INTERACTIONS BETWEEN GUT MICROFLORA AND DIGESTIVE MUCOSAL IMMUNITY, AND STRATEGIES TO IMPROVE DIGESTIVE HEALTH IN YOUNG RABBITS

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ABSTRACT

Enteric diseases frequently occur in rabbit around weaning leading to extensive use of antibiotics in rabbit breedings. In this context, breeders as well as consumers ask for alternative strategies that improve the health of animals. But the maintenance of gut health is complex and relies on a delicate balance between the mucosa (including the absorptive epithelium and the digestive immune system), the commensal microflora and environmental factors including diet. Firstly, immune and non-immune mechanisms of protection against pathogens in the gut were presented followed by installation and composition of the gut microflora in the rabbits and its role on health. Finally, several strategies to stimulate digestive immune system or favour beneficial flora to exclude enteric pathogens were discussed. Several nutrients were implicated in the development of immune response and could be used to improve immune ability of animals. Among which, dietary fatty acids ($\omega3/\omega6$ ratio) could be of interest in the rabbit. The role of dietary fibres on digestive health have been demonstrated in weaned rabbits, and strong relations between fibres supply and caecal microflora were evidenced. Some works also reported an influence of fibre level in the diet given to the young before weaning on health status of rabbits after weaning. Therefore, nutritional needs of suckling rabbits, more especially fibre requirements, to enhance subsequent gut health need to be deeply studied, in relation with needs of their mothers. Exogenous flora could also be added to the diet to stimulate the digestive immune system and prevent the development of enteric pathogens. Finally, vaccines permit protection of the host against specific pathogens.

Key words: gut, immunity, nutrition, microflora, probiotics, rabbit.

INTRODUCTION

Enteric diseases frequently occur in rabbit breeding, more especially in young around weaning. These troubles cause mortality and reduced growth rates with important economic losses. Antibiotics are frequently used to prevent or to treat such illness. Unfortunately, the long term and extensive use of antibiotics has lead to the appearance of worrying bacterial drug resistance and stressed the problem of food residues. European policy tries to put a ban on such practices, and consumers as well as breeders require alternative breeding strategies that improve the health of animals without using in-feed antibiotics.

The concept of "gut health" recently emerge and generally refer to three main components : the diet, the mucosa and the commensal flora (MONTAGNE *et al*., 2003). The mucosa is composed of the digestive epithelium, the gut-associated lymphoid tissue and the mucus overlying the epithelium. To prevent digestive disturbances, it seems necessary to favour conditions in the gut to stabilise the equilibrium bewteen host, microflora and environnement. More especially in the young rabbits in which microflora is not yet stabilize and the diet change from milk to solid feed. Data concerning the nutrition of the young rabbit (food behaviour and digestive capacities) were recently reviewed by GIDENNE and FORTUN-LAMOTHE (2002) and were not recalled here.

The aim of this paper focused on strategies to improve digestive health in young rabbits. Firstly, we review immune and non-immune mechanisms of protection against pathogens in the gut. Therefater, we describe installation and composition of the gut microflora in the rabbits and its role on health. The last section will deal with possible strategies to stimulate digestive immune system (feeding, exogenous flora or vaccines) or favour beneficial flora to exclude enteric pathogens (feeding or exogenous flora). Each time that is possible we refer to works on young rabbits. However, effects of nutrition on digestive immunity are poorly known in this species, as well as nutrionnal needs of young rabbits or factors controlling installation of gut microflora. Therefore, we also refer to works on weaned rabbits or on others species, thinking that could help to open to new research areas.

IMMUNE SYSTEM AND PROTECTION AGAINST PATHOGENS IN THE GUT

The digestive tract has to face at least two dilemma: ensuring a good nutrient absorption but with the exclusion of digestive micro-organisms (harmful and non harmful). The digestive tract also needs to differentiate between nutriments and non pathogenic micro-organisms from harmful micro-organisms, to ignore the first ones and to eliminate the second ones. This is called tolerance. The structure of the intestinal epithelium and the specific organisation and functioning of the digestive immune system are designed to solve these contradictions.

Non-immunological intestinal mucosal barriers

Many factors ensure the defence of the mucous membrane (and thus of the body) against pathogens. Some do not belong to the immune system. Firstly, the gap junctions, which permits cohesion between epithelial cells, prevent the intercellular passage of causative agents. Intestinal peristalsis make possible to reduce the interactions between causative agents and epithelial cells. Additionally, proliferation of pluripotent cells located in the crypts at the base of villi permits the permanent renewal of digestive epithelium. These pluripotent cells generate several cells type and some of them, such as Paneth cells or mucous cells, exhibit properties which strongly take part in the maintenance of epithelium integrity. They secrete in the lumen several substances (lactoferrin, lactoperoxidase and lysozyme) with a bacteriolytic or bacterostatic activity and antibiotic peptides such as the cryptidine (SCHRODER, 1999). The mucus forms a protective coating on the surface of enterocytes. Lastly, the resident digestive saprophyte flora induce competition for substrates (see further paragraph) and create an unfavourable environment for the growth of pathogens (KUDSK, 2002).

When these non-immunological mechanisms do not allow elimination of the causative agent, the immune system is activated. Indeed, the digestive mucosa, as well as all others mucous membranes of the body, is associated with a lymphoid tissue. This tissue, called GALT (for Gut Associated Lymphoid Tissue), ensure of course the defence of the host by neutralisation of pathogens but also the protection of mucous membrane by controlling the inflammatory response. It contains more immune system cells than the remainder of the body. It should be noted that some cells which do not belong to the immune system (such as enterocytes) are also involved in the mechanisms of immune defence (see below).

The digestive immune system

As for systemic immunity, it is necessary to distinguish the "innate" or "natural" primary immune response which is non-specific and represents the first line of defence against pathogens, from the "adaptive" or "acquired" immune response which is directed against a specific foreign element in the gut. These two systems generally act as synergy (see review of DROUET-VIARD and FORTUN-LAMOTHE, 2002). For both ways, the immune response includes several steps from recognition and / or capture to neutralisation or destruction of the undesired microorganism and involves processes of cellular information and co-operation. These different steps generally do not occur at the same place of the digestive tract, and for the specific immune response, ones distinguishe the induction sites (identification of agents and activation of cells starting the reaction against antigens) and the effector sites (elimination of undesirable agent).

It should be recalled that in the young rabbit a passive immunity occurs due to mother's immunoglobulins transmission by the colostrum and to a lesser extent by the milk. This protection has the advantage to be oriented against agents of its breeding environment but could delay the setting up of its own active response (response to a vaccine).

