Controlling the rabbit digestive ecosystem to improve digestive efficacy and health[§]

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ABSTRACT: The digestive ecosystem has several physiological roles: hydrolysis and fermentation of nutrients, immune system regulation, angiogenesis, gut development and acting as a barrier against pathogens. Understanding the digestive ecosystem and how to control its functional and specific diversity is a priority, since this could provide new strategies to improve the resistance of the young rabbit to digestive disorders and improve feed efficiency. This review first recalls some facts about the digestive microbiota composition in the main fermentation compartment, and its variability in rabbits with some new insights based on recent molecular approaches. The main functions of the digestive microbiota will then be explained. Finally some possible ways to control rabbit caecal microbiota will be described and a suitable timing for action will be defined.

Key words: Microbiota, Implantation, Digestive efficacy, Immune system development, Health, Rabbit.

INTRODUCTION

Mammals can be regarded as super-organisms as they are permanently colonized by a vast and rich community of microorganisms. There is a host / microbiota relationship based on a model of symbiosis that defines "the digestive ecosystem" where each partner benefits from the association. Indeed, microorganisms colonize and grow rapidly under the favourable conditions of the gut, while the rabbit obtains the products of microbial fermentation from materials that could otherwise not be digested. In rabbits this association is called a combined competition-cooperation model (Mackie, 2002). However, the balance of this ecosystem is fragile and may be disturbed during digestive disorders. In recent years a considerable research effort using the techniques of molecular biology and microbiology have helped define its composition, understand its functioning and its many physiological roles: hydrolysis and fermentation of nutrients, immune system regulation, motility effects, angiogenesis and intestinal trophism, and acting as a barrier against infectious agents.

Control of the microbiota could therefore improve digestive efficiency or immune status and thus digestive health. Improved digestive efficiency through optimization of the composition of the microbiota has a direct impact on feed costs, and would also increase the use of "fibrous" raw materials useless for human consumption. Similarly, improving digestive efficiency would reduce emissions to the environment. Note that unlike ruminants, reducing the emission of greenhouse gases is not a major issue of the rabbit industry since the growing rabbit produces little methane (Franz *et al*., 2011). Finally, control of the microbiota could limit digestive problems around weaning, firstly through its barrier effect and partly through its role as immune stimulator. In this review, we will endeavour to take stock of knowledge about the composition and functioning of the

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ecosystem in the rabbit caecum. This paper highlights the physiological roles of the microbiota and the benefits for the host. Furthermore we will evaluate the possibility of engineering the microbiota to produce a better outcome for the host. The applied objectives are to reduce the frequency of occurrence of digestive disorders and / or to improve feed efficiency.

SPECIFIC DIVERSITY OF THE RABBIT GUT ECOSYSTEM

The digestive tract of animals, and particularly of mammals, is a habitat very conducive to the development of microorganisms. Indeed, the transit speed is quite slow, the acidic to neutral pH (6 to 6.5) of the medium is associated with high humidity (75-95%) a high and stable temperature (35 to 40°C) and relatively low redox potential, (<200mv; Kimsé *et al*., 2009). The intestinal microbial community, called microbiota, is abundant, since it consists of about 100 to 1000 billion microorganisms per gram of digesta. Its diversity and complexity is very high, with about a thousand different species. Bacteria predominate, (10^{10} to 10^{12} bacteria/g ceacal content) (Gouet and Fonty, 1973; Forsythe and Parker, 1985; Combes *et al*., 2011), while the archaeal population is estimated at 10^{7} per g of content (express in copy 16S RNA gene; Combes *et al*., 2011). Regarding eukaryotes, the rabbit caecal digestive ecosystem appears to lack anaerobic fungi (Bennegadi *et al*., 2003) and yeast (Kimsé *et al*., 2012) although commensal yeasts have been found in the caecum (10^{6}/g Forsythe and Parker, 1985). Protozoa are absent from the caecal ecosystem (Bennegadi *et al*., 2003) except in animals suffering from coccidiosis (Lelkes and Chang, 1987).

Microbiota taxonomic composition

The taxonomic diversity of the rabbit digestive ecosystem was first studied by culture techniques (Fonty and Gouet, 1989). These studies, based on the functional aspect of microorganisms and their ability to grow on defined substrates, have shown that the adult rabbit hosts 10^{7} and 10^{6} CFU (colony forming unit) of cellulolytic bacteria per gramm of caecal contents and faeces, respectively. Populations of pectinolytic and xylanolytic bacteria are between 10^{9} and 10^{10} CFU bacteria per gramm in the colon and caecum. Cultivable species most frequently identified were *Eubacterium cellulosolvens* for cellulolytic bacteria and *Bacteroides ruminicola* for pectinolytic and xylanolytic bacteria (Boulahrouf *et al*., 1991). Moreover, the cultivable fraction of the rabbit digestive microbiota in healthy adults was characterized by the absence or low density of *Lactobacillus, Streptococcus* and *Escherichia coli* (Ducluzeau, 1969; Gouet and Fonty, 1973; Fonty *et al*., 1979; Yu and Tsen, 1993; Padilha *et al*., 1996). In the last ten years, molecular microbiology techniques have led to substantial progress in the knowledge of the microbial diversity of digestive ecosystems. These techniques are often based on the use of genes encoding RNA of the small 16S subunit of prokaryotic ribosomes (16S rDNA) (Deng *et al*., 2008). This molecule is a good marker of the diversity of prokaryotes. Indeed, it is ubiquitous (present in all prokaryotes), and contains highly conserved areas and other highly variable that distinguish families and genera of them. Moreover, it is easily detectable because of its large number of copies. Finally the 16S rDNA is a neutral marker of evolution: this molecule has evolved over time in the absence of selective pressure, thus it allows us to classify the microorganisms but also to understand their evolution (Case *et al*., 2007). There are several methods using 16S rDNA to study microbial diversity: quantification by real-

time PCR, cloning (Abecia *et al*., 2005; Monteils *et al*., 2008; Kušar and Avguštin, 2010) and molecular fingerprinting (DGGE, RFLP, CE-SSCP etc...) (Abecia *et al*., 2007a,b,7c; Chamorro *et al*., 2007; Gomez-Conde *et al*., 2007; Gómez-Conde *et al*., 2009; Michelland *et al*., 2010a; Michelland *et al*., 2011). More recently deep 16S rDNA pyrosequencing was developped, which could be considered as a 2nd generation 16S rDNA fingerprinting (Lamendella *et al*., 2012). It provides a more complete picture of the composition of gut microbial inhabitants than previous techniques and provides considerable knowledge about the identity of the dominant member of the community (Lamendella *et al*., 2012). Currently, the development of high-throughput 'omics' methods, make it possible to investigate all levels of biological information of complex microbial communities. Indeed, metagenomics, metatranscriptomics, metaproteomics and metametabolomics are employed to explore at a given time within an ecosystem the DNA sequences, the collectively transcribed RNA, and the translated proteins and the metabolites resulting from cellular processes respectively (Siggins *et al*., 2012). In rabbits, deep 16S rDNA pyrosequencing was recently performed and has described for the first time the relative abundance of the main genera present in the caecal ecosystem (Massip *et al*., 2012, Combes *et al*., submitted to JAS). Moreover, an initial study of functional metagenomic in rabbits has allowed the characterization of caecal cellulase enzymes as yet unknown (Feng *et al*., 2007).

Bacterial community inventories (46 clones: Abecia *et al*., 2005 588; 228 clones: Monteils *et al*., 2008) revealed that most of the identified sequences correspond to new uncultivated bacterial species not found in the databases (90% Abecia *et al*., 2005; 80% Monteils *et al*., 2008). These studies also showed the uniqueness of the rabbit caecal microbiota, since half of the sequences described in each study are phylogenetically close to each other. Phylogenetic analysis listed the overwhelming majority of the sequences in the *Firmicutes* (over 90% of sequences), while the *Bacteroidetes* represented only 4%. In agreement with these results, the *Firmicutes* population density of adult rabbits, as assessed by real-time PCR, was 10.8 $\log_{10}$ copies of 16S rDNA / g of caecal contents, while the genera *Bacteroides* and *Prevotella* density was ten times lower (9.7 $\log_{10}$ copies of 16S rDNA / g) (Combes *et al*., 2011). In the same way, deep 16S rDNA pyrosequencing of caecal content of the rabbit (63 d) showed a preponderance of the *Firmicutes* phylum (about 90%), followed by *Bacteroides* (4.6%), then *Actinobacteria* (0.9%) and *Proteobacteria* (0.7%). Within the *Firmicutes* phylum, the families of *Ruminococcaceae* and *Lachnospiraceae* were dominant (45% and 35% of whole sequences, respectively) (Massip *et al*., 2012).

