (P < 0.001) to 0.54 on day 49 (r^2 = 0.68; n = 64) (fig 2).

Caecal pH fell linearly (P < 0.001) throughout the experiment (fig 3), from 6.84 on day 15 to 5.59 on day 49 (r^2 = 0.86; n = 64). The significant coefficient of regression (P < 0.05) between the total VFA and ammonia concentration and the pH was exclusively due to the significant correlation between the pH and the total VFA concentration (r^2 = 0.59; n = 64) (P < 0.001) whereas the pH
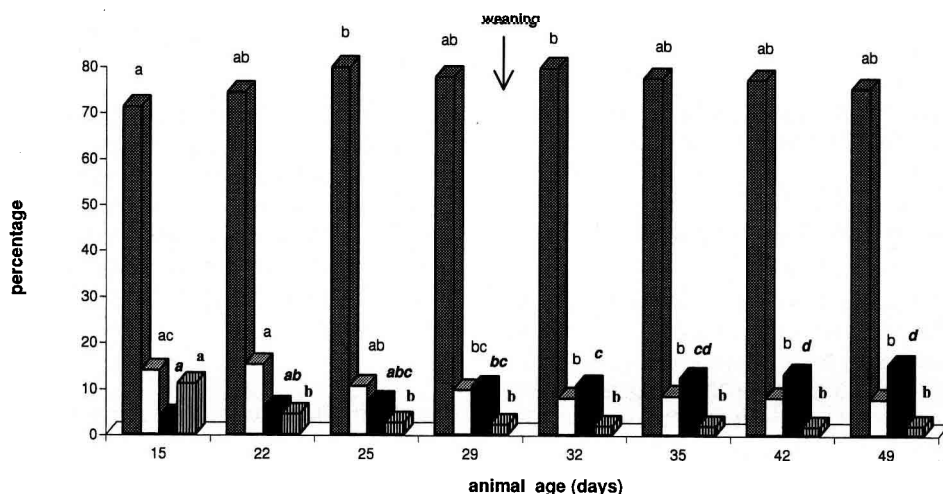

Fig 2. Evolution of the molar proportions of the volatile fatty acids (VFA), acetate ■, propionate ☐, butyrate ☐ and minor VFA ⦀ (branched-chain fatty acids (isobutyric + isovaleric and valeric acids), of the caecal content in the young rabbit between 15 and 49 d of age. Data are mean ± SEM (standard error of the mean). Mean values with common superscripts were not significantly different (P < 0.05).
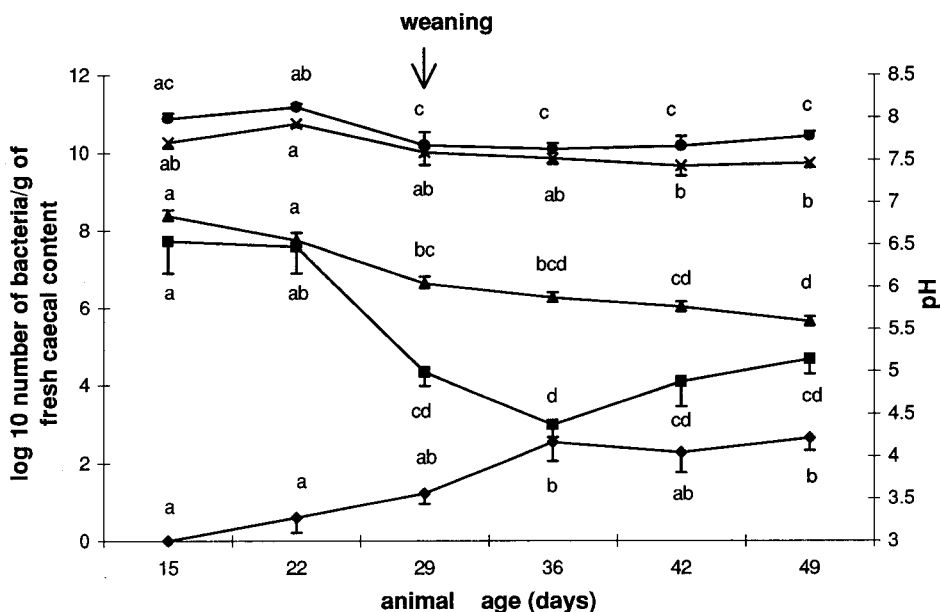
Fig 3. Evolution of the pH (▲) and the microflora: colibacilli flora (■), total anaerobic flora (●), cellulolytic flora (◆) and amylolytic flora (✖), of the caecal content in the young rabbit between 15 and 49 d of age. Data are mean ± SEM (standard error of the mean). Mean values with common superscripts were not significantly different ($P < 0.05$).

was not significantly correlated with the concentration of NH_3 ($P = 0.21$).

