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# The caecal ecosystem in the growing rabbit: impact of nutritional and feeding factors.

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## 1 Introduction

An ecosystem is defined by the biotope, i.e. the environment, and by the biocenosis, i.e. the organisms living in the biotope. Applied to the rabbit caecum, the biotope is the organ itself and its content that could be analysed through physical parameters, including the pH, the redox potential (Eh), the level of dry matter, viscosity etc., and through biochemical parameters such the concentrations in organic compounds (e.g. organic acids, proteins, enzymes, etc...) or the enzymatic activity of the flora (fibrolytic enzymes, etc..). The rabbit caecal biotope have been relatively extensively studied, mainly by nutritionists, although some parameters remain still fewly known, such the redox potential or the production of gases ($H_2$, $CO_2$, $CH_4$). In comparison, the microbial flora has been fewly studied, probably because there are fewer teams implicated in caecal microbiology, and also because the classical culture methods used are time-consuming and do not cover the whole digestive flora.

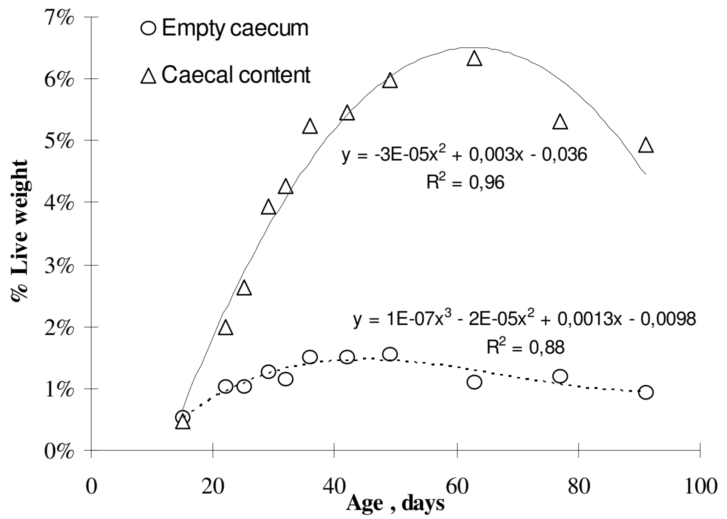
Consequently, the relationship among caecal biotope and biocenosis have been weakly described, and the effect of and nutritional or breeding/feeding factors has been fewly described. However in the last five years, this subject appears as a thematic priority for some teams implicated in rabbit nutrition, since it could provide some new strategies to improve the resistance of the young rabbit to digestive disorders. For instance, the favourable effect of dietary fibre on prevention of the digestive disorders in the growing rabbit have been demonstrated (Gidenne, 2003), even face to an experimental inoculation of a enteropathogenic *E. coli* (EPEC) strain (Gidenne and Licois, 2005). In return, the incidence of digestive disturbances is weak in adult rabbit, since a fibre deficiency hardly provokes diarrhoea as well inoculation of an EPEC strain (Licois *et al*., 1990). Since fibres are a major substrate for the commensal caecal flora, the understanding of this fibre effect on digestive health in the growing rabbit is based on microbial activity or balance, and two main hypothesis have been put forward, that could act in association : 1- the implication of the digestive maturation, including flora implantation, in the resistance to pathogens (Padilha *et al*., 1995) and corresponding to concept of "barrier flora" ; 2- the maturation of the immune system in interaction with flora (Fortun-Lamothe and Boullier, 2004). The concept of "barrier flora also include considerations about the stability and the biodiversity of the flora. It is assumed that a high degree of stability and a biodiversity would favour the digestive health. New approaches, using molecular microbiology tools are presently under development to assess these concepts (see 2.2.2).

Thus, this review aimed to recall some basis on the caecal biotope and biocenosis, and then to present some recent results illustrating how it would be possible to control the flora activity to improve the digestive health of the young rabbit.

## 2 The caecal ecosystem of the rabbit

### 2.1 Basics on the caecal biotope

The relative digesta content of the caecum increases linearly (figure 1) from 2 to 6 weeks of age, and then become the largest digestive compartment from 5-6 weeks of age (40% of the total digestive volume). An early weaning (21 vs 35d) stimulate the relative development (% of LW) of the caecum and of its content (Gallois *et al.*, 2005). The dry matter content of the caecum, which is low at 2 weeks of age (12-15%), increases to 23-26% the following week and then stabilises between 21 and 23% from 35 days of age.



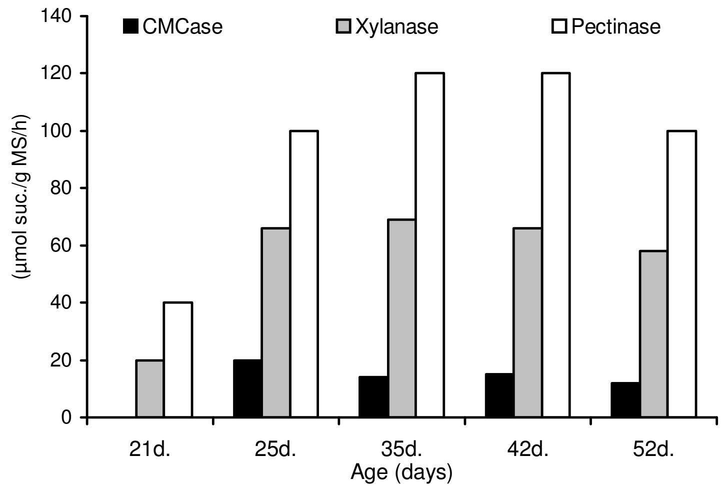
(from: Lebas and Laplace, 1972; Candau *et al.*, 1978; Padilha *et al.*, 1995, Gallois *et al.*, 2005).