- *Origin and morphology of the digestive immune system*

All the cells of the immune system are derived from pluripotent bone marrow stem cells. Differentiation and maturation of lymphoid cells occurs in primary lymphoid organs such as bone marrow, thymus, foetal liver and, in the rabbit, the vermiform appendix. After differentiation, lymphocytes migrate towards the secondary lymphoid organs (spleen, lymph nodes, and mucosa associated lymphoid tissues such as GALT) through the blood vessels and lymphatic system. They are then stimulated and proliferate. Mucosal digestive immune system is generally divided into organised and diffuse cells (Figure 1).
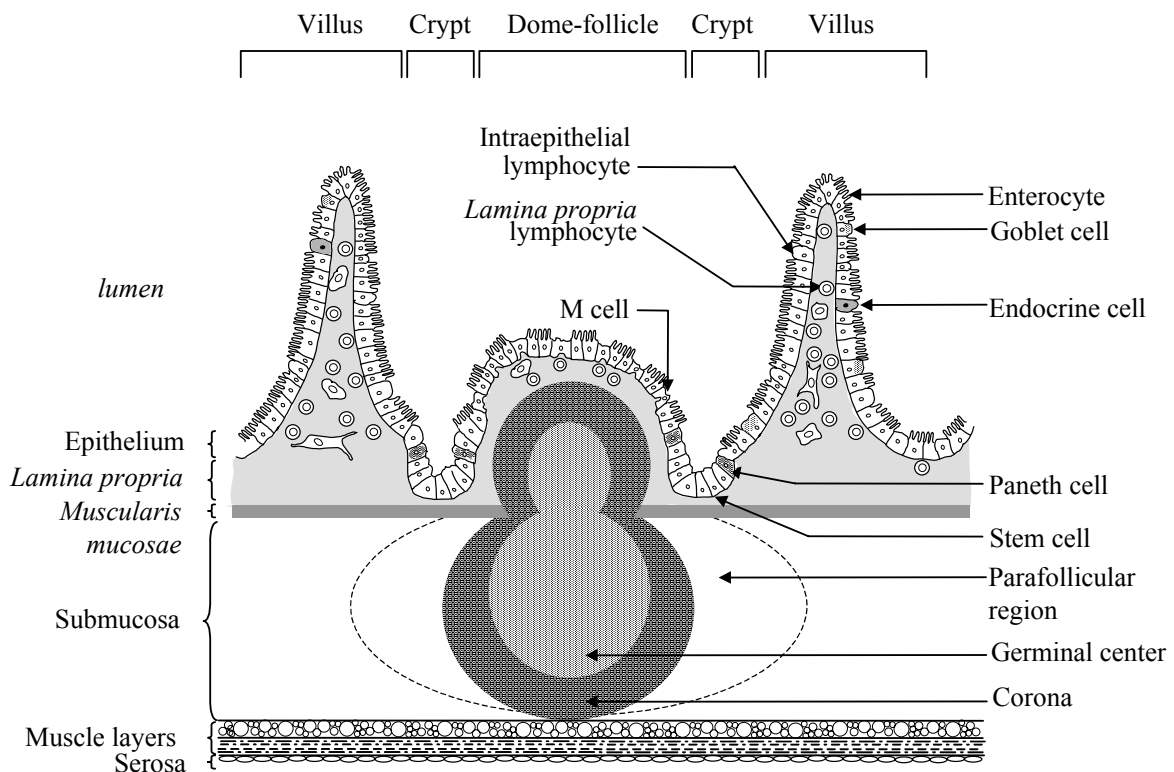

Figure 1 : Schematic spatial organization of the gut-associated lymphoid tissue.

The intestinal epithelium is organized into two distinct compartments (the crypt of Lieberkühn and the villi). Stem cells, located in the crypt, proliferate and differentiate into enterocytes, goblets cells and endocrine cells (which exit the crypt towards the villi) and paneth cells (which reside in crypts). The gut-associated lymphoid tissue is divided into diffuse cells (in the lamina propria or in the epithelium) or organized cells (lymphoid follicles). Dome-follicles are covered by a follicle-associated epithelium rich in M cells and contain a germinal center surrounding by corona.

Organised lymphoid tissue. In organised tissue, the lymphoid cells are grouped in lymphoid follicles classified according to their spatial organisation and composition. Lymphoid follicles could also contains some cells of non specific immune response (granulocytes, macrophages). Simple follicles are more-or-less spherical core of B

lymphocytes assembled within a matrix of loose connective tissue and dendritic cells. Simples follicles are sometimes partially encircled by a network of small lymphatic vessels (HEIN, 1999). Follicle-dome are usually larger and consists of identifiable body, containing a germinal center in which lymphocytes proliferate, and generally located under the muscularis mucosa, and corona and dome regions, which protrude into the *lamina propria*. Lymphoid follicles (simple or with a dome) may occurs anywhere in the gut, either singly, and thus be of microscopic size, or as aggregates, in structure of macroscopic size.

Specialised aggregation of lymphoid follicles, such as Peyer's patches have additional morphological characteristics, specific for each one of them. In the rabbit, there are between two to ten Peyer's patches along the small intestine (MAGE, 1998). The organisation of rabbit Peyer's patches is similar to that of other mammalian species. They are composed of numerous dome-follicles. The dome region extends into the lumen of the gut and the follicle germinal center abut the muscular wall. The dome-follicles contain primarily B cells producing IgM, and also macrophages and CD4-T cells (ERMAK *et al.*, 1994). The interfollicular regions, between dome and germinal center, are T-cell rich areas (HEIN, 1999).

The dome is covered by a specific epithelium, the FAE (Follicle Associated Epithelium). In contrast to surface epithelia of small intestine and colon which is specialised for absorption of digested nutrients and resorption of fluids, FAE is designed for uptake of macromolecules, particles and micro-organisms by transepithelial transport (NEUTRA, 1999). The FAE differs from the intestinal epithelium by the absence of mucus cells, the absence of secretion of the dimeric IgA receptor (PAPPO and OWEN, 1988) and of alkaline phosphatase activity (OWEN and BHALLA, 1983), by its capacity to bind lectins (NEUTRA *et al.*, 1987) and by the presence of large numbers of particular epithelial cells, M cells, which in the rabbit account for 50% of the cells of the epithelium (PAPPO, 1989). M cells are specialised epithelial cells types that occurs only over mucosal follicles. The apical surface of M cells are distinguished by morphological criteria : the absence of a typical brush border and the presence of variable microvilli or microfolds and inter-microvillar endocytic domains (NEUTRA, 1999). The basolateral surface of M-cell is deeply invaginated and forms an intra-epithelial pocket in which lymphocytes and macrophages are embedded. M cells collect a wide variety of antigens and micro-organisms and make them available to these lymphoid cells (SIEBERS and FINLAY, 1996). Their particular structure ensure that transytocis from M-Cells to subjacent cells (macrophages, B and T lymphocytes) is rapid and efficient (NEUTRA, 1999). Capture of the antigens by antigen presenting cells (macrophages or dendritic cells) of the domes allows activation of follicular lymphocytes and induction of the specific immune response.

The rabbit species is characterized by two additional structures having a very important role in the digestive immune system : the vermiform appendix, present at the caudal end of the cæcum, and sacculus rotondus located at the ileo-caecal junction (Figure 2). They contains each several-hundred of dome-follicles and their organisation in adults animals are quite similar to that of Peyer's patches. However, the villi of these two structures are

singular and this shape justified the name they received of "mushroom villi" (MAGE, 1998).