Archaea that reside in the digestive tract are all strictly anaerobic methanogenic. Integrated at the end of the food chain, they allow the elimination of H_2 from fermentation to provide methane (Jones *et al*., 1987). Methane is a powerful greenhouse gas (23 times as warming as CO_2) and also represents a loss of 6 to 8% of the energy and carbon ingested by the animal (Boadi *et al*., 2004). In rabbits, the data on the archaeal community are limited. Methanogenesis was first observed *in vitro* (Piattoni *et al*., 1996; Marounek *et al*., 1999; Yang *et al*., 2010; Belenguer *et al*., 2011) and more recently *in vivo* using respiratory chambers (Belenguer *et al*., 2011; Franz *et al*., 2011). The simplicity of the CE-SSCP profiles obtained for the archaeal community indicates a much lower species diversity than the bacterial population. Indeed, archaea diversity is low in the mammalian digestive ecosystems. The order that prevails is the order of *Methanobacteriales* with *Methanobrevibacter* as main genus (sometimes associated with some *Methanomicrobium*, *Methanobacterium* and *Methanosarcina* (Order:

Methanosarcinales) (Jarvis *et al*., 2000; Wright *et al*., 2007). The molecular inventory of the archaeal population recently made for rabbits (Kušar and Avguštin, 2010) confirmed the predominance of the genus *Methanobrevibacter* and suggested the presence of a new species specific to rabbit. *In vitro*, the amounts of methane excreted depend on the diet of the rabbits (Belenguer *et al*., 2011) or the nature of the substrate placed in the presence of inoculum (Yang *et al*., 2010). Great variability of methane excretion was observed *in vivo* between individuals (excretion of methane was detected only in two individuals out of sixteen: Belenguer *et al*., 2011). This suggests the existence of a genetic effect but also the existence for some non-methano-excreting rabbits of another route for the elimination of H_2, i.e. reductive acetogenesis. The amount of energy lost as methane is lower in rabbits than in dairy cows (1% vs. 6% of gross energy ingested Vermorel, 1995; Franz *et al*., 2011)

Microbiota structuring

Although the caecum is the primary fermenter in rabbits, a microbial population is also present in the proximal (stomach, small intestine) and distal (colon) segments of the gastrointestinal tract (Gouet and Fonty, 1979). The stomach of rabbits contains 10^4 - 10^6 CFU bacteria / g in adulthood. The small intestine contains 10 - 100 fold more bacteria. The colon has a population similar to that of the caecum (Gouet and Fonty, 1979), which is still 100 - 1000 times more than in the ileum. Bacterial diversity is higher in the ileum than in the caecum according to fingerprinting (Badiola *et al*., 2004; Martignon *et al*., 2010b). This difference is surprising since a faster passage of food particles in the ileum, would not be favourable to bacterial proliferation and diversity. Moreover, the bacterial density of soft faeces, which correspond to the caecal contents slightly modified, is of the same order of magnitude as that of the caecum (10^{11} bacteria / g). Conversely, the bacterial density of faeces, which are richer in large particles (> 0.3 mm), is 10 times lower than that in the caecum (Emaldi *et al*., 1978). Similarly, the structure of the archaeal and bacterial community of soft faeces is closer to that of the caecal content than that present in the faeces (Rodriguez-Romero *et al*., 2009; Michelland *et al*., 2010a,b). The feature of the spatial structure of the community is mainly due to the differences or similarities in chemical composition between the different digestive compartments. Indeed, the physicochemical factors of the ecosystem play a major role in the selection of species of microorganisms, each of which has specific physiological characteristics. Altogether, studies in different gastrointestinal segments of the rabbit suggest the use of soft faeces for monitoring the dynamics of the microbiota of the caecum, limiting thus surgery or sacrifice of the animal.

In the absence of induced perturbations, the bacterial community of the adult rabbit caecum remained stable over time (Michelland *et al*., 2010a; Michelland *et al*., 2011). In agreement with observations made in man (Zoetendal *et al*., 1998; Vanhoutte *et al*., 2004), the absence of temporal variations in the rabbit caecal microbiota adult shows a remarkable stability of the dominant microbial composition and indicates that the ecosystem has reached equilibrium.

The analysis of caecal microbiota by molecular fingerprint (CE-SSCP) did not reveal in the rabbit the existence of a pattern specific to each individual, stable in time or space (compartments) (Michelland *et al*., 2010a). Indeed, the inter- and intra-individual bacterial and archaeal communities are of similar magnitude (Michelland *et al*., 2010a,b). A high variability of the bacterial community composition between individuals has already been shown in chickens (Wielen *et al*., 2002), however there are

few studies that evaluate the intra individual variation (repetition of the same individual over time or in space). In humans, a pattern specific to each individual is found within the various segments of the colon (ascending, descending and transverse) (Zoetendal *et al*., 2002) or over time in the faeces (Vanhoutte *et al*., 2004). The lack of pattern or structure of the archaeal and bacterial community specific to the individual host in rabbits may have originated in the genetic similarity between animals from selected lines and the high standardization of rearing conditions and feeding. These parameters would tend to equalize the influence of the host on the composition of the bacterial community.

ROLES OF THE DIGESTIVE MICROBIOTA

Role in digestion and feed efficiency

One of the most obvious roles of the digestive ecosystem is its ability to hydrolyze and ferment nutrients. In rabbits and monogastric herbivores, digestion of nutrients takes place mainly in the small intestine through the digestive enzymes of the host. These enzymes hydrolyze most components with the exception of components of plant cell walls or fibres (lignins, cellulose, hemicelluloses, pectins, etc.) (Fonty and Gouet, 1989), which are hydrolyzed by bacterial enzymes. Because of the low microbial density and fast passage of digesta in the upper part of digestive tract, dietary fibre that enters the caecum is little modified. This fibre, plus the small intestine's undigested nutrients and endogenous secretions (mucopolysaccharides, cell debris, enzymes) are the main source of carbon for the microbiota. At the end of the ileal segment, fibre is the major constituent (70% dry matter Gidenne, 1992), while nitrogen compounds come next (15% dry matter) (Villamide *et al*., 2010). The metabolic activities of microbiota depend on the nature of incoming substrates and are organized in a trophic chain. The first step of the trophic chain corresponds to the hydrolysis of complex polymers by a variety of hydrolases (polysaccharidases, glycosidases, proteases, peptidases) provided by hydrolytic species in smaller compounds (monosaccharides, amino acids etc...). These soluble compounds are used by hydrolytic and fermentative species as energy sources. Fermentation processes lead to volatile fatty acid production (VFA: acetic acid, propionic acid and butyric acid), ammonia (NH_3) derived from proteolysis, intermediary metabolites (lactic acid, succinic acid, formic acid) and gas (CO_2, CH_4, H_2). All these fermentation reactions allow bacteria to obtain energy for their growth and their multiplication and maintenance of their cellular functions. In rabbits, the role of microbiota in the digestion was first studied through its enzymatic activity and fermentation products (VFA, NH3) (for review Gidenne *et al*., 2008; Carabaño *et al*., 2010). Pectinase, xylanase, cellulase and urease are the major enzymes of the microbial ecosystem in rabbits (Carabaño *et al*., 2010). The hierarchy of bacterial fibrolytic activities (pectinase> xylanase> cellulase) is consistent with that of the digestibility of fibre fractions (pectins> hemicelluloses> cellulose) (Gidenne *et al*., 2008). The fermentation products are important for the rabbit because the VFA and NH_3 are absorbed through the walls of the caecum and colon and are a source of energy for the host. VFA production can cover 30% to 50% of maintenance energy requirements of adult rabbits (Gidenne, 1994). The concentration of VFA in the caecum of an adult rabbit is around 75% acetate, 15% and 10% butyrate propionate. However, these proportions change depending on the age of the animal, the level of intake (Bellier *et al*., 1995) and feed quality, including rapidly fermentable fibre concentration (Gidenne

et al., 2004a). Unlike most herbivores, in rabbits, the ratio of propionate:butyrate is less than 1 because of the characteristics of the microbiota (Adjiri *et al*., 1992). Finally, the caecotrophy behaviour allows the animal to recycle some of the bacterial proteins. Depending on diet, soft faeces ingestion contributes about 15% of the total nitrogen ingested, but this proportion can reach 70% for a diet very low in nitrogen (Garcia *et al*., 2004).

The capacity of the microbiota to provide 30 to 50% of maintenance energy requirements for an adult rabbit emphasizes the significant impact of the caecal ecosystem on the overall digestive efficiency. In rabbits, 30 to 50% of the digestible fraction of digestible organic matter is digested in the caeco-colic segment (Gidenne, 1992; Gidenne *et al*., 2000). In mice, the involvement of the microbiota in feed efficiency has been proved by observing that axenic mice (without microbiota or "germ free") ate more than conventional mice to maintain body weight (Corthier, 2011). Also, when conventional microbiota were introduced into germ-free mice, there was a 60% increase in body fat, concomitant with a decrease in feed intake by 30% in two weeks (Backhed *et al*., 2004). Moreover, the transfer of the microbiota from obese mice to germ-free mice induced an increase in the energy extraction from ingested diet and a greater weight gain than that induced by the transfer of lean mice microbiota to germ-free mice (Turnbaugh *et al*., 2006). Thus, it is demonstrated that the microbiota is involved in feed efficiency in mice. In terms of composition, it has been shown in humans and mice that obese subjects had a ratio of Firmicutes / Bacteroides higher than in lean individuals (Ley *et al*., 2005; Ley *et al*., 2006) and less diversity (Turnbaugh *et al*., 2009). To our knowledge no study in rabbits has helped to connect the feed efficiency and characteristics of the composition of the microbiota.