**Figure 1:** Relative development of the caecum in the rabbit.

The caecal and colon mucosa wall change from 16 days of age with the appearance of ridges (Yu and Chiou, 1997; Sabatakou *et al.,* 1999), when the intestinal flora becomes established together with fermentation. The size and shape of the villi are largely dependent on feeding conditions (pure milk or solid feed).

The measurement of the microbial activity is essential to estimate the digestive efficacy of the flora, but it would also relate with the flora “health”.

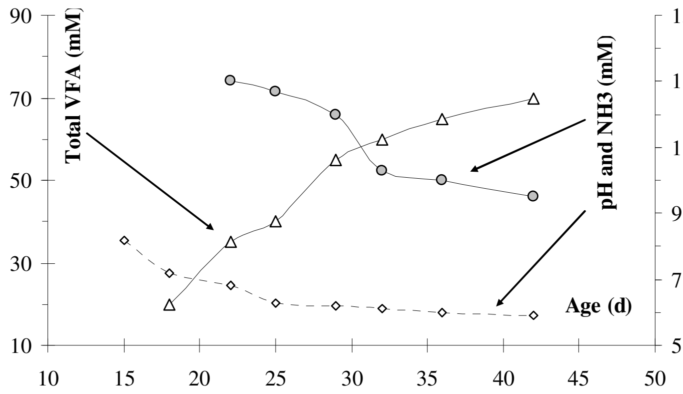
Historically, a fermentative activity was first identified in the rabbit caecum (Elsden *et* al., 1946), and then the cellulolytic activity of the rabbit caecal flora was evidenced in-vitro by Cools and Jeuniaux (1961). Emaldi *et al.* (1979) studied first metabolic activity of the caecal flora, i.e. its enzymic potential to hydrolyse the substrates entering the caecal ecosystem. Microbial activities could be classified according to the substrate degraded: ureolytic, proteolytic and fibrolytic. An important ureolytic activity is observed in the caecum, due to ureolytic aerobes and anaerobes (Crociani *et al.*, 1984; Forsythe and Parker 1985). Fibrolytic activity of the flora was studied more recently, by assessing the concentration of bacterial enzymes able to hydrolyse purified cellulose or wood xylanes or apple pectines (Jehl *et al.*, 1995). Bacterial fibrolytic activity is similar in the caecum and in the soft faeces, thus allowing to study within the same animals these criteria according to age or nutritional factors (Jehl *et al.*, 1996). Caecal fibrolytic activity is not detectable in young rabbits at 2 weeks of age, but is already 80% developed at 4 weeks of age (figure 2), since it depends of the presence of fibrous substrate in the caecum and obviously to the presence of a fibrolytic flora (see 2.2.1). Cellulolytic activity appears at 25d of age, and then remains at a low level. Reversely, xylanasic and pectinolyctic activity increases weakly between 21 and 52 d. of age. However, the development of the bacterial fibrolytic activity is subjected to large variations. For instance, Bennegadi *et al.* (2004) reported a decrease of the fibrolytic activity between weaning (28d) and 70d of age.



CMCase: carboxymethyl cellulase; data from: Gidenne *et al.*, 2000; Pinheiro *et al.*, 2001; Gidenne *et al.*, 2002; Debray, 2002; Gallois *et al.*, 2006.

**Figure 2**: Fibrolytic activity of bacteria in the rabbit caecum

Degradation of the digesta entering the caecum by microorganisms results mainly in production of volatile fatty acids, ammonia, carbon dioxide, methane and hydrogen. The caecal VFA profile is specific to the rabbit, with a predominance of acetate (C2 = 60 to 80 mmol/100mol) followed by butyrate (C4 = 8 to 20) and then by propionate (C3 = 3 to 10). Moreover, Adjiri *et al.* (1992) has shown *in vitro* (using a semi-continuous flow fermentor: "caecitec") that the VFA pattern was specific of the caecal flora, and not linked to the composition of the substrate fermented. The fermentative activity of the bacteria varies according to the circadian rhythm of the intake behaviour (Gidenne and Lebas, 2006) including a lower concentration of VFA (-25%) during the caecotrophy period compared to a higher VFA level found during the hard faeces excretion phase (Gidenne, 1986; Bellier *et* al., 1995; Bellier and Gidenne, 1996). This diurnal pattern of the caecal fermentative activity coincides with a similar rhythm of VFA absorption and metabolisation (Vernay, 1989). Consequently, fatty acids concentration in the caecum increases progressively (40 to 70 mmol/L, figure 3). Acetate levels (75-85%) are always higher than propionate (6-8%) or butyrate levels (6-10%). Nature of end-products change with age showing an inversion of propionate/butyrate ratio which becomes lower than 1 after 25-30 days of age (Padilha *et al.*, 1995; Zomborsky-Kovacs *et al.*, 2000 ; Gidenne *et al.*, 2002). Ammonia concentration in the caecum slightly falls with age (Gidenne and Fortun-Lamothe, 2002). Both increase in volatile fatty acids and decrease in ammonia concentration induce a fall in caecal pH from 15 to 42 days of age.



from: Candau *et al.*, 1978; Gidenne *et al.*, 2000; Debray, 2002; and unpublished results

**Figure 3**. Evolution of the fermentative activity with age,

Almost no results are available on gas production (hydrogen, methane) associated with fermentation, except an *in vitro* study evaluating that methanogenic activity was around zero until weaning, and rose after 36 d of age (Piattoni *et al.*, 1996). This would suggest that no methanogenic flora is developing before weaning, but this contrasted with the recent results showing that archaeal flora (known to produce methane) is predominant before weaning, compared to bacteria (Bennegadi *et al.*, 2003).