Diffuse tissue. Mucosal surfaces contain also numerous lymphoid cells, as well as cells of non specific immune response (granulocytes, macrophages) disseminated along the gut. Some lymphocytes are interspersed among epithelial cells and called IntraEpithelial Lymphocytes (IEL). The majority of these cells are T lymphocytes (80 to 90%). Others are located in the connective tissue of the *lamina propria*, and called *Lamina Propria* Lymphocytes (LPL). In contrast to the epithelial compartment, *Lamina Propria* Lymphocytes include 40 to 90 % of T cells and thus also a substantial population of B cells, a large proportion of these latter being plasma cells, secreting sIgA (ABREU-MARTIN and TARGAN, 1996).
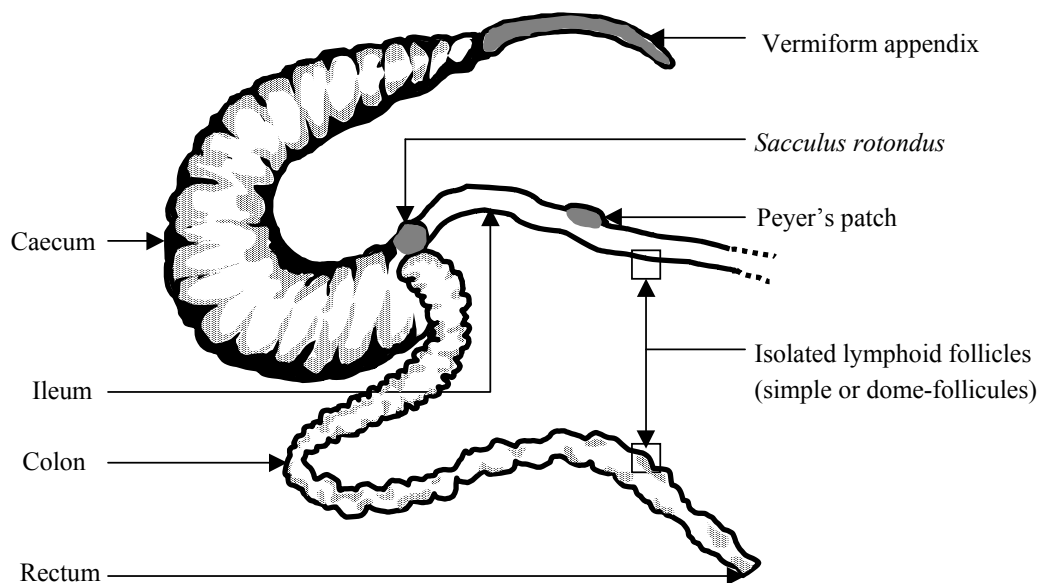

Figure 2 : Morphology of the organised lymphoid tissue in the gut of rabbit

- ***Functional organisation of the digestive immune system***

Non lymphoid cells. Macrophages and dendritic cells are strongly present in Peyer's patches, forming a dense layer of cells in the subepithelial dome, in close contact with M cells. Several non lymphoid cells are also widely distributed in the *lamina propria* of the gut (GRANUCCI and RICCIARDI-CASTAGNOLI, 2003). They are involved in direct bacterial uptake across mucosal surfaces. Their rapid kinetics of recruitment highlights their relevance as antigen sentinels at the mucosal sites. Dendritic cells could also play a role in the activation of B cells to produce IgA.

Activation and homing of lymphocytes. Peyer's patches, the vermiform appendix and the *sacculus rotundus* are specialized in the uptake of macromolecules and microorganisms from the gut lumen to the lymphoid cells (SIMECKA, 1998). M cells of the follicle associated epithelia of these three structures capture luminal antigens by macro-

pinocytose and transfer them intact to underlying dendritic and macrophage populations which express major histocompatibility complex Class II molecules (CMH-II) and process them. These latter (called antigen presenting cells) present by their CMH-II the processed antigen to CD4^{+} T cells. Naïve B cells recognize directly the intact antigen. After the B and T cells are sensitized to the antigen, they move towards the mesenteric lymph nodes where they mature and proliferate. Activated cells thus migrate through the thoracic duct into the circulation and home to the epithelium or *lamina propria* of the digestive tract, as well as to other mucosal sites, and form the effector tissue (Figure 3). The effector cells diffuse along the intestine tract allowing the development of the immune response throughout the digestive tract.
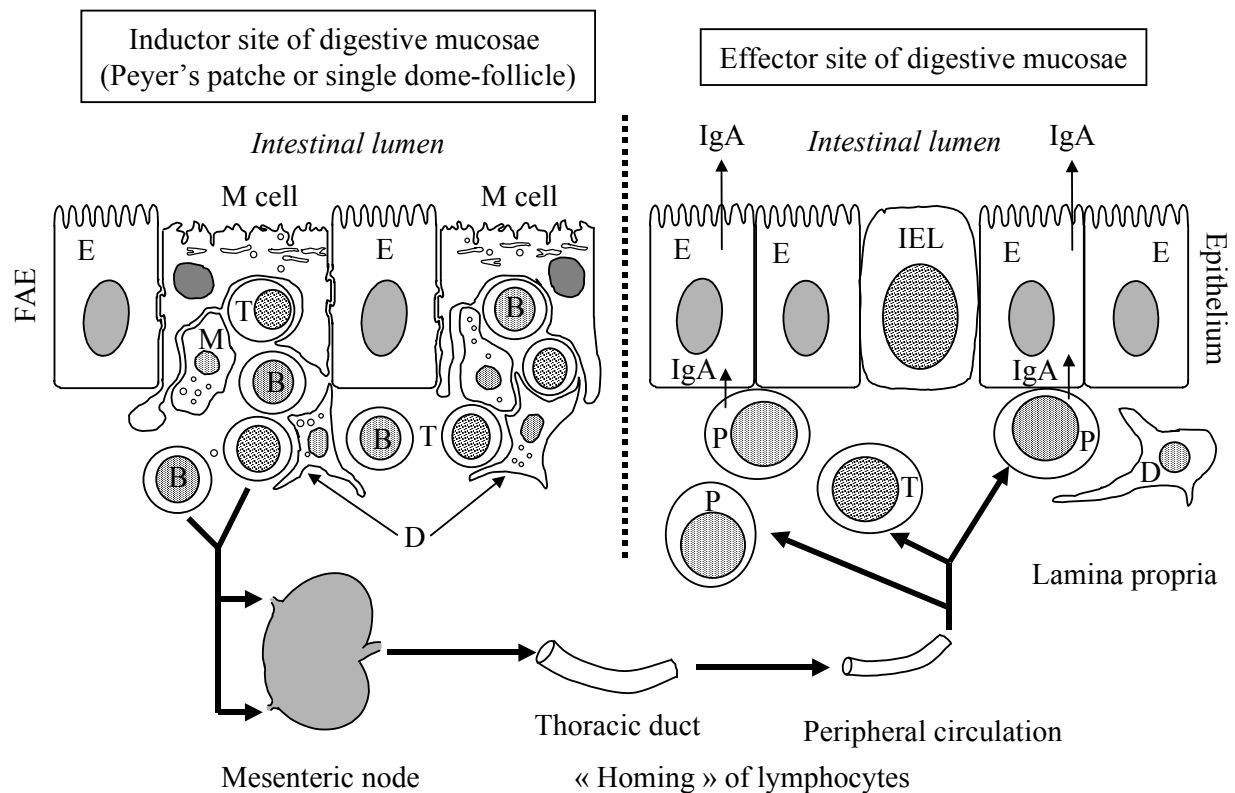

Figure 3 : Schematic functional organisation of the gut-associated lymphoid tissue.
E: enterocytes; T: lymphocytes; B : B lymphocytes; IEL : intraepithelial lymphocyte;
P : plasma B cell (secreting sIgA); D: dendritic cell ; FAE : follicle-associated epithelium.