Role in defence against infectious agents and in the intestinal immune system

The intestinal immune system of the rabbit (GALT for Gut Associated Lymphoid Tissue) is mainly located in the small intestine and colon, as in most mammals, but with two additional special structures: the *sacculus rotondus*, whichis located at the ileo-caecal junction and the vermiform appendix, located at the end of the caecum. The GALT contains more immune cells than the whole body (almost 70% in man; Corthier, 2011). In the small intestine GALT consists of organized lymphoid aggregates: Peyer's patches and isolated cells scattered in the lamina propria and the epithelium of the villi (for review Fortun-Lamothe and Boullier, 2007). The germ-free mouse model, compared to conventional mice, revealed the fundamental role of the intestinal microbiota on the development and functions of the GALT. Beside their barrier role, microbiota mainly stimulates immune organs and cell development, diversification of antibodies and mechanisms of oral tolerance.

Barrier role - The concept of barrier (or colonization resistance) is based on the fact that the microbiota permanently implanted in the digestive tract hinders the implantation of exogenous pathogenic bacteria (Berg, 1996). Indeed, in germ-free animals, the transport of antigen across the intestinal mucosa is increased. Different mechanisms have been proposed to explain the barrier effect: i) Commensal bacteria adherence to the mucosa can prevent attachment and entry of pathogenic bacteria. In rabbits, the filamentous bacteria that colonize the ileum reduce the attachment of enteropathogenic *Escherichia coli* (Heczko *et al*., 2000). ii) the microorganisms compete for nutrients to maintain their ecological niche and habitat by consuming all resources. iii) the bacteria are able to

inhibit the growth of competing bacteria by producing antimicrobial substances (Guarner and Malagelada, 2003).

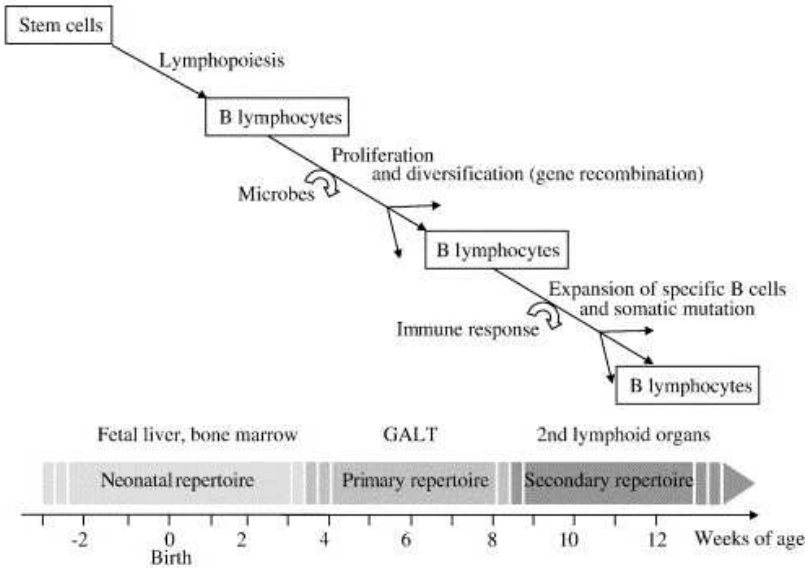
Role of microbiota in the maturation of intestinal mucosa and angiogenesis - The role of the microbiota on the development of the intestinal mucosa was demonstrated by comparing the intestinal epithelium of germ-free animals to conventional animals. The caecum of germ-free rabbits is enlarged by 6 - 10 times compared to that of conventional rabbits (Fonty *et al*., 1979; Coudert *et al*., 1988). In germ-free mice the turnover rate and the number of crypt cells were reduced compared to conventional animals, suggesting that the microbiota reduced cell proliferation in the colon (Guarner and Malagelada, 2003). In germ-free mice, GALT is poorly developed and is comparable to that of a newborn, with a low density of lymphoid cells in the intestinal mucosa, reduced Peyer's patch and low blood immunoglobulin concentration. Some Gram-negative bacteria species, such as *E. coli* and *Bacteroides*, appear to play an important role in this stimulation since their mere presence in the digestive tract of gnotoxenic mice is able to cause a stimulation equal to half of that measured with a complex intestinal microbiota. Indeed, the polysaccharide wall of these bacteria plays an important role in activating the immune system (Mazmanian *et al*., 2005). Furthermore, network of blood vessels of the intestinal villi of germ-free mice is only half as dense as in germ-free mice inoculated with conventional microbiota. In germ-free mice growth of the networks of blood vessel development was stopped prematurely (Stappenbeck *et al*., 2002).

Role in the diversification of the primary repertoire of antibodies - In rabbits, the diversification of the primary repertoire of antibodies continues after birth and is dependent on bacterial stimulation. This diversification begins before birth and ends at the age of 10-12 weeks (Figure 1). Up to 2-3 weeks of age the young rabbits have their narrow neonatal repertoire of antibodies. The establishment of the primary repertoire of antibodies takes place between 4 and 8 weeks of age by recombination processes of nucleotides, gene conversion and somatic hypermutation in the GALT and particularly in the vermiform appendix (Mage *et al*., 2006; Hanson and Lanning, 2008). The microbiota are essential to the production and diversification of the first antibody repertoire (Lanning *et al*., 2000) necessary for the animal to fight effectively against various pathogens. Inoculation of several intestinal bacteria in sterile rabbit vermiform appendix, showed that *Bacillus subtilis* and *B. fragilis* together stimulate B cell proliferation and diversification of genes encoding the immunoglobulin (Rhee *et al*., 2004). More recently, Severson *et al*. (2010) showed that the spores of *Bacillus* stimulated the GALT by a recognition mechanism of superantigen present at the surfaces of spores.

Role in the development of oral tolerance - Although the GALT is continually in the presence of a considerable amount of antigens such as food proteins and commensal microorganisms, it does not develop an immune response, suggesting a host tolerance towards these antigens. The establishment of tolerance mechanisms is also dependent on the presence of the microbiota and takes place early in the life of the host (Fortun-Lamothe and Boullier, 2007). In human medicine, the hygiene hypothesis is that the lack of stimulation or exposure to pathogens and symbiotic microorganisms (microbiota) or frequent use of antibiotics in young children increases the susceptibility of patients to develop allergic disorders and autoimmune diseases. This phenomenon is linked to impaired development of the immune system in relation to changes in the composition of the microbiota (Okada *et al*., 2010). This hypothesis was supported by

recent observations in pigs raised in three different health conditions (outdoor *vs.* building *vs.* in an isolator with antibiotic treatment). Thus, animals reared in isolators have an altered microbiota composition and a higher expression of genes involved in inflammatory immune response (Mulder *et al.*, 2009).

Figure 1 – Schematic representation of antibody repertoire development in rabbit from Fortun-Lamothe and Boullier (2007)



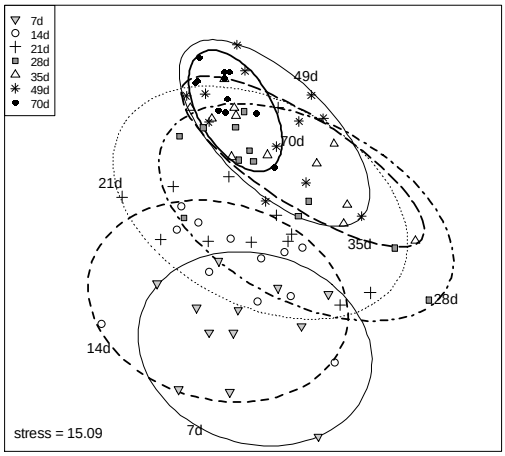
PLASTICITY OF MICROBIOTA

Microbiota implantation and ecological succession of species

Traditionally, the mammal gastrointestinal tracts have been considered sterile *in utero*, however recent studies demonstrated that meconium from healthy newborn were not completely sterile and that a prenatal mother-to-child efflux of commensal bacteria may exist (Jimenez *et al.*, 2008) but both number and diversity are low (Koenig *et al.*, 2011). Microbial colonization really begins at birth in contact with the mother and the immediate environment (birth canal, close to the nest and feed) (Berg, 1996). Like all mammals, the introduction of species is orchestrated by an ecological succession of species. In rabbits, this succession was first studied by culture techniques (Gouet and Fonty, 1973, 1979; Kovacs *et al.*, 2006) and recently molecular tools (Combes *et al.*, 2011). At two days old, the bacterial density is already high in the caecum (10^9 16S RNA copies / g) and increases to reach its maximum at 21 days of age (10^{11} - 10^{12} copies of rDNA 16S.g-1). At this point, the rabbit is still suckling, but has already begun to eat solid food (Gidenne *et al.*, 2010c). During the first weeks of life, the caecal bacterial community is composed of equal numbers of strict anaerobes and facultative anaerobes; then the abundance of the latter falls rapidly and may disappear in some individuals after weaning (Gouet and Fonty, 1979). Bacteria of the *Bacteroides Prevotella* groupwere detected from 2 to 3 days of age (Kovacs *et al.*, 2006; Combes *et al.*, 2011) to reach a peak at 21 days (10^{10}-10^{11} copies of rDNA 16S.g-1 Combes *et al.*, 2011). Moreover, seven days after birth, archaea are present in the caecum at a significant level (10^5 copies of 16S rDNA / g) (Combes *et al.*, 2011). The implantation

of archaea seems to occur later than that of bacteria since it reaches its maximum density at 35 days of age. Molecular fingerprints of the bacterial community allowed, the dynamics of the establishment of the bacterial community present in the caecum to be described (Combes *et al*., 2011). The caecal bacterial community is gradually changing, with a shift in terms of composition and relative abundance (Figure 2). A gradual establishment of an increasingly diverse community is observed, that seems to reach a climax at 70 days of age (Combes *et al*., 2011).