More recent studies have dealt with two other approaches for evaluating the microbial activity in digestive ecosystems. The measurement of the ATP level as an indicator of the energetic metabolism of the bacteria, but it showed too-high inter-individual variability to be of interest for studies in the rabbit (Bellier and Gidenne, 1996). The evaluation of the microbial biomass using an internal marker of bacteria, such as DAPA (diaminopimelic acid), was initially developed for ruminants. A recent application of this method in the rabbit indicated a good relationship between biomass formed and fibre intake (Jehl and Gidenne, 1996; Gidenne *et al.*, 2004a). Further studies are required to confirm the validity of this approach; however these techniques are time-consuming and costly.

The caecal microbial activity can also be addressed through its contribution to the supply of nutrient via the ingestion of soft faeces. Piattoni *et al.* (1995) evaluate that around 50% of the caecal nitrogen were of bacterial origin, using a new indicator based on RNA/crude protein ratio. The nitrogen produced by the flora and recycled in soft faeces was evaluated recently using a method based on the measurements of the purine/bacterial nitrogen ratio (Garcia *et al.*, 1995), in isolated caecal bacterial preparation. According to this technique, the bacterial contribution to the total nitrogen intake ranged between 12 and 24%. More recently, new methods were developed to assessed the nitrogen metabolism and the impact of the caecal flora, using either purines derivatives (Balcells *et al.*, 1998) or labelled nitrogen sources ($NH_4Cl$-$N^{15}$; Bellenguer *et al.*, 2005).

## 2.2 Elements on the caecal biocenosis

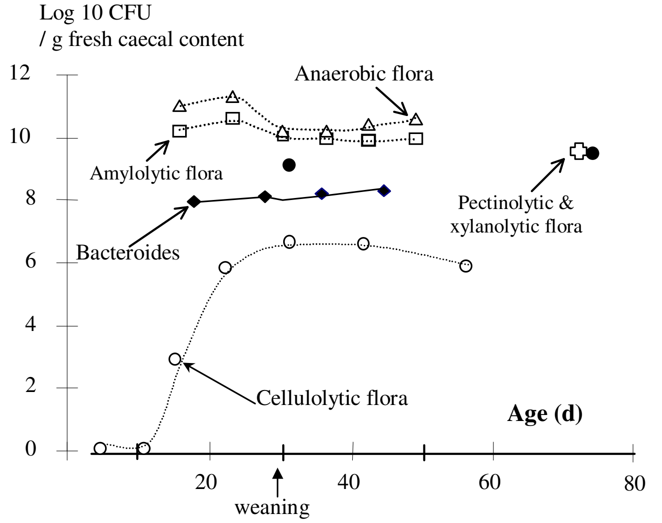
Gut flora remains relatively poorly understood, probably because the flora has a great diversity. For instance, in the man, the digestive tract harbours about $10^{14}$ bacteria belonging to more than 400 different species, almost all being strictly anaerobes (99%) ; in addition, 70-80% of this microbial biomass is not-cultivable (Suau *et al.*, 1999). Our knowledge on colonisation of the rabbit gut was mainly obtained with cultured-based techniques. However some more recent studies using molecular biology-based techniques revealed numerous uncultivable bacterial species (Abecia *et al.*, 2005a).

Contrary to the ruminants the normal flora of the rabbit does not contain any anaerobic fungi or protozoa (Bennegadi *et al.*, 2003). Other eukaryotes can be present and multiply especially in diseased animals (e.g. coccidia). The presence of yeast in caecal content was fewly studied. However, one species of yeast (*Saccharomycopsis guttulatus*) would be “commensal” of the rabbit caecum, and was reported by Peeters (1988).

### 2.2.1 Approach of the caecal biocenosis using the culture methods

Until the end of the first week of life, the anterior part of the digestive tract of the young rabbit is virtually sterile (unlike that of the pig or the rat). In 2 or 3-day-old animals, the total number of bacteria in the cæcum varies considerably according to individuals (Gouet and Fonty, 1973). At the end of the first week of life, the cæcum harbours already an abundant bacterial flora ($10^7$ to $10^9$ bacteria/g) which increases from the 2nd week ($10^9$-$10^{10}$ bacteria/g) with a lower inter-individual variations. During all this period, the number of facultative anaerobic bacteria is sometimes equivalent to that of the strictly anaerobes. From the third week, the number of facultative anaerobic bacteria falls dawn to $10^2$-$10^4$ and it is not rare that this microbiota is absent after weaning (Ducluzeau, 1969), whereas the strictly anaerobic flora remains stable to $10^9$-$10^{11}$ bacteria/g (figure 4). In the colon, the microbiota follows an evolution identical to that of the cæcum but the total number of bacteria is there systematically lower (Gouet and Fonty, 1979). In addition, Emaldi *et al.* (1979) have shown that the total microbiota of the cæcal contents and that of the soft faeces are similar (approximately $10^{11}$ bacteria/g), whereas in hard faeces, the number of bacteria is 10 times weaker.

Amylolytic flora is present as soon as 15 days of life, i.e. before the rabbit consume starch, at a high level $10^{10}$-$10^{11}$ bacteria/g and then, does not decrease (figure 4).



from: Boulahrouf *et al.*, 1991; Zomborsky-kovacs *et al.*, 2000; Gidenne and Licois, 2005.

**Figure 4**: Implantation of some bacterial population in the caecum of the growing rabbit.

The facultative anaerobic flora is simple in the rabbit, dominated by streptococci until 14 days of age. Enterobacteria appears when solid feeding begins. The absence of the genus *Lactobacillus* in the rabbit flora is noteworthy and original. The strictly anaerobic, non-sporulating bacteria, especially Gram-negative bacilli (*Bacteroides*) dominate the digestive flora in every segment of the intestine. Sporulating bacteria are 100-1000 times less numerous than the *Bacteroides* and belong to the genera *Clostridium, Endosporus* and *Acuformis*. The streptococci (*S. faecium, S. faecalis*) reaches a maximum in rabbits aged 7-14 days and then declines with age. *Escherichia coli* is generally absent in 2-3 day old rabbits. This type of bacteria appears in specific pathogen free rabbits at 7 days (Padilha *et al.,* 1995), reaches a maximum at the end of the third week (up to $10^7$/g) and then falls sharply. This trend is practically independent of the feeding regimen of the young: milk followed by solid feed, or exclusively milked until 42 days (Padilha *et al.,* 1999). Padilha (1996) considered that there are in fact rabbits without detectable *E coli* (less than $10^2$/g) and others with populations reaching $10^4$-$10^5$/g. Ten to 20% of 15-22 day old rabbits have no detectable *E. coli* and this proportion reaches 30% in older rabbits (studied up to 49 days.)