Intraepithelial lymphocytes. According to GUY-GRAND and VASSALLI (1993) most of the intraepithelial lymphocytes localised between the enterocytes (90%) are T-CD8^{+} type. The presence of intracytoplasmic granulations rich in perforin, granzymes and Fas-Ligand evidence their cytotoxic activity. This is thought to be an important rapid response mechanism in host defence against pathogens (MAC DONALD, 1999; ABREU-MARTIN and TARGAN, 1996). All IntraEpithelial Lymphocytes have also the ability to secrete cytokines associated with Th1 and Th2 function. In this way, they may be capable of regulating other lymphocyte populations as well as epithelial cells function

such as expression of Major Histocompatibility Complex class I molecules and secretory component (ABREU-MARTIN and TARGAN, 1996).

Lamina propria lymphocytes. On the opposite, T cells located in the *lamina propria* present a low cytotoxic activity and primarily exhibit a helper and inducer phenotype (AKBAR *et al.*, 1988; TROUT and LILLEHOJ, 1996). The activated T cells could produce cytokines of Th2 type IgA-stimulating (IL-4, IL-5, IL-6, IL-10 and IL-13), or Th1 type Ig-A inhibiting cytokines (IFNγ, TNFβ, IL-2). In this later case, they are considered as T suppressor cells. Nevertheless, the development of mucosal immune responses is highly controlled and depend on the kind (food or microorganisms) and dose (low or high) of antigens. Thus, it is preferentially orientated toward a non-inflammatory response of Th-3 pattern against food antigens and a Th-1 or Th-2 pattern against virus, bacteria and high dose food antigens (KAGNOFF, 1993). The B cells are transformed under the appropriate T cells cytokine milieu to plasma cells secreting Ig-A. Reciprocally, *lamina propria* B cells may play a very significant role in regulating *lamina propria* T cells. It is the interaction between T and B cells and balance of cytokines which control Ig-A production for transport and mucosal protection (KUDSK, 2002; ABREU-MARTIN and TARGAN, 1996; KRAMER *et al.*, 1995).

Secretory IgA. As with other mucous membranes, the predominant type of immunoglobulin secreted by the B lymphocytes of the gut is IgA (70 to 90 % of all immunoglobulin present in normal intestine mucosa, Lamm; 1997). In contrast to IgA in serum, secretorys IgA are present in dimeric or polymeric form and are thus resistant to intraluminal proteolysis (BOUVET and FISCHETTI, 1999). Secretorys IgA produced by *lamina propria* plasma cells are transported by enterocytes into the bowel lumen. Unlike other immunoglobulins classes, they do not activate complement or inflammatory responses, which makes them ideal for protecting mucosal surfaces. Their major function seem to protect digestive epithelium by cross-linking microorganisms or macromolecules, thus facilitating their elimination by peristalitism or mucociliari movements, and preventing their contact with the surface of epithelial cells. This phenomenon is called immune exclusion (CORTHESY and KRAEHENBUHL, 1999). sIgA can prevent attachment of microorganisms to the epithelium, indirectly by limiting their diffusion across the mucus layer when coated with IgAs, or directly blocking the microbial sites that mediate attachments. IgA can also neutralize microorgansims that are internalized by epithelial cells in mucosal tissues (GEBBERS and LAISSUE, 1989; CORTHESY and KRAEHENBUHL, 1999). Secretory IgA may adhere selectively to M cells in the lumen of the gut and present them to the underlying lymphoid follicles (NEUTRA, 1999; ABREU-MARTIN and TARGAN, 1996). They represent a key effector of a correctly regulated intestinal immune response.

Interaction between epithelial cells and mucosal immune cells

Some data suggests that interactions of lymphocytes with intestinal epithelium are very important. Lymphocytes, particularly those of B cell lineage, can induce enterocytes into M cell like-cells of the Follicle Associated Epithelium (KERNÉIS *et al.*, 1997). Additionnally, some intraepithelial T lymphocytes (with γδ T cell receptors) seem to be

generated neither in the thymus nor in the lymph nodes, but locally in cryptopatches of the digestive epithelium. These cells interact with epithelial cells and protect the mucosa by killing infected cells and attracting other immune cell to combat infection (ISOLAURI *et al.*, 2001).

Enterocytes have been shown to possess processing and presentation pathways. They express Major Histocompatibility Complex molecules, and are able to produce some cytokines. Antigens could be transported between the epithelium (paracellular pathways) or through the epithelium (transcellular pathways; SHAO *et al.*, 2001). The pathway of transport across the digestive mucosa depends on antigen and will dictate the type of immune response generated. Therefore, intestinal epithelial cells serve an immunoregulatory function and are strongly implicated in the digestive mucosal immunity (ABREU-MARTIN and TARGAN, 1996).

Oral tolerance

The mucosal immune system has developed under the dual evolutionary pressures of protecting the host from invasion of pathogens while permitting the transfer of food antigens. Oral tolerance is defined as the decreased ability to stimulate a systemic response to antigens previously encountered in the gut (SIMECKA, 1998). The intestinal mucosa normally contacts many different food, micro-organisms and chemical products. There would be no advantage, and indeed a great disadvantage, if the host were to respond systematically to multiple antigens that cross the mucosal surface. The mechanisms underlying the induction of oral tolerance are not totally understood and no data exists on rabbit species. Several studies suggest that tolerance training is directly linked to the interaction between the microbiota and the digestive immune system (see probiotic § below). Studies from other species suggest that Peyer's Patches are necessary for the induction of oral tolerance. When non-harmful macromolecules (for example non digested nutriment) or non-pathogenic microorganisms are uptake by M cells, a Th-3 response is induced. This Th-3 response is characterised either by the secretion of specific IgA or by the anergy of the lymphocytes specific for this antigen. The anergy prevents lymphocytes activation and the development of the immune response. Such a regulation seems to involve the release of cytokines such as TGFβ (WEINER, 2001). On the opposite, when the regulation of the response is not correctly controlled, pro-inflammatory Igs like IgG are secreted by non-anergised lymphocytes. These IgG activate the complement cascade and granulocytes and lead to lesions in the intestinal epithelium. These lesions allow the contact between normally excluded components (non digested nutriments and micro-organisms) and lymphoid cells from the effector sites. The inflammatory response increases and the tolerance is broken (ROITT and DELVES, 1998). The oral tolerance is strictly necessary to avoid uncontrolled response and development of inflammatory bowel syndromes.

INFLUENCE OF GUT MICROFLORA ON DIGESTIVE PHYSIOLOGY AND HEALTH

The rabbit is both a monogastric and a herbivore. Its digestive system is adapted to fermentation of vegetable cell wall owing to an important gut microflora located in colon and cæcum. Several reviews describe the digestive system and physiology of the rabbit

(GIDENNE, 1997; CARABANO and PIQUER, 1998, GIDENNE and FORTUN-LAMOTHE, 2002). Therefore, the present paragraph focus on gut microflora and its role in digestive physiology to better understand the relation between diet, microflora and health developed in the last section.

Gut microflora of rabbits

Microbial colonisation begins after birth, but the development of the intestinal microbiota is a gradual process. The maternal intestinal flora and surroundings are the main source of bacteria colonising the new born's intestine. GUARNER and MALAGELADA (2003) relate that the initial colonisation is very relevant to the final composition of the permanent flora in adults. Indeed, pioneer bacteria can modulate expression of genes in host epithelial cells, thus creating a favourable habitat for themselves, and can prevent growth of other bacteria introduced later in the ecosystem. BERG (1996) recalled the definition of autochtonous flora (or indigenous flora) as "resident microorganisms present in all communities of a particular animal species". They can grow anaerobically in the gastrointestinal tract and are always present in adults. They colonize particular gastro-intestinal niches, contribute to a stable climax and are often associate intimately with mucosal epithelium.