Microbiological parameters

The colibacilli flora count, which was around 10^8 *E coli* per gram of caecal content 15 d before weaning, decreased linearly ($r^2 = 0.58$; $n = 32$) ($P < 0.001$), reaching values of 10^3–10^4 bacteria/g (fig 3) in weaned rabbits. The *E coli* count was significantly correlated to the pH before ($r^2 = 0.56$; $n = 32$; $P < 0.001$) but not after ($P = 0.31$) weaning.

High levels of total anaerobic flora was observed (10^{11} bacteria/g) until 25 d of age and then decreased up to weaning ($P < 0.05$) but did not change according to the

age, at around 10^{10} bacteria/g, from day 29 until the end of the observation period. Amylolytic flora had a parallel evolution but at slightly lower levels. In contrast, cellulolytic flora (fig 3), which was absent 15 d before weaning, could be detected at day 22 and reached values higher than 10^2 bacteria/g after weaning.

Results of the discriminant analysis

Simple variance analysis with comparison of means (Tukey's test) revealed that for the majority of the parameters studied there was a clear difference between the periods before and after weaning at 29 d. The discriminant analysis (fig 4) revealed relation-
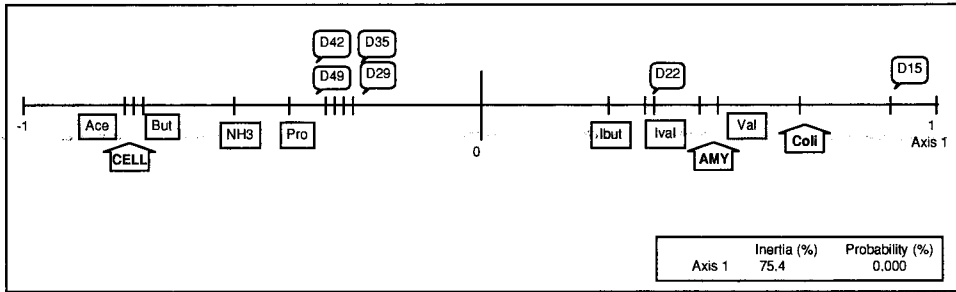
Fig 4. Relationships between the age of the rabbit, the microflora and the fermentary parameters from the caecum, between 15 and 49 d of age. Discriminant analysis. (▢) = D15, D22, D29, D36, D42 and D49 = sacrifice days corresponding to the animal's age (⌂). Caecal microflora: amylolytic (AMY), cellulolytic (CELL) and colibacilli (Coli) flora (▢). Caecal fermentary parameters: acetic acid (Ace), propionic acid (Pro), butyric acid (But), isobutyric acid (Ibut), isovaleric acid (Ival), valeric acid (Val) and ammonia (NH₃).

ships between ages, the different flora of the caecal content and the fermentation parameters in the period between 15 and 49 d of age. There was a clear difference between days 15 and 22 and then between day 22 and the other sacrifice days, which corresponded to the post-weaning period. Colibacilli flora was strongly opposed to cellulolytic flora and associated with mother-fed animals and amylolytic flora. The latter was strongly linked to the production of valeric, isovaleric and, to a lesser extent, isobutyric acids. On the other hand, cellulolytic flora was associated with animals older than 4 weeks and was probably linked to the production of acetic and butyric acids and ammonia and less with the production of propionate.

Other parameters which do not appear on the graph were omitted because they did not play any part in the discrimination between age groups.

DISCUSSION

This work is original in studying in parallel the evolution of intestinal microflora (anaer-

obic: total, amylolytic and cellulolytic flora; and colibacilli flora) and that of the fermentation products.

The analysis of zootechnical criteria revealed the appropriate growth of the animals with a mean feed-conversion after weaning of 2.4. The relative decrease in weight gain observed at day 32 was probably a result of the stress of weaning.

Few authors have studied the parameters of weight of the caecum from the birth of the rabbit (Alus and Edwards, 1977; Wu, 1986). They described slow caecal growth up to the age of 10 d, followed by very rapid growth, in agreement with our results and those of Lebas and Laplace (1972) and Candau et al (1978). On the other hand, to our knowledge, there have been no other studies of caecal parameters following 15 d of age.