Fibrolytic flora increases slowly to reach $10^7$/g according to Boulharouf *et al.,* (1991), and only $10^3$ in SPF rabbits according to Padilha *et al,* (1995). So long as the rabbits are fed only on milk, the cellulolytic flora does not appear, even in rabbits 35-42 days old (Padhila *et al.,* 1999). However, these studies addressed only the colonisation rate of "bacteria" able to grow on a specified substrate, in *in-vitro* conditions (e.g. roll tubes techniques, see figure 4). For instance, Boulahrouf *et al.* (1991) have shown that caecal bacteria are able to grow on cellulose paper substrate or on citrus pectin or on beechwood xylan. Similarly, ureolytic proteolytic and amylolytic bacteria were isolated from the caecum and caecotrophes (Crociani *et al.* 1984; Emaldi *et al.*, 1979; Padilha *et al.*, 1995). However, this approach is time consuming, and it remains difficult to follow the implantation dynamic or relationship with nutrient intake. Furthermore, only some cultivable bacteria are identified.

Some of the strains involved in fibrolysis have been identified in the caecum: *Eubacterium cellulosolvens, Bacteroides sp.* (cellulose), *Bacteroides ruminicola, Butyrivibrio fibrisolvens* (pectins and xylanes; Boulharouf *et al.,* 1991). However from 42 days of age xylanolytic and pectinolytic bacteria become established at a higher density than the cellulolytic bacteria ($10^8$-

$10^{10}$ *vs* $10^5$-$10^6$). Furthermore, fibrolytic bacteria found in the rumen (*Ruminococcus albus*) or in the caecum of the rat (*Flavefasciens intestinalis*) have also been found in the rabbit caecum, using 16SrRNA hybridation methods (Bennegadi *et al.*, 2003).

### 2.2.2 The caecal biocenosis analysed through molecular tools

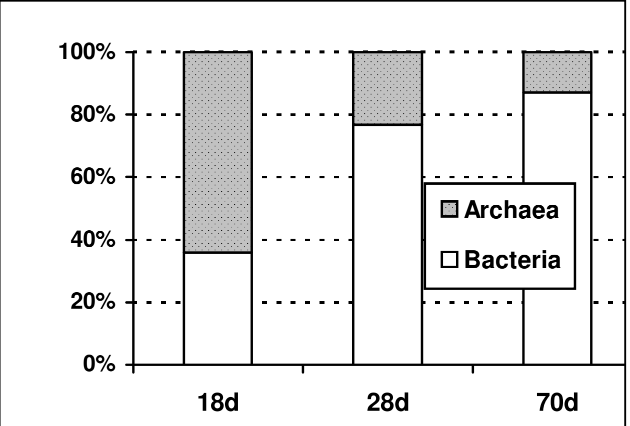


**Figure 5:** Balance among archea and bacterial population in the caecal ecosystem, accordind to age of the rabbit.

Microbiologists presently agree to estimate the uncultivable flora, not reached with cultured-based techniques, would represent from 80 to 90% of the gut flora. Thus, molecular techniques appear essential to understand the complexity of the flora, and more particularly to explore the concepts of biodiversity and stability according to nutritional and feeding factors. Several molecular techniques for microbiology have been developed in the last decade to obtain better knowledge of the bacterial diversity with no culture requirements. Most of them are based on the identification 16S-rRNA/16S-DNA and their analysis by several methods, such restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism 'SSCP), etc. The 16S-rRNA gene, located in the chromosome of all the prokaryotic cells, present several interesting characteristics to allow studies of complex microbiota: it has some highly conserved areas and also some hyper-variable areas. The first characteristic allows the use of some universal primers that lead to the amplification (PCR procedure) of the 16S area of bacteria. The second characteristic enables the resolution between bacteria (Suau *et al.*, 1999).

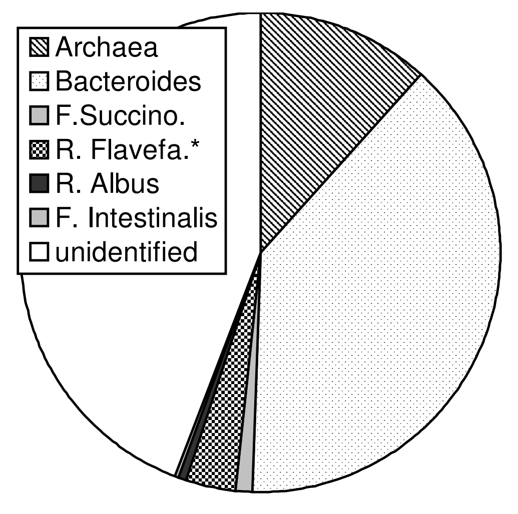


**Figure 6:** Elements of the caecal flora in the rabbit at weaning (28d old)

By using dot-blot hybridization with 16S rRNA targeted oligonucleotides probes, Bennegadi *et al.* (2003) stated that bacteria and archaea represent respectively 73% and 22% of the total microbial communities in the caecum at weaning (figure 5), and were in reverse ratio after weaning. They also demonstrate the predominance of the Flexibacter-Cytophaga-Bacteroides group and the presence of four cellulolytic species, usually identified in the rumen: *Fibrobacter succinogenes*, *F. intestinalis*, *Ruminococcus albus* and *R. flavefasciens*, this last species being dominant (figure 6). However, these fibrolytic species covered only 5% of the caecal flora, since the main part of the flora remains uncultivable. Recently, Abecia *et al.* (2005a) performed a molecular inventory based on 16S RRNA genes from caecal content of one adult rabbit, and found only one sequence with a 97% degree of similarity with previously known sequences in database. This suggest, that numerous bacterial species remain still unknown in the caecal ecosystem.