Several studies demonstrated that bacteria are the main constituant of the gut microbiota in rabbit (GOUET and FONTY, 1979; BOULHAROUF et $al.$, 1991). More recently, detection and quantification of microbial population were assessed by hybridisation with 16S rRNA targeted oligonucleotides probes (BENNEGADI et $al.$, 2003). The results obtained confirmed that bacteria is the dominant population. However, the authors also reported an important communitie of archea (22% and 12% at 28 and 70 days of life, respectively). Some authors reported the presence of yeast (*Saccharomycopsis guttulata*; PEETERS, 1987) and protozoa (FORSYTHE and PARKER, 1985; LELKE, 1986). Anaerobic fungi were absent in rabbit.

During the first two weeks of life, strictly anaerobic and facultative anaerobic flora are present in similar proportion (10^7-10^{10} bact/g). The facultative anaerobic bacteria, mainly *Streptoccus sp.* and *Escherichia coli*, reached a maximum level at the 2nd or 3rd week of life and then decreased to be **residual** or absent after weaning. The strictly anaerobic, non-sporulating bacteria, especially Gram-negative bacilla (*Bacteroides*) **dominate** the digestive flora in every segment of the intestine. Sporulating bacteria (*Clostridium, Endosporus* and *Acuformis*), 100 to 1000 times less numerous than the *Bacteroides*, are considered to belong to the **sub-dominant** flora. The absence of the genus *Lactobacillus* in the rabbit flora is original.

The bacteria involved in fibrolysis (hydrolysis of cellulose, xylanes, pectins, etc) only become established after 15 days of age, when intake of solid food begins and a fibrous substrate enters the caecum (Figure 4). Then the fibrolytic flora increases slowly to reach 10^7 bact/g at 25 days of age in conventional rabbits (BOULHAROUF et $al.$, 1991). It should be noted that 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).
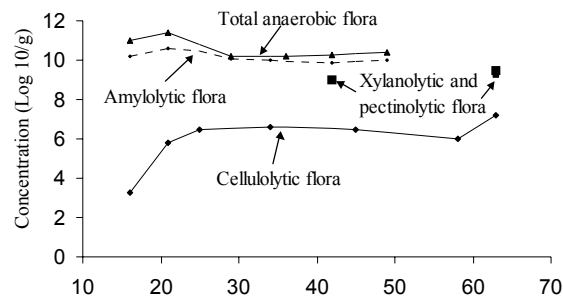
Figure 4 : Establishment of the rabbit caecal flora between 2
and 10 weeks of age (from Gidenne and Fortun-Lamothe, 2002).

Comparison of gut microbiota composition in conventional and Specified Pathogen Free (SPF) leads to contradictory results. BENNEGADI *et al.* (2003) reported a higher proportion of fibrolytic populations in SPF than in conventional rabbits. It has been suggested that a better balance of the caecal ecosystem in favour of commensal fibrolytic populations may explain the higher resistance of SPF rabbits for digestive troubles when fed a fibre deficient diet (BENNEGADI *et al.*, 2001). PADHILA *et al.,* (1995) reported a low level of cellulolytic bacteria (10^3 bact./g) in specific-pathogens-free rabbits than in conventionnal animals.

Role of the gut microflora on digestive physiology in rabbits

Several studies demonstrates that microflora have important and specific morphologic, metabolic, trophic and protective functions.

- ***Architectural and trophic effects***
Bacteria have a direct impact on the morphology of the gut. Indeed, germ-free animals exhibit a larger caecum size than conventional animals (BERG, 1996). This enlargemnt is due in large part to accumulation of undegraded mucus. Some strains of microbiota (*Ruminococcus sp., Bifidobacterium sp.*) produce a variety of glycosides hydrolases which permit hydrolysis of mucus produced by epithelial cells (FALK *et al.*, 1998). Some data suggest that intraluminal bacteria affect cell proliferation in the small intestine and colon. The crypts contained fewer cells and the rate of production of crypt cells is reduced in the colon of rats bred in germ-free conditions than do in animals colonised by conventional flora (GUARNER and MALAGELADA, 2003).

- ***Metabolic effects***
The major, and the most studied, function of caeco-colic microflora in rabbit is the fermentation of the digesta which has escaped intestinal absorption and endogenous mucus produced by epithelia. The flora exhibit various enzymes and biochemical pathways that are distinct from the host's constitutives resources. Caecal flora exhibit a strong proteolyctic and ureolytic activity (review : GIDENNE, 1997) which lead to production of ammonia, and also a series of other potentially toxic substances including amine, phenols and indols. Pectinase, xylanase and cellulase are the main bacterial enzymes implicated in the fibres fermentation (MAROUNEK *et al.*, 1995; GIDENNE et JEHL, 2000, PINHEIRO *et al.*, 2001). There is a remarkable capacity for degrading pectins and

hemicelluloses, attributable to the substantial establishment of this flora compared with the cellulolytic flora (Figure 5). Degradation of complex carbohydrates generate short term fatty acids (Volatile Fatty Acids). The caecal volatile fatty acid is specific to the rabbit, with a predominance of acetate (60-80%), followed by butyrate (8-20%) and then propionate (3-10%; GIDENNE, 1997).
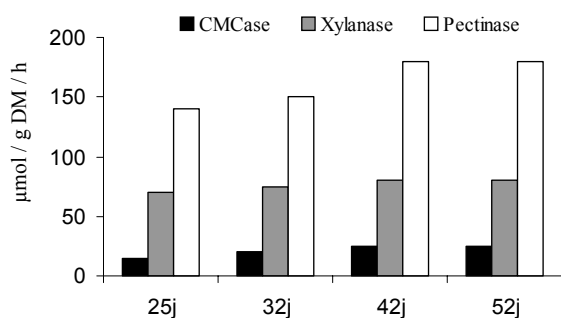

Figure 5 : Evolution of fibrolytic activity in the caecum in the young rabbit (from Gidenne and Fortun-Lamothe, 2002).
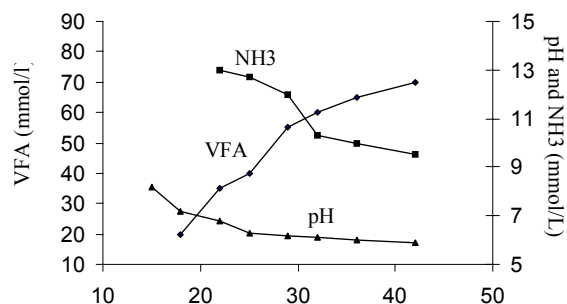CMCase : Carboxy-methyl cellulase

Figure 6 : Development of caecal fermentative activity in the young rabbit (from Gidenne and Fortun-Lamothe, 2002).
VFA : Volatile Fatty Acids

The caecal concentration of fermentation end-products (ammonia and volatile fatty acids) controls the acidity of the caecal content (pH) and reflects the fermentative activity of the flora (Figure 6). The volatile fatty acids level in caecum is weak at two weeks of life, in relation with the low implantation of fibrolytic flora at this age and absence of substrate. It increases progressively from the beginning of solid feed intake (16-18 days) to reach 60-70 mmoles/l at 45 days of age in conventionnal rabbits (GIDENNE and FORTUN-LAMOTHE, 2002). In specified-pathogens-free rabbits PADHILA *et al.* (1995) observed lower fatty acids levels in caecum (34 mmoles/l at weaning).