Figure 2 – Age-related variability of the composition of the bacterial communities in the rabbit caecum. Each point represents an individual's microbiota: the closer the points are together, the more similar are the microbiota (Combes *et al*., 2011)



Defining time windows of permissiveness

In mammals, the colonization of the gut begins at birth. Indeed, at this time of life, there is probably little or no barrier to the installation and development of microorganisms. According to Curtis and Sloan (2004), the digestive community of a newborn mammal is a subset of a wider meta-community including all species capable of living in the digestive tract. For example, communities whose environment is similar, have different compositions because they are formed by random sampling from the meta-community around them (mother, bedding, cage, air, etc.). Indeed, the composition of caecal microbiota of young rabbits is highly variable between individuals up to 49 days of age (Figure 2) (Combes *et al*., 2011). Conversely, at 70 days of age the caecal microbiota composition is very homogeneous between individuals (Figure 2). This observation supports the lack of individual specificity of the microbiota (see above). But it also allows us to define an action window (0-49 days) during which it would be possible to modify the microbiota. This action window corresponds to a period of permissiveness in which the barrier effect of the microbiota or host immunity allows the installation of new species, beneficial or pathogenic to the host.

Three scenarii to engineer the microbiota can be proposed from this analysis of the microbiota implantation dynamics: (1) Modify the initial composition: the element of chance in the initial composition of the microbiota can be considered as a possible period for manipulation of the original composition; this manipulation period would take place in the nest; (2) Modify the ecological succession of species: the high variability within age groups persisted up to 49 days, which in rabbits is a period of high digestive health risk. Since the rabbit consumes solid food from 17 days of age (Padilha

et al., 1995; Fortun-Lamothe and Gidenne, 2000), the path of a nutritional modulation of the microbiota could be relevant. 3 – Maturation acceleration: We have shown that whatever the initial microbiota composition, the phenomenon of ecological succession seems to lead to a bacterial community which is very similar between individuals (Combes *et al*., 2011). Therefore, a course of action might be to speed up the installation process so as to accelerate progress towards a climax community. This could correspond to a stable community, as in adult rabbits which are less subject to digestive troubles.

POTENTIAL WAYS TO ENGINEER THE RABBIT DIGESTIVE ECOSYSTEM

Influence of the immediate environment on colonization

The immediate environment at birth plays a role in the initial colonization of the digestive tract. One extreme illustration of this is observed in animals submitted to germ-free breeding. Indeed, if the birth occurs in a totally sterile environment, there will be no microbial colonisation, and a rabbit without microbiota cannot survive for long. Moreover, the composition of the microbiota of rabbits raised in a pathogen-free system (SPF) differs from those raised in conventional farming: fibrolytic population density is greater in SPF rabbits (Bennegadi *et al*., 2003).

The meta-community of the immediate environment that serves as a reservoir for colonization of the digestive tract of young rabbits came from the birth canal of the rabbit, gastrointestinal tract, and fur (direct contact and hairs deposited in the nest). Moreover, during nursing, the doe leaves some faecal pellets in the nest that are eaten by the pups (Moncomble *et al*., 2004; Kovacs *et al*., 2006). This behaviour may contribute to the early implantation of the microbiota in neonates. The prevention of ingestion of maternal faeces by the pups delayed the implantation of *Bacteroides* compared to pups which had access to mother's faeces in the nest. However, this difference did not persist after eight days of age (Kovacs *et al*., 2006). The influence of the caecal microbiota of the nursing mother rather than the biological mother on the pup's caecal microbiota's initial composition was demonstrated by Abecia *et al.* (2007c). DDGE analyses showed that at 26 days of age composition of microbiota of fostered pups was closer to the cohabiting pups than to that of their own non-fostered brother.

Finally, the breeding environment (nest box hygiene, atmosphere) and the breeder (handling of pups for fostering for example) are also sources for microbial colonization of the digestive tract. In pigs separated from their mothers and receiving a milk substitute, the structure of microbiota is more dependent on the environment in which they are raised than their genetic origin (Thompson and Holmes, 2009). In pigs, the composition of faecal microbiota and ileal mucosa microbiota is influenced by the type of farming (outdoor *vs*. building *vs*. in an isolator with antibiotic treatment). Under these conditions, these differences in microbiota composition persisted until the end of the experiment (56 days old) (Mulder *et al*., 2009). In humans, it was shown that birth route (cesarean or vaginal), type of milk (breast milk *vs* infant formula) or antibiotic use influence the initial composition of the microbiota (Penders *et al*., 2006). However, the effect of this initial microbiota composition on the final composition of the microbiota in adults has not been demonstrated.

Influence of nutrition

The food is a key factor affecting the balance of microbial populations in the digestive tract. It conditions the supply of nutrients and energy to the ecosystem. During the biodegradation of the food, it acts on the physicochemical parameters of the medium such as pH, redox potential, metabolite concentrations, and the size and density of particles. In turn, these parameters determine the balance of microbial communities (Fonty and Chaucheyras-Durand, 2007). Although the effect of diet on the microbiota including fibre intake has been the subject of numerous studies (for review Gidenne *et al*., 2008), it remains unclear due to limitations of traditional techniques of microbiology. In most studies only the major taxonomic groups (Bennegadi *et al*., 2003) or functional groups (Boulahrouf *et al*., 1991) were considered.

Effect of weaning - Suckling rabbits using a cow's milk substitute induced significant qualitative and quantitative changes in caecal microbiota between 0 and 11 days of life (Fonty *et al*., 1979). However, in this experiment none of the rabbits fed cow's milk survived beyond 14 days. In rabbits, weaning *ie* the transition to solid food is progressive. From 17-18 days of age, consumption of solid food takes place gradually while the proportion of milk ingested decreases (Gidenne and Lebas, 2006). When rabbits were subjected to an exclusively milk diet (without access to solid food) until weaning, development of the caecum and pectinolytic and xylanolytic activity were lower and the biodiversity index was lower at 30 days than in controls. Nevertheless, differences fade at 37 days (Combes *et al*., 2008). Furthermore, ingestion of milk appears to delay colonization by cellulolytic bacteria without affecting the population of *E. coli* (Padilha *et al*., 1996; Padilha *et al*., 1999). Weaning seems to have a beneficial effect on the maturation of the caecum and colon. Early weaning increases the weight of the organs and their contents without any effect on mucosal morphology (Gallois *et al*., 2005) or strictly anaerobic bacteria (Kovács *et al*., 2012), stimulates fermentation activity (Kovács *et al*., 2012) and accelerates the maturation of GALT (for review Carabaño *et al*., 2010).

Effect of the feed intake level - Dietary restriction is one of the most effective non-drug ways to protect the rabbit against non-specific enteropathy (Gidenne, 2003; Gidenne *et al*., 2012). However the mechanisms of action remain to be elucidated (Martignon *et al*., 2010a). The morphology of the intestinal mucosa, the maltasic and fibrolytic activity, concentration of VFA and finally the structure and diversity of caecal microbiota were not affected by a reduction of 25% in the food intake after weaning (Gidenne and Feugier, 2009; Martignon *et al*., 2010a). Conversely, Abecia *et al*. (2007b) showed that the structure of the caecal microbiota was influenced by the level of intake of does nursing 5 or 9 rabbits.

Effect of the quantity and quality of the fibres - Feeding rabbits with a fibre-deficient diet results in a higher frequency of enteropathy (Gidenne *et al*., 2004a; Gidenne *et al*., 2010b). A reduction in indigestible fibre leads to: i) Alterations in the fermentation profile (decrease of VFA, a sharp increase in propionate and increase and decrease of acetate and butyrate), ii) A change in enzyme activity (decreased fibrolytic activity), and iii) A change in the composition of the microbiota: the structure of the caecal bacterial community (composition and relative abundance of species) is altered (Michelland *et al*., 2011) but not its diversity (Rodriguez-Romero *et al*., 2009; Michelland *et al*., 2011). The quantities of the major bacterial divisions studied decrease (Bennegadi *et al*., 2003; Michelland *et al*., 2011). All these microbial and environmental changes are observable on the second day after the change of diet and remained stable throughout this new