Some recent studies addressed the question of the caecal flora biodiversity in the rabbit. First results indicated that the biodiversity would change with the age and with the weaning. Suckling rabbits at 16 days of age present similar caecal biodiversity that their mother (figure 7), however when the rabbits begin to eat solid feed the biodiversity decrease (García *et al.,* 2005). After that, it increases in parallel to solid feed intake. However, according to García *et al.* (2005), the degree of similarity of caecal microbiota between the mother and its corresponding litter may be very low (only 10% between the mother and the young rabbits at 16 days of age) possibly due to differences in the food ingested (solid food vs milk). However, when young rabbits from different litters are compared, the brothers present the maximum of similarity (47%).

The weaning also produces a decrease (from 2000 to 600 identified sequences) in the caecal biodiversity. The interpretation of these findings in terms of stability or intestinal health security is not easy and should be complemented by more precise information about the changes produces in the commensal bacteria and potential pathogens. The doe and the litter at 16 days of lactation has the same biodiversity but the degree of similarity of ceacal microbiota is only 10%. In parallel, Abecia *et al.* (2006a) using the DGGE, reported that the differences in caecal microflora (Hamming Distance Analysis) within pups of the same litter was lower than among doe. It was also shown that the bacterial population of the offsprings was largely dependent on the microbial diversity of their mothers. These findings agree with the great influence of the litter origin in the incidence of digestive pathologies in the growth trials.

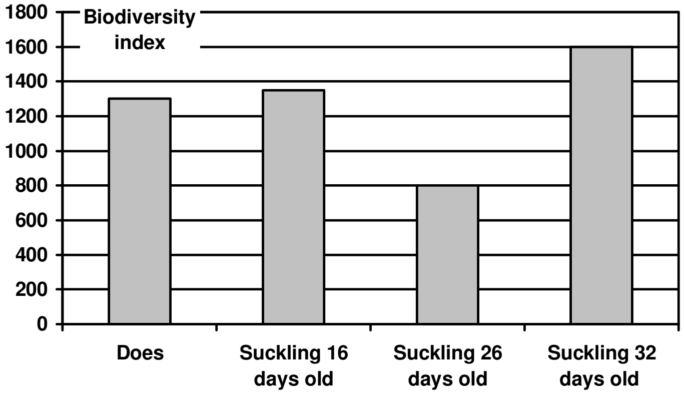


**Figure 7.** Caecal biodiversity of the caecal flora in doe and their litters at different ages.

## 3 Impact of feeding and nutrition on the caecal ecosystem of the rabbit

### 3.1 Nutrient intake and control of the caecal ecosystem

Nutritional factors act on caecal ecosystem at least at two levels: by supplying appropriate quantity and quality of nutrients to the flora, and ensuring good physico-chemical conditions in the caecal biotope, including also motility and digesta transit. Obviously, the microbial activity depends closely upon the nutrient supply, as only a few hours of starvation lead to a sharp decrease in caecal VFA level and to a reversed C3:C4 ratio (Vernay and Raynaud, 1975; Gidenne and Bellier, 1992; Piattoni *et al.*, 1997).

Studies concerning the dietary effect on caecal digestive physiology are often restricted to measuring the fibre digestibility with sometimes a description of the fermentation pattern and/or fibre rate of passage measurements. For instance, a correct assessment of the ileal flow of nutrients, which is of first importance in evaluating the dietary effect on caecal metabolism, remains very difficult and time-consuming, and is rarely performed. Thus it should be kept in mind that the results listed in the following review present a lack of precision to obtain a comprehensive view of the impact of nutritional factors. Effects of nutritional factors upon CMA are here presented only for major nutrients and also for some dietary additives.

### 3.1.1 Impact of fibre and starch intake

A large number of authors deal with the effect of the fibre intake on microbial activity and relationship with the digestive health of the rabbit, since a too lower fibre intake increases the incidence of digestive disorders.

Increasing the fibre intake (and lowering that of starch), without modifying the quality of fibre, either increase or has no effect on the fibrolytic activity and on the caecal VFA concentration, while a lower butyrate molar proportion is generally registered. Low fibre diets would also modify the caecal mucosa and cause flattened colon villi (Yu and Chiou, 1996). Fibre digestibility, which reflects the microbial digestion, is not greatly affected by a lower level of fibre, while the quantity of fibre degraded decreased (combination of a lower feed intake and of a lower dietary fibre level). In fact, we can suppose that the quantity of fibre entering the caecum is not a limiting factor for the fermentation processes, probably because the digesta retention time in the caecum is relatively short allowing the degradation of mainly the easily digestible fibre fractions such as pectins or hemicelluloses. However, for very low fibre level (ADF lower than 10%) some increase in the efficiency of fibre degradation was noticed (De Blas *et al.*, 1986; Bellier and Gidenne, 1996) and may be attributed to a prolonged fibre retention in the caeco-colic segment. Besides, the level of fibre seemed not influence the energetic supply from caecal fermentation (Gidenne, 1994). In association to changes in microbial activity it is suspected that nutrition also modulate the microbial population balance, as suggested by Belenguer *et* al. (2000). For instance, using 16S-rRNA probes, Bennegadi *et* al. (2003) reported that a low fibre intake reduced the archaeal community and the R. albus population, for conventional growing rabbit.