Gut microorganisms also play an important role in vitamin synthesis, more especially B vitamins, which are available for the host through caecotrophy (LEBAS, 2000).

Influence of the gut microflora on health

Several studies demonstrated that a close relationship exists between gut microflora and health of host. The gut microbiota is an important constituent in the intestine's defense barrier, as shown by increased antigen transport across the gut mucosa in the absence of an intestinal microbiota. The role of indigenous microorganisms includes both a protection against pathogens (the barrier effect) and a strong implication in the development and maturation of digestive mucosa immunity.

- *The barrier effect*
One of the most important beneficial effects of the indigenous gastro-intestinal microflora is to make it more difficult for exogenous pathogenic bacteria to colonize the digestive tract (BERG, 1996). The increased antigen transport across the gut mucosa in the absence of an intestinal microbiota support this latter assertion. Several

mechanisms have been implicated in the barrier effect. Adherent non-pathogenic bacteria can prevent attachment and subsequent entry of pathogens into the epithelial cells. Furthermore, bacteria compete for nutrient availability in ecological niches and maintain their collective habitat by consuming all resources. Finally, bacteria can inhibits the growth of their competitors by producing antimicrobial substances (GUARNER and MALAGELADA, 2003).

- ***The development of mucosal lymphoid tissue and oral tolerance***
Gut colonisation acts as an important antigenic stimulus for the maturation of the gut-associated lymphoid tissue. Indeed, animals bred in a germ-free environment have low densities of lymphoid cells in the gut mucosa, Peyer's patches are small, and circulating concentrations of immunoglobulins in the blood are low. Microbial colonisation of the digestive tract also affects the composition of the gut-associated lymphoid tissue : it increases the number of intraepithelial lymphocytes and immunoglobulins producing cells, both in follicles and *lamina propria* (GUARNER and MALAGELADA, 2003). Gut flora is also implicated in the induction of major histocompatibility complex II molecules in the small intestinal epithelial cells and acquisition of their cytotoxic activity (UMESAKI and SETOYAMA, 2000).

The intestinal bacterial flora seems also implicated in oral tolerance. The systemic response to a specific antigen can be abrogated after ingesting the same antigen. But, conventional intestinal flora is necessary for the persistence of systemic hyporesponsiveness to an antigen (GABORIAU-ROUTHIAU and MOREAU, 1996). Oral tolerance in germ free animals could be induced by reconstitution of conventional flora, but this procedure is only effective in neonates and not in older individuals (SUDO *et al.*, 1997). Therefore the interaction between gut-associated lymphoid tissue and flora early in life seems to be crucial for appropriate development of complex mucosal and systemic immunoregulatory circuits (ISOLAURI *et al.*, 2001).

Studies involving gnotobiotic rodents have shown that the presence of limited kinds of intestinal bacteria is responsible for the development of the gut immune system such as secretory IgA, major histocompatibility complex and intraepithelial lymphocytes. Some bacteria, such as segmented filamentous bacteria and clostridia seem important to induce to the development of immune response (UMESAKI and SETOYAMA, 2000). The components of the bacterial cell wall, such as peptidoglycan and lipopolysaccharide, have been shown to play an important role in the activation of immune system (HAMANN *et al.*, 1998).

- ***The diversification of antibody repertoire***
The immune system can identify a great number of antigens and produces a wide antibody repertoire either secreted by B cells or as receptors of T cells. Both soluble (immunoglobulins) and cellular (membranous) antibody are constituted of heavy and light chains which contain each one a constant and a variable region. In vertebrates, three mechanisms allow the diversification of antibody repertoire : the multiplicity of genes coding for variable regions (in theory all possible combinations of the various shapes of heavy and light chains), somatic recombination (rearrangement between several gene segments, called *VDJ* segments)) associated with random nucleotide

additions at the junction points, and for immunoglobulins only, somatic mutations (change of one or several bases in DNA chain). Each species use these different strategies in a more or less important way.
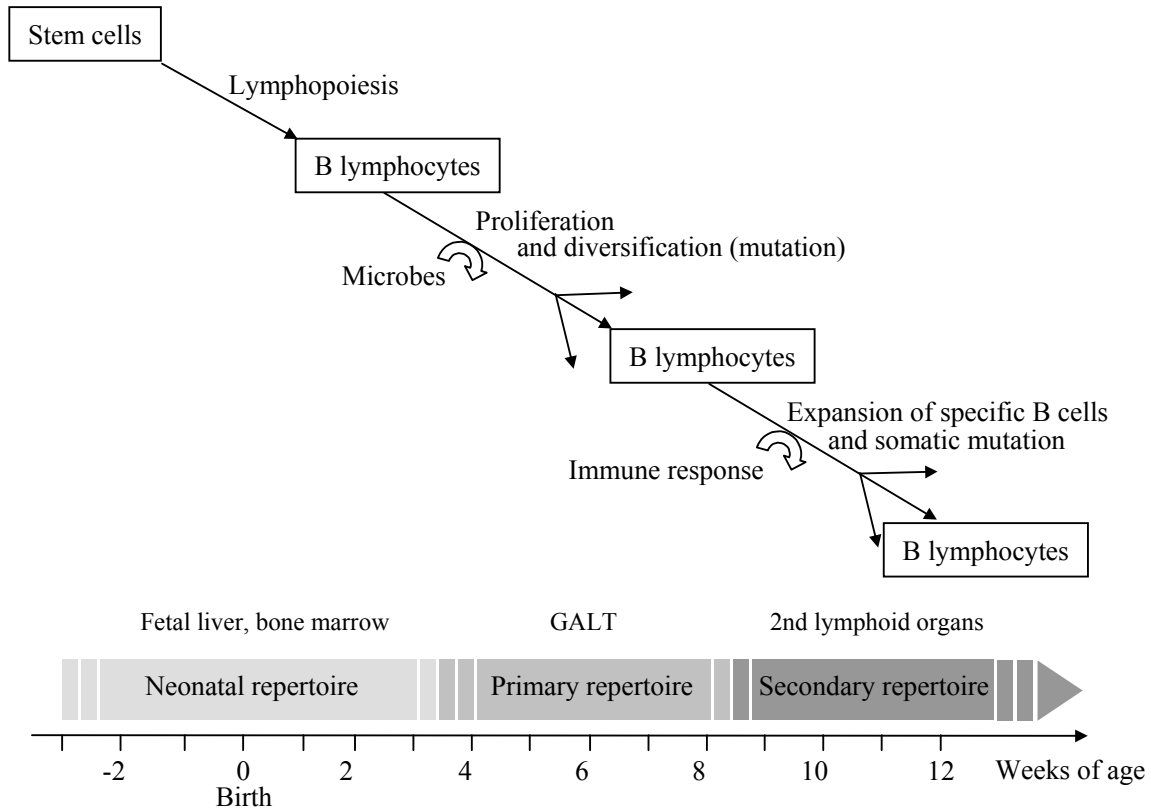

Figure 7 : Schematic representation of antibody repertoire development in rabbit.