dietary period (Michelland *et al*., 2011). These results also showed that the bacterial community of the rabbit caecum is able to change and adapt rapidly to reach a new equilibrium in response to a nutritional disturbance (e.g. fibre deficiency).
Fibre quality is one of the most prevailing factors. Several studies have shown that an intake of rapidly fermentable fibre (pectins and hemicelluloses) stimulates fibrolytic activity and the VFA concentration in the caecum (Gidenne *et al*., 2010a). The most rapidly fermentable fibres such as pectins are probably the most decisive for the caecal microbial activity, as shown by Garcia *et al*. (2002). Moreover, several studies have shown the favourable effect of digestible fibre on the digestive health of the rabbit (Perez *et al*., 2000; Gidenne *et al*., 2004b). The inclusion of fibres called "soluble" (criterion NDSF), e.g. from beet pulp, also reduces mortality and improves the intestinal mucosa. However, the influence of the level of NDSF on the structure of caecal microbiota remains uncertain (Gomez-Conde *et al*., 2007; Gómez-Conde *et al*., 2009) since the animals were given antibiotics in their drinking water (apramycin sulfate and tylosin tartrate).
Effect of the level of protein intake - The protein concentration of the food and its amino acid content have an effect on rabbit digestive health (for review Carabaño *et al*., 2009; Gidenne *et al*., 2010b). Thus reducing the protein content (21% vs. 18%: Chamorro *et al*., 2007) or arginine supplementation (Chamorro *et al*., 2010) reduced mortality and affected the fingerprint of the ileal and/or caecal bacterial community (RFLP). Arginine supplementation reduced the frequency of detection of *Clostridium* spp and *Helicobacter* spp RFLP compatibility profiles (Chamorro *et al*., 2010). Similarly, lowering the dietary crude protein content led to a reduction in the frequency of detection of *Clostridium* spp RFLP compatibility profiles (Chamorro *et al*., 2007)
Effects of prebiotics - A prebiotic is defined as a "non-digestible food ingredient that positively affects the host by selectively stimulating the growth and/or activity of one or a limited number of intestinal bacteria" (Gibson and Roberfroid, 1995). Prebiotics are mostly short chain carbohydrates (or oligosaccharides) that are not hydrolyzed in the small intestine, and thus arrive unchanged in the caecum and colon. Prebiotics are thus a rapidly fermentable substrate and lead to the production of lactic acid and VFA. Three modes of action are attributed to prebiotics: i) stimulation of the growth of beneficial bacteria for the host, ii) competition by masking the binding sites of pathogenic bacteria to the mucosa and iii) binding to pathogenic bacteria. The two most studied prebiotics are fructo-oligosaccharides (FOS) and manno-oligosaccharides (MOS). FOS stimulates the growth of *Bifidobacteria* and *Lactobacilli*, both of which are considered beneficial bacteria to the host (Gibson and Roberfroid, 1995; Kim *et al*., 2011). The MOS used in chicken, veal and pork would reduce the risk of digestive tract colonization by pathogenic microorganisms by a mechanism of competitive exclusion. Indeed, mannose binds to type 1 fimbriae, which corresponds to a filament that many bacteria use to bind to host cells. Thus, in chickens supplemented with MOS, salmonellae bind to mannose, thus reducing the carriage density (Oyofo *et al*., 1989). Depending on the dose used, supplementation with FOS and MOS decreased the density of *Clostridium perfringens* and *E. coli* in chickens (Kim *et al*., 2011). Moreover, MOS supplementation would alter the structure of the bacterial community of chickens (Corrigan *et al*., 2011). In rabbit, studies on the influence of prebiotics concerned mainly growth performance and caecal fermentation activity but the results are contradictory even for the same type of prebiotic (for review Falcao-e-Cunha *et al*., 2007). According to Falcao-e-Cunha (2007), this lack of consensus may be attributed to variation in experimental factors

between studies, but also because of the nature of rabbit feed, which is rich in fibre and thus may contain significant amounts of oligosaccharides. Recently, an effect of MOS on the structure of the mucosa was observed with an increase in the size of ileal villi (Mourao *et al*., 2006), while inulin did not appear to affect the counts of anaerobic bacteria and *E. coli* (Bónai *et al*., 2010).

Effects of probiotics - Probiotics are living microorganisms used as feed additives for animals and humans that can modulate the activities of the digestive microbiota to improve the health or performance of the host. They consist of one or more species of live microorganisms, with or without culture residues. To act on the digestive caecal ecosystem, the probiotic must arrive alive at its site of action and thus survive the acid attack of the rabbit stomach (pH <2). Yeast (Kimsé *et al*., 2012), and most of the lactic acid bacteria and spores of the genus *Bacillus* are able to resist to stomach acid. Because of the barrier effect exerted by the microbiota, but also of the ecological conditions, which are not optimal for its maintenance and growth, a probiotic microorganism cannot develop in a sustainable manner in the gastrointestinal tract. To maintain the probiotic at a sufficient level, it must be evenly distributed.

The biological effects of probiotics are generally highly dependent on the microorganism strains used, on their ability to maintain metabolic activity in the digestive environment and on their cellular concentration (Fonty and Gouet, 1989). In rabbits, according to the literature reviewed by Falcao-E-Cunha (2007), the addition of a probiotic tends to improve growth performance when the breeding conditions are not optimal. Accordingly, recent results confirmed the favourable effect of live yeast on rabbit health (Kimsé *et al*. 2012). Concerning the action of probiotics on microbiota, Amber *et al*., (2004) showed that the addition of *Lactobacillus acidophilus* increased the number of cellulolytic bacteria and reduced ureolytic bacteria. Furthermore, the addition of yeast (*Saccharomyces cerevisiae*) increased the proportion of *Ruminococcus albus* (Gidenne *et al*., 2006), but did not alter the structure or the diversity of the bacterial community (Kimsé *et al*., 2012).

Some probiotics (lactic acid bacteria) have the ability to adhere to epithelial cells of the host, thus slowing a possible colonization by pathogenic bacteria. Probiotics are also able to produce antimicrobial factors (bacteriocin), or metabolites (lactic acid) or enzymes creating ecological conditions more favourable to the indigenous population. *In vitro*, it was shown that probiotics modulated the host immunity. Finally, probiotics have a direct action on the environmental conditions favourable to the activity of the microbiota (change of pH, redox etc.). In rabbits, the addition of yeast led to an increase of redox without altering the pH (Kimsé *et al*., 2012).

Effect of antibiotics - Since the ban on the use of antibiotics as growth promoter (in 2006), these are currently used therapeutically and can be used on veterinary prescription only. Two major risks exist additionally to the presence of residues in animal products. The first risk is that the presence of antibiotics in the gut of the animal might select resistant bacteria, which can then be transferred to other animals of the same species, other animal species and humans. This transmission can be direct, in the case of contact with the animal, or indirect if the bacteria are released into the environment. This has been particularly highlighted by the emergence of *E. coli* strains resistant to apramycin in humans, although this antibiotic is not used in human medicine (Barton, 2000). The second risk is that an antibiotic use before 8 weeks in rabbits would alter the digestive microbiota, and thus the diversification of the antibody repertoire (secreted by B cells or T cell receptors) (see Figure 1). This has led researchers in

human medicine to formulate the "hygiene hypothesis". Therefore, it seems important to avoid all practices which might limit the development of the microbiota such as exposure to antibiotics directly or indirectly, such as treatment of mothers.
In rabbits, the effect of antibiotics on the microbiota depends on the molecule used Abecia (2007a). The administration of bacitracin (100 ppm), but not that of tiamulin (100 ppm), reduced fermentation activity of the lactating female (Abecia *et al*., 2007b). Conversely, the molecular fingerprints (DGGE) performed on the caecal contents showed that tiamulin, but not bacitracin, modified the structure of the bacterial community. In rabbits after weaning, the administration of 100 ppm and 120 ppm apramycin tylosin reduced mortality but also reduced the microbiota diversity (Chamorro *et al*., 2007). Conversely, a medicated feed containing 500 mg / kg oxytetracycline and 50 mg / kg tiamulin did not change the cellulase and pectinasic activity, caecal counts of anaerobic bacteria or *E. coli* (Bónai *et al*., 2010).

Influence of host genetics on microbiota

To study the influence of host genetics is equivalent to answering the following question: is there a genetic effect on implantation and / or the final composition of the microbiota of the host? In humans, the microbiota of individuals within the members of one family is closer than between individuals from different families (Zoetendal *et al*., 2001). This similarity may result from a genetic effect but the effect of a common environment cannot be ruled out. Indeed, the study of Abecia *et al*. (2007c), tended to show that the influence of genetic origin played little part in the colonization of the caecal microbiota of young rabbit, since the community structure of fostered pups is closer to that of their cohabiting pups than to that of their non-fostered biological brother. Similarly, in pigs separated from their mothers at birth and nursed artificially, bacterial communities from individuals bred in the same pen were more similar between themselves than to their brothers raised in a different pen (Thompson *et al*., 2008). In contrast, the composition of the microbiota of obese mice (ob/ob) differs from those of the thin line (ob/+) or wild strain (+/+) with an increase in the ratio Firmicutes / Bacteroides (Ley *et al*., 2005). The microbiota of monozygotic twins are more similar than are the microbiota of identical dizygotic twins (Steward *et al*., 2005). Finally, greater similarity between the microbiota of mouse pups born to mothers' sisters is observed compared to the microbiota of mouse pups born to unrelated mothers (Hufeldt *et al*., 2010). All these three last observations suggest that if the transfer of microbiota from one generation to another is through contact between parents and offspring, the host genetic plays a role.

CONCLUSION

Recent technological advances in molecular microbiology have provided new knowledge on the composition of the microbiota in humans and animals. However, in rabbits knowledge of these organisms is still patchy. The metagenome analysis tool in the rabbit could provide valuable information about the relationship between the functions of the microbiota and digestive problems. In this context one can also imagine the development of a new probiotic in which the key functions necessary to maintain homeostasis would be integrated. Studies so far indicate a relative plasticity of the digestive ecosystem in rabbits before 49 days of age. From them, three hypotheses of modification of the ecosystem were presented in this review i) a control of implantation

in the nest, ii) the possibility of controlling the microbiota in the period around weaning and / or iii) an acceleration of microbiota maturation. All these hypothesis open promising research avenues that may lead to changes in farming practices (weaning age, early access to food), nutrition (quantity and quality of fibre, prebiotic and probiotic) and genetics. Moreover, it may be important to avoid all practices which might limit the development of the microbiota, such as exposure to antibiotics directly or indirectly, e.g. by treatment of mothers, to ensure optimal development of the immune system of young rabbits. However although the final objective, which is to optimize ecosystem services to the host in terms of health and feed efficiency is determined, it must be recognized that the composition in term of species and/or functional gene of the targeted microbiota is not yet known.