The quality of fibre, particularly their fermentescibility, is able to modulate the microbial activity. For instance, increasing the intake of pectins or hemicelluloses generally stimulates the flora activity (Garcia *et* al., 2000; Gidenne and Bellier, 2000; Gidenne *et al*., 2004a). In a collaborative study, Garcia *et al.* (2002) reported that dietary uronic acids concentration is positively correlated to the caecal VFA and propionic levels. A supply of digestible fibre could thus stimulate the CMA and favour its establishment in the growing rabbit. This assumption was supported by the results of Candau *et al.* (1978) who reported an earlier stabilisation (at 5 wk of age) of the caecal fermentation for rabbits fed a diet based on beet pulp compared to a lucerne-based diet (at 7 wk). Interactions between the establishment of the caecal fermentation pattern and dietary fibre supply were also reported by Bellier (1994). As reported by Jehl and Gidenne (1996), the intake of digestible fibre (compared to starch) favoured the in-vivo caecal fermentative activity (+25%) and also the daily bacterial biomass production (+80%). However the biomass recycled in soft faeces (13% of the feed intake) was not affected by the diet, as it was also found by Garcia *et al.* (1995).

Another way to analyse the role of cell-wall polysaccharides that are rapidly fermented is to determine the Neutral Detergent Soluble Fibre residue "NDSF" (Hall *et al.*, 1997), which corresponds to the cell wall polysaccharides soluble in neutral detergent solution (= sum of water soluble and insoluble pectins + β-glucans + fructans + oligosaccharides[DP>15] ). Although the level of NDSF is moderate in rabbit feeds, a reduction of its level (12% vs 8%) could be unfavourable on digestive health of the early-weaned rabbit, possibly due to changes in caecal microbiota balance. Reversely, a higher level of NDSF would improve the mucosal morphology and functionality and its immune response. Besides, soluble fibre reduced the proportion of animals with *Clostridium perfringens* in the caecum and other pathogens as Campylobacter both in the ileum and in the caecum. Accordingly, mortality due to REE was reduced with a diet with 12% soluble fibre (Gómez-Conde *et al.*, 2004a,b, 2005, 2006).

Starch not completely hydrolysed in the small intestine is then quickly fermented by the caecal flora. The effect of the starch intake could be addressed, by modifying the level and the nature of starch. For instance, Pinheiro (2002) studied the effect of crude potato starch "PS" (highly resistant to intestinal digestion) on the caeco-colic digestion and on microbial activity. Increasing the dietary level of PS (0-7-14%) tripled the ileal starch concentration (Pinheiro and Gidenne, 2000) and ileal flow, but did not affect the fibre digestion in the caecum, nor the bacterial fibrolytic activity, not the caecal fermentative activity (VFA level and proportions). Similarly, Gidenne *et al.* (2005) reported no significant effect of the nature of starch (wheat vs maize vs pea) on the caecal fermentative traits of the growing rabbit. Thus, starch would not play a major role in the control of the caecal microbial activity, at least in the growing rabbit (over 6 weeks old). Nevertheless, in the young rabbit around the weaning period (< 6 wks old), since the starch digestion in the intestine would less efficient, the starch flow entering the caecum would be greater and may affect the caecal ecosystem.

### 3.1.2 Impact of protein intake

The quality of the dietary protein source affects evidently the protein ileal flow, that is fermented by the caecal flora and converted to ammonia that represents the main nitrogen source for the microbial protein synthesis. The caecal ammonia level ranges generally between 4 and 18 mM/L. Other metabolic pathways contribute to the supply of ammonia to the caecum. About 25% of the caecal ammonia pool originate from the catabolism of blood urea absorbed by the caecal wall (Forsythe and Parker, 1985a) and then converted to ammonia by ureolytic flora (Emaldi *et al.*, 1979; Crociani *et al.*, 1984). In addition, some urea (15% of the urea degradation rate) was provided through the ileal digesta. As judged from rumen levels, Carabaño *et al.* (1988) estimated that caecal ammonia level is not a limiting factor for the microbial protein synthesis. It could be of interest to replace dietary protein by non-proteic nitrogen sources. For instance, urea is efficiently utilised if directly infused into the caecum (Salses *et al.*, 1977). But numerous studies have demonstrated that urea supply is useless, because 90% of the urea is absorbed before reaching the caecum (Candau *et al.*, 1980). However, Makkar *et al.* (1990) reported that the enzymatic cellulolytic activity should be higher for a diet supplemented with urea. More recently, Crociani *et al.* (1991) pointed out that biuret could be efficiently utilised by the growing rabbit, and shown the presence in the caecum of an aerobic facultative biureolytic flora.

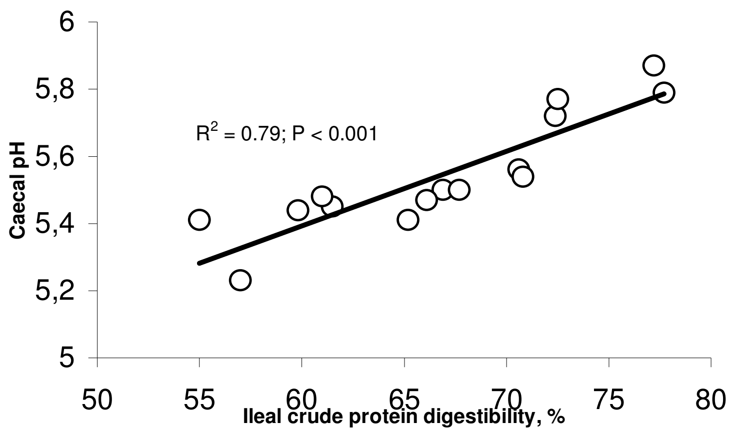


**Figure 8**: Relationship among ileal digestibility of protein and the caecal pH, in 35d old rabbits (n = 15), weaned at 25d

Few studies were performed to assess microbial activity according to the dietary supply of protein. Increasing the dietary crude protein level from 12.8 to 16% led to a rise in caecal nitrogen concentration, but the effect on caecal pH are controversial, and VFA caecal concentrations tended to increase with increasing dietary protein level (Fraga *et al.* 1984; Al-Bar and Al-Aghbari, 1996). An excess of dietary protein could also favour the proliferation of *Clostridia* in the adult rabbit and could also slightly increase the prevalence of *E. coli* (Cortez *et al.*, 1992).