Rabbits generate their antibody repertoire in three stages (KNIGHT and CRANE, 1994; Figure 7). First, a ***neonatal antibody repertoire*** is established by B cells generated during B lymphopoiesis early in rabbit life (before 3 weeks of life). It is limited by preferential usage of a gene segment (V_{H1}) for the heavy chain and utilisation of multiple V_κ genes for light chain. However, this limitation is partially compensated by a high degree of N nucleotide addition at the *VD* and *DJ* junctions. The ***primary antibody repertoire*** develops between 4 and 8 weeks of age in gut-associated lymphoid tissue. The B cells undergo proliferative expansion and *VDJ* gene diversification by two mutational processes 1/ a gene conversion-like mechanism that replaces tracts of nucleotide sequence in the rearranged V_H gene segment with homologous sequences from upstream V_H gene segment donors and 2/ somatic hypermutation, which distributes single nucleotide changes throughout the *VDJ* gene. Several experimental data suggested that intestinal microbial flora are required during development of the primary antibody repertoire. (LANNING *et al.*, 2000a,b; VAJDY *et al.*, 1998). The primary repertoire, which provides the rabbit a diverse collection of antibody specificities, is subsequently modified in adult life during antigen-specific immune responses by somatic mutation of

immunoglobulin genes to give the **secondary repertoire**. Since B lymphopoiesis is limited in adult rabbits (JASPER *et al.*, 2003), it is suggested that B-cell pool and antibody repertoire is maintained through self-renewing and a long lifespan of B cells (KNIGHT and WINSTEAD, 1997).

STRATEGIES TO IMPROVE DIGESTIVE HEALTH IN THE YOUNG RABBIT

The epithelial cells, GALT and commensal flora interact with each other forming a dynamic but delicate equilibrium. A balanced system is necessary to ensure both nutrients absorption and protection against pathogens. Diet has a strong influence on this balance. Firstly, it contains substrates for host's digestive mucosal cells and/or gut flora. Additionally, it strongly influences the physical and chemical properties of the intestinal content and the digestive transit time. These latter factors could affect the integrity of the intestinal epithelium and the capacity of micro-organisms to attach to enterocytes and/or pass troughout the epithelial barrier.

In the young rabbits (15-35 days of age), the equilibrium between gut mucosa (absorptive epithelium and GALT) and gut microflora is more especially fragile. Data reported in the previous section suggested that several factors explain this situation : 1/ the gut microflora is not completely stabilised until 30-40 days of age; 2/ immune competency is still immature since immune structure are not fully developed and diversification of antibody repertoire occurs tardily in this species (4-8 weeks of age) and is dependant of gut flora; and 3/ a strong modification of the diet occurs during this period since animals change (more or less progressively, depending on age at weaning) from exclusively milk intake to exclusively solid feed intake.

In this section we discuss several strategies which could favour conditions that create and stabilise the balance between host and microflora and prevent disturbance of the gut function (absorption and protection).

Nutrients to stimulate the digestive immunity

Several works were devoted to the determination of nutrient requirements for growth or reproduction in rabbits, leading to nutritional recommendations according to physiological status of animals (DE BLAS and WISEMAN, 1998). In these studies, the influence of the nutriment considered, on the resistance of the animal to infections is rarely taken into consideration. However, the dietary level which maximizes growth or reproduction does not necessarily induce the greatest defense capacity in the animals (see Figure 8). Indeed, during the course of immune response, a lot of cytokines are liberated which lead to stimulation of local (and sometimes then general) endocrine and nervous cells. These phenomenons induce cellular, metabolic and behavioral changes, which alter the distribution of nutrients in favour of the immune response to the detriment of other metabolic uses, such as growth and milk production (KLASING and LESHCHINSKY, 2000). Thus one observes a decline in physical activity and food ingestion (up to 70%), an increase in body temperature (fever), and modifications of host metabolism. As a consequence, zootechnical performance of the sick animals are generally sharply

decreased (JOHNSON, 1997). It is therefore interesting to review the possible influence of nutrition on immune system and resistance of animals to infectious diseases.
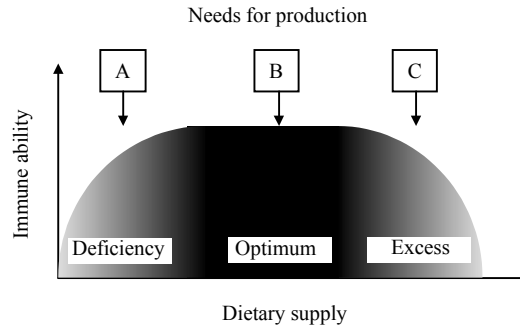

Figure 8 : Relations between the nutrional needs for production and immune system functionning.

For one nutrient, the nutrionnal need for growth (indicated with an arrow) could be lower (A), equal (B) or higher (C) than those for an optimal functionning of immune system (from Klasing and Leshchinsky, 2000).

- ***Mechanisms of nutritional effects on immunity***
Nutrients have ***direct role*** on immunity as they serve as substrates and enzymes co-factors for cellular multiplication during immune response (phagocytes, lymphocytes) and for synthesis of effective molecules (antibody, complement, nitric oxyde, lysozyme) or informant molecules (cytokines, inflammatory mediators; KLASING and LESHCHINSKY, 2000; Figure 9). Furnitures of susbtrates concerned mainly amino acis, fatty acids and glucose while co-factors for enzymatic activities are often vitamins or minerals.

Nutrients could also have an ***indirect effect*** on immune response by modifying the intra- and extra-cellular communication pathways (cytokines) or limiting undesirable effects of effective molecules. For example, the amount and profile of fatty acids in the diet determines the type of fatty acids which are incorporated into the cell membranes and hence the fluidity of the membranes and the type of eicosanoid secreted as informant molecules. Certains anti-oxidants (vitamins E and C, β-carotene) may limit the undesirable effects of destructive molecule (nitric oxide, hydrolases..) on membraneous components (phospolipids). Finally, the immune system is also regulated by numerous hormones (gastrin-releasing peptide, GH, IGF1, insulin, thyroides hormones…) most of which are responsive to nutrionnal factors (glucose, protein/energy ratio…; GENTON and KUDSK, 2003).