REFERENCES – **Abecia** L., Fondevila M., Balcells J., Edwards J.E., Newbold C.J., McEwan N.R., 2005. Molecular profiling of bacterial species in the rabbit caecum. FEMS Microbiol. Lett., 244, 111-115. **Abecia** L., Fondevila M., Balcells J., Edwards J.E., Newbold C.J., McEwan N.R., 2007a. Effect of antibiotics on the bacterial population of the rabbit caecum. FEMS Microbiol. Lett., 272, 144-153. **Abecia** L., Fondevila M., Balcells J., Lobley G.E., McEwan N.R., 2007b. The effect of medicated diets and level of feeding on caecal microbiota of lactating rabbit does. J. Appl. Microbiol., 103, 787 - 793. **Abecia** L., Fondevila M., Balcells J., McEwan N.R., 2007c. The effect of lactating rabbit does on the development of the caecal microbial community in the pups they nurture. J. Appl. Microbiol., 103, 557-564. **Adjiri** D., Bouillier Oudot M., Lebas F., Candau M., 1992. Simulation in vitro des fermentations caecales du lapin en fermenteur à flux semi-continu. 1. Rôle du prétraitement du substrat alimentaire. Reprod Nutr Dev, 32, 351-360. **Amber** K.H., 2004. Effect of feeding diets containing yucca extract or probiotic on growth, digestibility, nitrogen balance and caecal microbial activity of growing new zealand white rabbits. In Proc.: 8th World Rabbit Congress,Puebla, Mexico 7-10 september, 4. **Backhed** F., Ding H., Wang T., Hooper L.V., Koh G.Y., Nagy A., Semenkovich C.F., Gordon J.I., 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. USA, 101, 15718-15723. **Badiola** I., Perez de Rozas A.M., Roca M., Carabano R., Gomez M., Garcia J., Blas C.d., 2004. Characterization of the microbial diversity of rabbit intestinal tract by restriction fragment length polymorphism. Proceedings of the 8th World Rabbit Congress, September 7-10, 2004, Pueblo, Mexico, pp. 746-751. **Barton** M.D., 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Reviews, 13, 279-299. **Belenguer** A., Fondevila M., Balcells J., Abecia L., Lachica M., Carro M.D., 2011. Methanogenesis in rabbit caecum as affected by the fermentation pattern: in vitro and in vivo measurements. World Rabbit Sci, 19, 75-83. **Bellier** R., Gidenne T., Vernay M., Colin M., 1995. In-vivo study of circadian variations of the cecal fermentation pattern in postweaned and adult-rabbits. J. Anim. Sci., 73, 128-135. **Bennegadi** N., Fonty G., Millet L., Gidenne T., Licois D., 2003. Effects of age and dietary fibre level on caecal microbial communities of conventional and specific pathogen-free rabbits. Microbial Ecology in Health and Disease, 5, 23-32. **Berg** D., 1996. The indigenous gastrointestinal microflora. Trends in microbiology, 4, 430-435. **Boadi** D., Benchaar C., Chiquette J., Masse D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows. Can. J. Anim. Sci., 84, 319-335. **Bónai** A., Szendro Z., Matics Z., Febel H., Kametler L., Tornyos G., Horn P., Kovacs F., Kovacs M., 2010. Effect of inulin supplementation and age on growth performance

and digestive physiological parameters in weaned rabbits. World Rabbit Sci, 18, 121-129. **Boulahrouf** A., Fonty G., Gouet P., 1991. Establishment, counts and identification of the fibrolytic bacteria in the digestive tract of rabbit. Influence of feed cellulose content. Current microb., 22, 1-25. **Carabaño** R., Piquer J., Menoyo D., Badiola I., 2010. The digestive system of the rabbit. In: De Blas, C., Wiseman, J. (Ed.), Nutrition of the rabbit. CABI, pp. 1-18. **Carabaño** R., Villamide M.J., Garcia J., Nicodemus N., Llorente A., Chamorro S., Menoyo D., Garcia-Rebollar P., Garcia-Ruiz A.I., De Blas J.C., 2009. New concepts and objectives for protein-amino acid nutrition in rabbits: a review. World Rabbit Sci, 17, 1-14. **Case** R.J., Boucher Y., Dahllof I., Holmstrom C., Doolittle W.F., Kjelleberg S., 2007. Use of 16S rRNA and rpoB Genes as Molecular Markers for Microbial Ecology Studies. Appl. Environ. Microbiol., 73, 278-288. **Chamorro** S., de Blas C., Grant G., Badiola I., Menoyo D., Carabano R., 2010. Effect of dietary supplementation with glutamine and a combination of glutamine-arginine on intestinal health in twenty-five-day-old weaned rabbits. J. Anim Sci., 88, 170-180. **Chamorro** S., Gomez-Conde M.S., Perez de Rozas A.M., Carabano R., De Blas J.C., 2007. Effect on digestion and perfomance of dietary protein content and of increased substitution of lucerne hay with soya-bean protein concentrate in starter diets fo young rabbits. Animal, 1, 651-659. **Combes** S., Cauquil L., Gidenne T., 2008. Impact of an exclusive milk vs milk and dry feed intake till weaning on intake, growth, and on the caecal biociversity and fibrolytic activity of the young rabbit. In Proc.: 9th World Rabbit Congress, ,Verona, Italy June 10-13, 607-611. **Combes** S., Michelland R.J., Monteils V., Cauquil L., Soulie V., Tran N.U., Gidenne T., Fortun-Lamothe L., 2011. Postnatal development of the rabbit caecal microbiota composition and activity. FEMS Microbiol. Ecol., 77, 680-689. **Corrigan** A., Horgan K., Clipson N., Murphy R.A., 2011. Effect of Dietary Supplementation with a Saccharomyces cerevisiae Mannan Oligosaccharide on the Bacterial Community Structure of Broiler Cecal Contents. Appl. Environ. Microbiol., 77, 6653-6662. **Corthier** G., 2011. Bonnes bactéries et bonne santé. Carnet de sciences. Quae (Ed), Versailles, France, pp 128. **Coudert** P., Licois D., Besnard J., 1988. Establishment of a specified pathogen free breeding colony (SPF) without hysterectomy and hand-rearing procedures. In Proc.: Proc. 4th Congress of WRSA,Budapest 10-14 october, 2, 137-148. **Curtis** T.P., Sloan W.T., 2004. Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. Curr. Opin. Microbiol., 7, 221-226. **Deng** W., Xi D., Mao H., Wanapat M., 2008. The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. Mol. Biol. Rep., 35, 265-274. **Ducluzeau** R., 1969. Influence de l'espèce zoologique sur la microflore du tractus digestif. Rev. Immuno., , 33, 345-384. **Emaldi** O., Crociani F., Matteuzzi D., Proto V., 1978. A note on the total viable counts and selective enumeration of anaerobic bacteria in the caecal contents, soft and hard faeces of rabbit. J. appl. Bacteriol., 46, 169-172. **Falcao-e-Cunha** L., Castro-Solla L., Maertens L., Marounek M., Pinheiro V., Freire J., Mourao J.L., 2007. Alternatives to antibiotic growth promoters in rabbit feeding: A review. World Rabbit Sci, 15, 127-140. **Feng** Y., Duan C.-J., Pang H., Mo X.-C., Wu C.-F., Yu Y., Hu Y.-L., Wei J., Tang J.-L., Feng J.-X., 2007. Cloning and identification of novel cellulase genes from uncultured microorganisms in rabbit cecum and characterization of the expressed cellulases. Appl. Microbiol. Biotechnol., 75, 319-328. **Fonty** G., Chaucheyras-Durand F., 2007. Les écosystèmes digestifs. Lavoisier (Tec & Doc) (Ed), Paris, France, pp 311. **Fonty** G., Gouet P., 1989. Fibre-degradating microorganisms in the monogastric digestive trac. Anim. Feed Sci. Technol., 23, 91-