In the case of the young rabbits, having high protein requirements, an excessive replacement of digestible fibre by protein increase the health risk for diarrhoea, as shown in a large scale study (Gidenne *et al.*, 2001). A hypothesis to explain it could be a higher availability of substrates for microbial growth, with prevalence of pathogenic species, when animals are fed with high protein diets Accordingly, a higher ileal flux of protein increased the caecal acidity (figure 8: Gutiérrez *et al.*, 2003; Nicodemus *et* al., 2003, 2004; Gómez-Conde *et al.*, 2004a,b and personal comm.), and may favour pathogenic species. This event would be more important in young rabbits due to the unachieved digestive maturation. Besides, some harmful genera, as *E. Coli* or Clostridia, can use amino acids as substrate for growth. For instance, Clostridium would increase when the diet contained an excess of protein (Catala and Bonnafous, 1979; Haffar *et al.*, 1988).

### 3.1.3 Impact of lipids intake

Unsaturated fat escaping digestion in the small intestine of the monogastric is subjected to hydrogenation (increase of the degree of saturation) by the caeco-colic flora. However, as the fat level is generally low in rabbit feed (1 to 4%), fat hydrogenation by the caecal flora may be presumed to be of relatively low importance. Information is lacking about the effect of dietary fat addition on caecal microbial activity. However, the efficacy of fibre degradation would be improved in case of fat addition (Santoma *et al.*, 1987; Fekete *et al.*, 1989; Falcao E Cunha *et al.*, 1996), and the weight of the caecal wall and of digesta content would be higher (Falcao E Cunha *et al.*, 1996).

Besides, it has been recently found that some medium-chain fatty acids, such caprylic and capric acid (as triacylglycerol form), exhibit antimicrobial activity for some bacteria of the caecal digestive flora (Marounek *et al.*, 2002), and would have favourable impact on digestive health of the growing rabbit (Skrivanova and Marounek, 2005). However, Gallois (2006) did not found a significant effect of a dietary incorporation (+2%) of medium chain triglycerides (mix of C8 and C10) on the bacterial fibrolytic activity or VFA pattern in the caecum of the young rabbit.

### 3.1.4 Impact of nutritional factors on caecal biodiversity.

Recently, three collaborative studies between microbiologist and nutritionist (CRESA, Barcelona and UPM Madrid) assessed the effect of nutrition on intestinal microbiota in a context of a farm with Epizootic Rabbit Enteropathy, using the T-RFLP method. The level and type of fibre and protein were studied since they are in relation with the digestive problems in rabbits (Nicodemus *et al.*, 2004, Goméz-Conde *et al.*, 2004; Chamorro *et al.*, 2005, Gómez-Conde *et al.,* 2006). The results were obtained on healthy rabbits, weaned at 25 days and supplemented with antibiotics (Zn bacitracin and Apramicine) or without any antibiotic, and they are summarised in the Table 1.

A low mortality was observed with 30 % of insoluble fiber (NDF) and 12% of soluble fibre and 16% of protein. Except for the study of dietary fibre level (Nicodemus *et al.*, 2004), the reduction of mortality occurred in parallel of a reduction in the proportion of animals having *Clostridium perfringens*. However, this effect was more evident in ileal (protein level) or caecal samples (type of fibre) depending on the type of substrate available to microbiota. Same opportunistic harmful bacteria associated to the intestinal mucosa, such as *E. Coli*, *Campylobacter*, *Yersinia* or *Helicobacter* seem to be more sensitive to changes in the diet when the sample is taken at the ileum than in the caecum. So a correct selection of the sampling intestinal place is important to detect significant effects of the diet on potential pathogenic bacteria.

**Table 1**. Effect of type of diet on Biodiversity (35-39d old), the proportion of animals where potential pathogenic bacteria were detected and on the mortality in early weaned (25d) rabbits.

| | Biodiversity | | *Clostridium perfringens* | | Other bacteria | | Mortality |
|---|---|---|---|---|---|---|---|
| | Ileum | caecum | Ileum | Caesium | Ileum | Caecum | |
| Decrease in the level of fibre (25 vs 30% NDF) (1) | Increase | Decrease | No effect | No effect | Increment of Bacteroides | Decrease of *Bacteroides* and *Ruminococos* | Increase |
| Increase of particle size (large vs normal) (1) | Decrease | Decrease, low fibre diets (25 % NDF) | No effect | No effect | Decrease of *E. coli, Helicobacter* or *Yersinia* | Little effect | No Effect |
| Increase of soluble fibre (2) | No effect | No effect | No effect | Decrease | Decrease *Campylobacter* | Decrease *Campylobacter* | Decrease |
| Level of protein (16 vs 18% CP) (3) | Decrease | No effect | Decrease | No effect | Decrease *Campylobacter* | | Decrease |
| Type of protein (alfalfa vs soybean concentrate + fibre) (3) | No effect | No effect | No effect | No effect | Decrease *Clostridium* spp. | | No effect |

From : Nicodemus *et a*l.(2004). 2: Gómez- Conde *et al.* (2004, 2006). 3: Chamorro *et al*. (2005).