The total VFA concentration, which was very low at day 15, greatly increased up to 25 d and then stabilized at a relatively low level, in comparison with the values obtained in vivo by Bellier et al (1994) in cannulated rabbits of 42 d of age (70–80 mmol/L), by Prohaszka (1980) (82 mmol/L) and Morisse et al (1990) (55 mmol/L) in healthy 6- to 8-

week-old non-cannulated rabbits and by Vernay et al (1984) in adults (70–76 mmol/L). The content of the feed (few cereals) and its relative low digestibility might explain, at least in part, this difference. This is in agreement with the results of Morisse et al (1985) and Carabaño et al (1988) but in contrast with those obtained by Gidenne et al (1991). Another explanation might be the rate of lignin in the diet. Morisse et al (1985) found that the concentration of the total VFA from the caecum was reduced from 84 to 34 mmol/L in diets containing 3.4 and 7% lignin respectively. On the contrary, Gidenne (1986) observed that total caecal VFA concentration increased by 11% when the lignin content of the diet increased from 7.4 to 16% DM, but this author underlined the role of the quality of the lignin used. From a methodology point of view, a partial explanation for the low total VFA concentration found in our study might be the slaughter time. According to Bellier and Gidenne (1992), who have studied the circadian changes of VFA level in the caecum, the lower values for this trait were found just for the 0 to 4 pm period (around 57 vs 82 mmol/L at the maximum). Carabaño et al (1988) obtained a VFA concentration of 47.7 mmol/L for a diet containing 19.9 acid detergent fibre (ADF) on dry matter, in 60-day-old-rabbits sacrificed at 6 pm. However, it is interesting to note that a total VFA content lower than 40 mmol/L, ie approximately 50% lower than the values frequently reported in conventional rabbits, agrees with the results of Boot et al (1985). They observed lower levels of VFA in 'germ-free' rabbits inoculated with a controlled rabbit flora than in conventional rabbits (43.3 against 88.1 mmol/L respectively). They suggested that this effect could be due to the absence of a particular cellulolytic bacterium in the inoculated flora. The rabbits used in our study were, in fact, SPF animals obtained in special conditions (Coudert et al, 1988). Under these conditions it cannot be ignored that caecal flora might have been modified. This might explain the slower implantation of cellulolytic flora that was seen in our animals with lower values than those found by Gouet and Fonty (1979) and Boulahrouf et al (1991).

The evolution of molar proportions of VFA indicated a preponderance of C3 and of BCFA and valeric acid at 15 days of age when the young rabbits were still exclusively milk fed. As soon as the young rabbits started to take pelleted food (from day 17–18) the level of total VFA concentration tripled (day 22). The C3/C4 ratio became progressively inverted over time whereas the acetate proportion was not affected by age. A similar, but slower evolution was observed in vivo by Bellier (1994). It is however necessary to add that the type of diet is likely to influence the evolution of caecal fermentation in the growing rabbit over time.

It is noteworthy that the C3/C4 ratio, which was higher than 1 before weaning, was not accompanied by any clinical signs (diarrhoea or death) in contrast to what is generally observed in weanlings (Morisse et al, 1985; Gidenne et Jehl, 1994).

The consistent decrease in pH between days 15 and 49 was at least in part related to variations in total VFA, but not to ammonia content. The latter varied little over time apart from a dramatic fall at the time of weaning which probably should be attributed to changes in feeding patterns. In fact, it seems that the flora before weaning was predominantly proteolytic as judged by the NH_3 and BCFA values.

The level of colibacilli flora, which was very high in 15-day-old animals (10^8–10^9 bacteria/g of caecal content), strongly decreases until weaning, in agreement with the data of various studies (Smith, 1961; Licois et al, 1992). Moreover colibacilli flora tended to stabilize between days 29 and 49, whereas pH decrease significantly during the same period. We did not find, therefore, at least after the weaning, a relationship between pH and E coli count.

From these observations it seems that the event of weaning (in this case, the separation of the young rabbits from their mother on day 29) is not a disturbing factor regarding the different parameters analysed. In fact the passage from the milk to the solid feeding, which generally begins around the 18 to 20th day and lasts about a week, is the period corresponding to the main changes observed in this study. This suggests that a nutritional control of the flora occurs before weaning, as soon as the animals consume solid food.

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