107. **Fonty** G., Gouet P., Riou Y., 1979. Effect of milk composition on the gastrointestinal microflora of the rabbit. Ann. Biol. Anim. Bioch. Biophys, , 19, 567-571. Forsythe S.J., Parker D.S., 1985. Nitrogen metabolism by the microbial flora of the rabbit. J. Appl. Bacteriology, 58, 363-369. **Fortun-Lamothe** L., Boullier S., 2007. A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. Livest Sci, 107, 1-18. **Fortun-Lamothe** L., Gidenne T., 2000. The effects of size of suckled litter on intake behaviour, performance and helth status of young and reproducing rabbits. Ann. Zootech., 49, 517-529. **Franz** R., Soliva C.R., Kreuzer M., Hummel J., Clauss M., 2011. Methane output of rabbits (Oryctolagus cuniculus) and guinea pigs (Cavia porcellus) fed a hay-only diet: Implications for the scaling of methane production with body mass in non-ruminant mammalian herbivores. Comp. Biochem. Physiol., A: Mol. Integr. Physiol., 158, 177-181. **Gabriel** I., Mallet S., Leconte M., Fort G., Naciri M., 2006. Effects of whole wheat feeding on the development of coccidial infection in broiler chickens until market-age. Anim. Feed Sci. Technol., 129, 279-303. **Gallois** M., Gidenne T., Fortun-Lamothe L., Le Huerou-Luron I., Lallès J.P., 2005. An early stimulaiton of solid feed intake slightly influences the morphological gut maturation in the rabbit. Reprod. Nutr. Develop., 45, 109-122. **Garcia** A.I., de Blas J.C., Carabano R., 2004. Effect of type of diet (casein-based or protein-free) and caecotrophy on ileal endogenous nitrogen and amino acid flow in rabbits. Anim. Sci., 79, 231-240. **García** J., Gidenne T., Falcao-e-Cunhac L., Blas C.d., 2002. Identification of the main factors that influence caecal fermentation traits in growing rabbits. Anim. Res., 51, 165-173. **Gibson** G., Roberfroid M., 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125, 1401-1412. **Gidenne** T., 1992. Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. Brit J Nutr, 67, 133-146. **Gidenne** T., 1994. Estimation of volatile fatty acids and of their energetic supply in the rabbit caecum: effect of the dietary fibre level. In Proc.: VIème Journées de la Recherche Cunicole,Paris 6 & 7 déc., 2, 293-299. **Gidenne** T., 2003. Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. Livest. Prod. Sci., 81, 105-117. **Gidenne** T., Bennegadi-Laurent N., Bayourthe C., Monteils V., Fonty G., 2006. Post-weaning maturation of rabbit caecal microbial communities: impact of live yeast intake. In Proc.: RRI-INRA conference, gut microbiology 2006, 5th biennial meeting, Aberdeen, 21-23 June. ,Aberdeen, Scotland 21-23 june 2006, Repr. Nutr. Develop., 46 (suppl.1), S95 (P166) **Gidenne** T., Carabano R., Garcia J., Blas C.d., 2010a. Fiber digestion. In: De Blas, C., Wiseman, J. (Ed.), Nutrition of the rabbit. CABI, pp. 179-199. **Gidenne** T., Combes S., Fortun-Lamothe L., 2012. Feed intake limitation strategies for the growing rabbit: effect on feeding behaviour, welfare, performance, digestive physiology and health: a review. Animal, FirstView, 1-13. **Gidenne** T., Combes S., Licois D., Carabaño R., Badiola I., Garcia J., 2008. Ecosystème caecal et nutrition du lapin : interactions avec la santé digestive. INRA Prod. Anim., 21, 239-250. **Gidenne** T., Feugier A., 2009. Feed restriction strategy in the growing rabbit. 1. Impact on digestion, rate of passage and microbial activity. Animal, 3, 501-508. **Gidenne** T., Garcia J., Lebas F., Licois D., 2010b. Nutrition and feeding strategy: interactions with pathology. In: De Blas, C., Wiseman, J. (Ed.), Nutrition of the rabbit. CABI, pp. 179-199. **Gidenne** T., Jehl N., Lapanouse A., Segura M., 2004a. Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of

rapidly fermentable polysaccharides. Brit J Nutr, 92, 95-104. **Gidenne** T., Lebas F., 2006. Feeding behaviour in rabbits. In: Bels, V. (Ed.), Feeding in domestic vertebrates. From structure to behaviour. CABI publishing, Wallingford, UK, pp. 179-209. **Gidenne** T., Lebas F., Fortun-Lamothe L., 2010c. Feeding behaviour of rabbits. In: De Blas, C., Wiseman, J. (Ed.), Nutrition of the rabbit. CABI, pp. 233-252. **Gidenne** T., Mirabito L., Jehl N., Perez J.M., Arveux P., Bourdillon A., Briens C., Duperray J., Corrent E., 2004b. Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit. Anim. Sci., 78, 389-398. **Gidenne** T., Pinheiro V., Falcao E Cunha L., 2000. A comprehensive approach of the rabbit digestion: consequences of a reduction in dietary fibre supply. Livest. Prod. Sci., 64, 225-237. **Gómez-Conde** M.S., de Rozas A.P., Badiola I., Pérez-Alba L., de Blas C., Carabaño R., García J., 2009. Effect of neutral detergent soluble fibre on digestion, intestinal microbiota and performance in twenty five day old weaned rabbits. Livest Sci, 125, 192-198. **Gomez-Conde** M.S., Garcia J., Chamorro S., Eiras P., Rebollar P.G., Perez de Rozas A., Badiola I., de Blas C., Carabano R., 2007. Neutral detergent-soluble fiber improves gut barrier function in twenty-five-day-old weaned rabbits. J. Anim Sci., 85, 3313-3321. **Gouet** P., Fonty G., 1973. Evolution de la microflore digestive du lapin holoxénique de la naissance au sevrage. Ann. Biol. anim. Biochim. Biophys., 13, 733-735. **Gouet** P., Fonty G., 1979. Changes in the digestive microflora of holoxenic rabbits from birth until adullthood. Ann. Biol. Anim. Bioch. Biophys., 19, 553-566. **Guarner** F., Malagelada J.-R., 2003. Gut flora in health and disease. The Lancet, 361, 512-519. **Hanson** N.B., Lanning D.K., 2008. Microbial induction of B and T cell areas in rabbit appendix. Dev. Comp. Immunol., 32, 980-991. **Heczko** U., Abe A., Finlay B.B., 2000. Segmented filamentous bacteria prevent colonization of enteropathogenic Escherichia coli O103 in rabbits. J. Infect. Dis., 181, 1027-1033. **Hufeldt** M.R., Nielsen D.S., Vogensen F.K., Midtvedt T., Hansen A.K., 2010. Family relationship of female breeders reduce the systematic inter-individual variation in the gut microbiota of inbred laboratory mice. Laboratory Animals, 44, 283-289. **Jarvis** G.N., Strömpl C., Burgess D.M., Skillman L.C., Moore E.R.B., Joblin K.N., 2000. Isolation and identification of ruminal methanogens from grazing cattle. Curr. Microbiol., 40, 327-332. **Jimenez** E., Marín M.L., Martín R., Odriozola J.M., Olivares M., Xaus J., Fernández L., Rodríguez J.M., 2008. Is meconium from healthy newborns actually sterile? Res. Microbiol., 159, 187-193. **Jones** W.J., Nagle D.P., Whitman W.B., 1987. Methanogens and the diversity of archaebacteria. Microbiol Rev., 51, 135-177. **Kim** G.B., Seo Y.M., Kim C.H., Paik I.K., 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult Sci, 90, 75-82. **Kimsé** M., Bayourthe C., Monteils V., Fortun-Lamothe L., Cauquil L., Combes S., Gidenne T., 2012. Live yeast stability in rabbit digestive tract: Consequences on the caecal ecosystem, digestion, growth and digestive health. Anim. Feed Sci. Technol., 173, 235-243. **Kimsé** M., Monteils V., Bayourthe C., Gidenne T., 2009. A new method to measure the redox potential (Eh) in rabbit caecum: relationship with pH and fermentation pattern. World Rabbit Sci, 17, 63-70. **Koenig** J.E., Spor A., Scalfone N., Fricker A.D., Stombaugh J., Knight R., Angenent L.T., Ley R.E., 2011. Succession of microbial consortia in the developing infant gut microbiome. Proceedings of the National Academy of Sciences, 108, 4578-4585. **Kovács** M., Bónai A., Szendrő Z., Milisits G., Lukács H., Szabó-Fodor J., Tornyos G., Matics Z., Kovács F., Horn P., 2012. Effect of different weaning ages (21, 28 or 35 days) on production, growth and certain parameters of the digestive tract in rabbits. Animal, 6, 894-901. **Kovács** M.,