With respect to the microbiota structure, a less **biodiverse** microbiota seemed to favour a higher proliferation of *Clostridium Perfringens* in piglets. Pigs parenterally nourished seem to favour a higher proliferation of Clostridium Perfringens with respect to those fed enterally. This tendency was not confirmed in rabbits fed enterally with several diets. Increments of the animals with presence of C. Perfringens were observed in parallel to the increments in the biodiversity (Chamorro *et al.*, 2005) or without changes in this index (Gómez Conde *et al.*, 2006). However, ileal biodiversity seemed more correlated with the mortality. An increment of ileal digestibility was associated with a higher mortality. So this index would be a used as predictor of the intestinal health if this results are confirmed.

## 3.2 Feeding strategy and control of the caecal ecosystem

Breeding techniques that modify the intake pattern are also able to modulate the digestive function, such age at weaning or feed restriction.

### 3.2.1 Age at weaning and role of milk/solid feed intake

Solid feed intake is correlated to the milk intake, and thus the digestive maturation is also modified. For instance, feeding young rabbits exclusively with milk, beyond the classical weaning age (till 42d), sharply inhibit the implantation of the fibrolytic flora, and the fermentative profile is specific of a proteolytic metabolic activity (with high $NH_3$ level), associated with a very low volatile fatty acid concentration (<10 mM/l) and a high pH (Padilha *et al.*, 1999). Similarly, Zomborszky-kovács *et al.* (2000) reported a slower development of the caecal microbial activity in double-suckled kits compared to single-suckled. Reversely, an early removal of milk (from 18-25 days of age) by an early weaning, or a lower milk intake stimulated the solid feed intake, would led to a higher concentration of volatile fatty acids and a lower caecal pH compared with animals of the same age which are receiving only milk (Maertens and Piattoni, 2001; Xiccato *et al.*, 2003). However, Gallois *et al.* (2006) did not found a significant effect of an early weaning (21 vs 35d) on the bacterial fibrolytic activity or on the caecal VFA level.

### 3.2.2 Impact of the feed intake level

Usually, studies on intake regulation aims to analyse the effects on the carcass quality of the growing rabbit, or to analyse the digestive efficiency. But, more recently some studies deal with the relationship between intake level and digestive trouble incidence, including a study with an experimental ERE infection. The effect of a quantitative linear reduction of the feed intake level (100 to 60%) on digestive health and growth of the rabbit was measured through a large-scale study (6 experimental units, 2000 rabbits per treatment, Gidenne *et al*., 2003). During feed restriction, the mortality and morbidity rates were significantly reduced (resp. from 12 to 3.5%, and from 12 to 6% for ad-lib+90% feeding level vs 70+60%). The feed restriction during 20d after weaning reduced proportionally the growth rate. Thereafter, returning to an ad-libitum feed intake led to a compensatory growth and to a higher feed efficiency. Moreover, Boisot *et al*. (2003) also demonstrated a similar positive effect of feed restriction when rabbits were challenged with ERE inoculum. Further similar results were also obtained more recently by reducing the intake level through a time restriction for water consumption (Boisot *et al.,* 2004; Verdelhan *et al.*, 2004).

Physiological mechanisms explaining such a favourable effect of reducing the intake level on diarrhoea incidence remained to be deeply studied. However, 5 hours after meal distribution, the caecal VFA level increased linearly with the feed restriction level (from 100 to 60%) and the caecal pH decrease, but no changes in fibrolytic activity were found (Gidenne *et al.*, 2004b). This suggest that feed intake would have an impact on the caecal ecosystem, particularly on the circadian variation of the fermentations, that favours a better resistance to digestive troubles. Further studies on the relationship among intake and caecal flora is required.

Recently, Abecia *et al*. (2006b) studied the caecal microflora of doe (through DGGE) having a litter of 5 or 9 kits and thus having a low or a high feed intake. The effect of the feeding level on flora similarity was identified when there was no antibiotic treatment. This effect disappeared with Thiamulin treatment but not with bacitracin.

### 3.2.3 Control of the caecal flora through probiotics or antibiotics

Probiotic is a feed supplement product containing live microbial which would beneficially affects the host animal by improving its intestinal microbial balance (Fooks and Gibson, 2002). While numerous studies analysed the potential impact of probiotics on growth mortality or caecal fermentation pattern, very few tried to evaluate their impact on the commensal flora of the rabbit. Literature did not report significant impact of a dietary addition of live bacteria on caecal ecosystem, although addition of *bacillus toyoi* seemed to reduce the E. coli flora (Hattori *et al*., 1984). Live yeast would have a more clear effect on caecal microflora, since they could modify the Redox potential by consuming the oxygen in the medium as shown in rumen (Marden and Bayourthe, 2005). For instance, Gidenne *et al.* (2006) reported that dietary addition of *Saccharomices cerevisiae* increased slightly the proportion of R. albus (through 16S rRNA hybridation method) but not other fibrolytic bacteria.

Abecia *et* al. (2006a, b) found that an antibiotic treatment could increase the differences in flora pattern among doe, and therefore influence the flora of the litter (using DGGE technique and Hamming Distance Analysis). Thiamulin had a marked effect on biodiversity than bacitracine. In return, the latter improved the fibre digestibility but did not affect the total caecal flora or the cellulolytic or amylolytic one (Abecia *et* al. 2005b).

## 4 Conclusions and perspectives

Numerous studies from nutritionists teams addressed the effect of the activity of the flora (through indirect methods, such fermentation patterns, enzymology, etc.) on the gut and more largely on animal performances and health. However, in domestic mammals, the gastrointestinal microbiota is a very complex community, where only the cultivable flora was partially known. In the past decade, the development of new molecular tools revolutionised the microbiological concepts. These techniques are still developing, and provide a more extensive view of the gut flora. Also new connections among microbiologists and nutritionists are emerging. For the rabbit, several teams are engaged in these topics, with a specific aim : understanding the maturation of the commensal flora in relation to the nutrition and breeding techniques to improve the prevention of the digestive disorders, particularly in the young.

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