Szendrõ Z., Milisits G., Biro-Nemeth E., Radnai I., Posa R., Bonai A., Kovacs F., Horn P., 2006. Effect of nursing method and faeces consumption on the development of bacetroides, lactobacillus and coliform flora in the caecum of the newborn rabbits. Reprod. Nutr. Dev., 46, 205-210. **Kušar** D., Avguštin G., 2010. Molecular profiling and identification of methanogenic archaeal species from rabbit caecum. FEMS Microbiol. Ecol., 74, 623-630. **Lamendella** R., VerBerkmoes N., Jansson J.K., 2012. "Omics" of the mammalian gut - new insights into function. Curr. Opin. Biotechnol. **Lanning** D., Zhu X., Zhai S.-K., Knight K.L., 2000. Development of the antibody repertoire in rabbit: gut-associated lymphoid tissue, microbes, and selection. Immunol. Rev., 175, 214-228. **Lelkes** L., Chang C., 1987. Microbial dysbiosis in rabbit mucoid enteropathy. Lab. Anim. Sci, 37, 757-764. **Ley** R.E., Backhed F., Turnbaugh P., Lozupone C.A., Knight R.D., Gordon J.I., 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA, 102, 11070-11075. **Ley** R.E., Turnbaugh P., Klein S., Gordon J.I., 2006. Microbial ecology: human gut microbes associated with obesity. Nature, 444, 1022 - 1023. **Mackie** R.I., 2002. Mutualistic Fermentative Digestion in the Gastrointestinal Tract: Diversity and Evolution. Integr. Comp. Biol., 42, 319-326. **Mage** R.G., Lanning D., Knight K.L., 2006. B cell and antibody repertoire development in rabbits: The requirement of gut-associated lymphoid tissues. Dev. Comp. Immunol., 30, 137-153. **Marounek** M., Fievez V., Mbanzamihigo L., Demeyer D., Maertens L., 1999. Age and incubation time effects on in vitro caecal fermentation pattern in rabbits before and after weaning. Arch Anim Nutr, 52, 195-201. **Martignon** M.H., Combes S., Gidenne T., 2010a. Digestive physiology and hindgut bacterial community of the young rabbit (Oryctolagus cuniculus): Effects of age and short-term intake limitation. Comp. Biochem. Physiol., A: Mol. Integr. Physiol., 156, 156-162. **Martignon** M.H., Reperant E., Valat C., 2010b. Digestive response of young rabbits to an experimental reproduction of colibacillosis according to two feeding strategies. In Proc.: The Prato Conference on the Pathogenesis of Bacterial Diseases of Animals,Monash Prato Campus, Prato, Italy 6-9 October. **Massip** K., Combes S., Cauquil L., Zemb O., Gidenne T., 2012. High throughput 16S-DNA sequencing for phylogenetic affiliation of the caecal bacterial community in the rabbit - Impact of the hygiene of housing and of the intake level. In Proc.: Symposium on Gut Microbiology. Gut microbiota: friend or foe?,Clermont-Ferrand - France 17 - 20 june, . **Mazmanian** S.K., Liu C.H., Tzianabos A.O., Kasper D.L., 2005. An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System. Cell, 122, 107-118. **Michelland** R., Combes S., Monteils V., Cauquil L., Gidenne T., Fortun-Lamothe L., 2011. Rapid adaptation of the bacterial community in the growing rabbit cæcum after a change of dietary fibre supply. Animal, in press. **Michelland** R.J., Combes S., Monteils V., Cauquil L., Gidenne T., Fortun-Lamothe L., 2010a. Molecular analysis of the bacterial community in digestive tract of rabbit. Anaerobe, 16, 61-65. **Michelland** R.J., Monteils V., Combes S., Cauquil L., Gidenne T., Fortun-Lamothe L., 2010b. Comparison of the archaeal community in the fermentative compartment and faeces of the cow and the rabbit. Anaerobe, 16, 396-401. **Moncomble** A.S., Quennedey B., Coureaud G., Langlois D., Perrier G., Schaal B., 2004. Newborn rabbit attraction toward maternal faecal pellets. In Proc.: International Society for Developmental Psychobiology, 37th annual meeting,Aix-en-Provence, France 45, 277. **Monteils** V., Cauquil L., Combes S., Godon J.-J., Gidenne T., 2008. Potential core species and satellite species in the bacterial community within the rabbit caecum. FEMS Microbiol. Ecol., 66, 620-629. **Mourao** J.L., Pinheiro V., Alves A., Guedes C.M., Pinto L., Saavedra M.J., Spring P.,

Kocher A., 2006. Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits. Anim. Feed Sci. Technol., 126, 107-120. **Mulder** I., Schmidt B., Stokes C., Lewis M., Bailey M., Aminov R., Prosser J., Gill B., Pluske J., Mayer C.-D., et al., 2009. Environmentally-acquired bacteria influence microbial diversity and natural innate immune responses at gut surfaces. BMC Biol., 7, 79. **Okada** H., Kuhn C., Feillet H., Bach J.F., 2010. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin. Exp. Immunol., 160, 1-9. **Oyofo** B., DeLoach J., Corrier D., Norman J., Ziprin R., Mollenhauer H., 1989. Prevention of Salmonella typhimurium colonization of broilers with D-mannose. Poult Sci. , 68, 1357-1360. **Padilha** M.T.S., Licois D., Coudert P., 1996. Frequency of the carriage and enumeration of Escherichia coli in caecal content of 15 to 49 day old rabbits. In Proc.: 6th World Rabbit Congress,Toulouse, France July 09-12, 3, 99-102. **Padilha** M.T.S., Licois D., Gidenne T., Carré B., 1999. Caecal microflora and fermentation pattern in exclusively milk-fed young rabbits. Reprod Nutr Dev, 39, 223-230. **Padilha** M.T.S., Licois D., Gidenne T., Carré B., Fonty G., 1995. Relationships between microflora and caecal fermentation in rabbits before and after weaning. Reprod Nutr Dev, 35, 375-386. **Penders** J., Thijs C., Vink C., Stelma F.F., Snijders B., Kummeling I., van den Brandt P.A., Stobberingh E.E., 2006. Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. Pediatrics, 118, 511-521. **Perez** J.M., Gidenne T., Bouvarel I., Arveux P., Bourdillon A., Briens C., Le Naour J., Messager B., Mirabito L., 2000. Replacement of digestible fibre by starch in the diet of the growing rabbit. II. Effects on performances and mortality by diarrhoea. Ann. Zootech., 49, 369-377. **Piattoni** F., Maertens L., Demeyer D., 1996. In vitro study of the age-dependent caecal fermentation pattern and methanogenesis in young rabbits. Reprod Nutr Dev, 36, 253-261. **Rhee** K.-J., Sethupathi P., Driks A., Lanning D.K., Knight K.L., 2004. Role of Commensal Bacteria in Development of Gut-Associated Lymphoid Tissues and Preimmune Antibody Repertoire. J Immunol, 172, 1118-1124. **Rodriguez-Romero** N., Abecia L., Balcells J., Martinez B., Fondevila M., 2009. Comparison of bacterial profile from caecal content and caecotrophes in growing rabbits fed on two levels of indigestible fibre. In Proc.: XXXIX Jornadas de Estudio, XIII Jornadas sobre Produccion Animal, Zaragoza, Espana, 12 y 13 de mayo de 2009., 784-786. **Severson** K.M., Mallozzi M., Driks A., Knight K.L., 2010. B Cell Development in GALT: Role of Bacterial Superantigen-Like Molecules. The Journal of Immunology, 184, 6782-6789. **Siggins** A., Gunnigle E., Abram F., 2012. Exploring mixed microbial community functioning: recent advances in metaproteomics. FEMS Microbiol. Ecol., 80, 265-280. **Stappenbeck** T.S., Hooper L.V., Gordon J.I., 2002. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. Proc. Natl. Acad. Sci. USA, 99, 15451-15455. **Steward** J.A., Chadwick V.S., Murray A., 2005. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. . J Med Microbiol, 54, 1239–1242. **Thompson** C.L., Holmes A.J., 2009. A window of environmental dependence is evident in multiple phylogenetically distinct subgroups in the faecal community of piglets. FEMS Microbiol. Lett., 290, 91-97. **Thompson** C.L., Wang B., Holmes A.J., 2008. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J, 2, 739-748. **Turnbaugh** P.J., Hamady M., Yatsunenko T., Cantarel B.L., Duncan A., Ley R.E., Sogin M.L., Jones W.J., Roe B.A., Affourtit J.P., et al., 2009. A core gut microbiome in obese and lean twins. Nature, 457, 480-484. **Turnbaugh** P.J., Ley R.E., Mahowald M.A., Magrini V., Mardis E.R.,

Gordon J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature, 444, 1027-1131. **Vanhoutte** T., Huys G., De Brandt E., Swings J., 2004. Temporal stability analysis of the microbiota in human feces by denaturing gradient gel electrophoresis using universal and group specific 16S rRNA primers. FEMS Microbiol. Ecol., 48, 437-446. **Vermorel** M., 1995. Emissions annuelles de méthane d'origine digestive par les bovins en France. Variations selon le type d'animal et le niveau de production. INRA Prod. Anim., 8, 265-272. **Villamide** M.J., Nicodemus N., Fraga M.J., Carabano R., 2010. Protein digestion. In: De Blas, C., Wiseman, J. (Ed.), Nutrition of the rabbit. CABI, pp. 39-55. **Wielen** P.W.J.J., Keuzenkamp D.A., Lipman L.J.A., Knapen F., Biesterveld S., 2002. Spatial and Temporal Variation of the Intestinal Bacterial Community in Commercially Raised Broiler Chickens During Growth. Microbial Ecology, 44, 286-293. **Wright** A.-D.G., Auckland C.H., Lynn D.H., 2007. Molecular Diversity of Methanogens in Feedlot Cattle from Ontario and Prince Edward Island, Canada. Appl. Environ. Microbiol., 73, 4206-4210. **Yang** H.J., Cao Y.C., Zhang D.F., 2010. Caecal fermentation patterns in vitro of glucose, cellobiose, microcrystalline cellulose and NDF separated from alfalfa hay in the adult rabbit. Anim. Feed Sci. Technol., 162, 149-154. **Yu** B., Tsen H.Y., 1993. Lactobacillus cells in the rabbit digestive tract and factors affecting their distribution. J. Appl. Bact., 75, 269-275. **Zoetendal** E.G., Akkermans A.D.L., Akkermans van-Vliet W.M., De Visser J.A.G.M., De Vos W.M., 2001. The host genotype affects the bacterial community in the human gastrointestinal tract. Microbial Ecol Health Dis 13 129–134. **Zoetendal** E.G., Akkermans A.D.L., De Vos W.M., 1998. Temperature Gradient Gel Electrophoresis Analysis of 16S rRNA from Human Fecal Samples Reveals Stable and Host-Specific Communities of Active Bacteria. Appl. Environ. Microbiol., 64, 3854-3859. **Zoetendal** E.G., von Wright A., Vilpponen-Salmela T., Ben-Amor K., Akkermans A.D.L., de Vos W.M., 2002. Mucosa-Associated Bacteria in the Human Gastrointestinal Tract Are Uniformly Distributed along the Colon and Differ from the Community Recovered from Feces. Appl. Environ. Microbiol., 68, 3401-3407.