- ***Role of different nutrients on the immune response***
Relations between nutrients and immunity were recently reviewed by FORTUN-LAMOTHE and DROUET-VIARD (2002). We reported here the main conclusions with specific mention for digestive mucosal immunity.
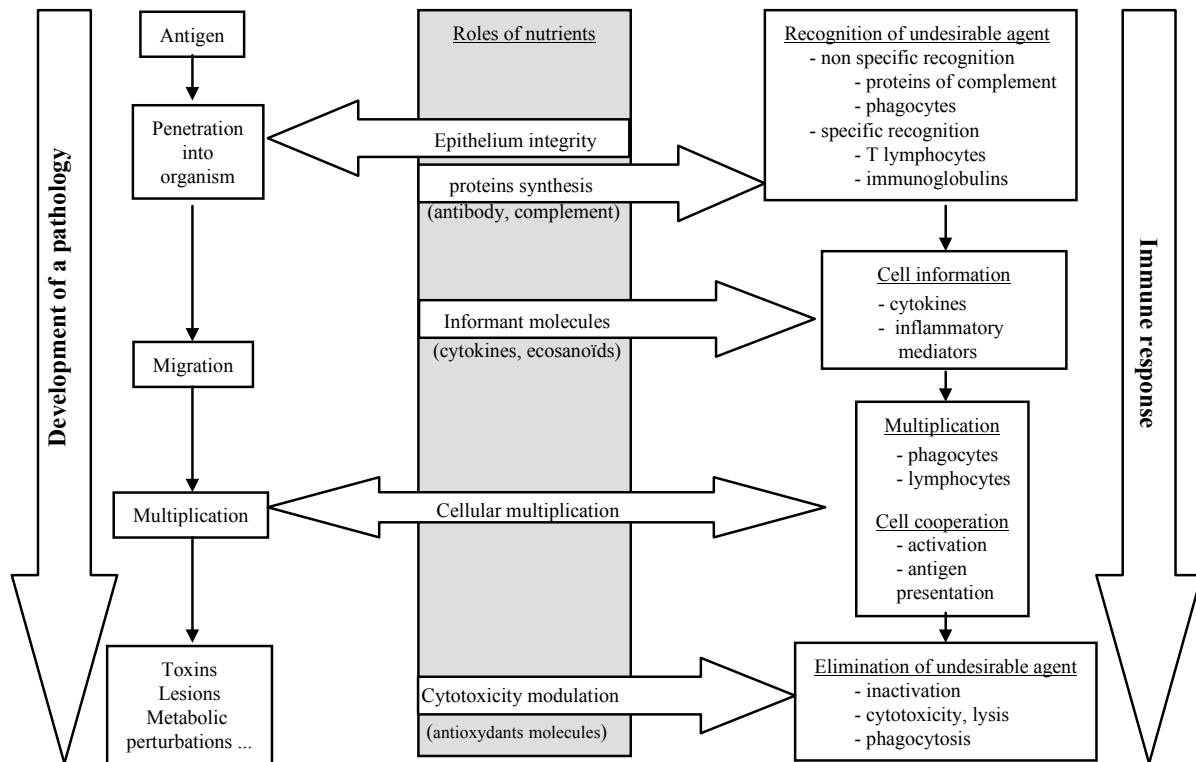
Figure 9 : Directs (substrates supply) and indirects roles (modulation of infection or of immune response) of nutrients on immune response (From Fortun-Lamothe and Drouet-Viard, 2002).

Remark : this figure doe not indicate some time axis, and differents steps of pathology developpement or immune response which face together are not necessarily contemporary.

Lipids and fatty acids. Influence of lipids on immune response is probably the most documented relation between nutrition and immunity, undoubtedly because of their implication in human diseases in occidental countries (cardiovascular diseases, diabete). The amount and type of dietary fat can modulate immune function both at systemic (reviews : CALDER, 1998; YAQOOB, 2003) and intestinal levels (MIURA *et al.*, 1998). Indeed, fatty acids are structural components of cells membranes and signalling molecules and precursors for the synthesis of eicosanoids (a family which includes leucotriens, inflammation mediators molecules). Both excess or deficiency of them could be harmful to immune system. Absorption of long-chain fatty acids enhances migration of T lymphocytes to Peyer's patches (MIURA *et al.*, 1998). Fat absorption could also influences indirectly the mucosa immune system increasing cytokine release from intestinal epithelial.

The quality of fatty acids have an effect on immune system. More especially the level of polyunsaturated fatty acids of the ω6 or ω3 series (fatty acids with double bonds, the first of which being on atom carbon 6 or 3, respectively, from the methyl end) seems of primary importance for immunomodulation. The metabolism of the ω6 or ω3 series fatty

acids is competitive because metabolic pathways of elongation or desaturation use the same set of enzymes. The major-end product of the ω6 and ω3 pathways are respectively arachidonic acid (precursor of leucotriens) and ecosa- or decosapentaneoic acid (ZIBOH, 2000). Arachidonic acid, from ω6 fatty acids, leads to the production of pro-inflammatory eicosanoids (LT4 and PGE$_2$). These pro-inflammatory molecules induce beneficial immune reactions, playing an immuno-regulatory role, but can also leads to harmful reactions if they are mobilised too intensely, reducing the production of cytokines. Metabolism of ω3 fatty acids could counterbalance theses negative effects by the formation of less biologically active leucotriene (LT$_5$; CHAPKIN *et al.*, 2000).

Proteins and amino acids. Most of the inflammation and immune reaction mediators are peptides (cytokines, immunoglobulins, complement proteins, DNA synthesis for lymphocyte proliferation). These proteins present a very different profile from the proteins involved in body growth or milk production. This leads to specific amino acid requirements for the immune response (LE FLOC'H, 2000). That confers a key role to level (quantity) and quality (amino acids) of proteins in the diet to optimise immune response. Immuno-modulatory role of arginine, glutamine and taurine have been demonstrated and discussed (EVOY *et al.*, 1998; REDMOND *et al.,* 1998; SHANG *et al.*, 2003. LAI *et al.*, 2004). But specific recommendations for amino acids to optimise immune response in rabbits are not known.

Minerals and vitamins. Several minerals are recognised as having great importance in immunity: zinc, copper, selenium magnesium and iron (SCRIMSHAW and SANGIOVANNI, 1997; GALAYAN *et al.*, 1999). Nearly all the vitamins are involved in the functions of the immune system cells: phagocytosis, synthesis of molecules regulating the leucocytic function (interleukins), and production of immunoglobulins (KOLB, 1997). In the rabbit, the caecal flora synthesises large amounts of water-soluble vitamins which are rendered available by caecotrophy. It is generally agreed that all the vitamin requirements of group B and C are met in this species. However, the course of caecotrophy is frequently disturbed in the sick animal, especially in the case of digestive disorders such as enteritis. In this case, a deficiency of vitamins of the B group and of vitamin C could arise, lowering the resistance to attack and delaying recovery. Therefore, supplementation of vitamins B and C could be of interest during infectious episode knowing that the risk of poisoning from an excess of these vitamins seems to be slight (LEBAS, 2000).

Feeding strategies to favour commensal microflora and prevent digestive troubles in the rabbits

GIDENNE (1997) previously review the impact of nutritional factors on caecal activity. Therefore, here we focussed mainly on recent results about influence of nutrition on microbial population or activity and digestive health in rabbits. Preliminarily, it is necessary to keep in mind that to have a direct action on gut microflora, dietary nutrients must escape to intestinal digestion. However, intestinal digestive capacity evolved under ontogenic factors and is not stabilised during establishment of gut microflora.

Additionnaly, the "ideal" composition of the gut microbiota to maximise the resistance to digestive troubles in the rabbit is not defined.

- *Fibres*

Several studies demonstrated that low fibres intake increases the frequency of digestive troubles in weaned rabbit (GIDENNE *et al.*, 2000; BENNEGADI *et al.*, 2001). Indeed, the digestive system of the rabbit is adapted to high intake of plant cell walls and dietary fibres are the main constituent of a rabbit feed (14 to 18 % of crude fibre). The whole tract digestibility coefficients of fibres depend on their chemical nature and vary from 10 to 76 % (GIDENNE, 2003). Therefore, an important fraction of intake fibres enter the caecum as substrates for microbial fermentation. Numerous works were thus devoted to clarify the respective role of each classes of fibres (sequential procedure analysis of Van Soest *et al.*, 1991) on microbial activity and health of animals : low-digested fibre such as lignin and cellulose (digestibility coefficients < 20%) and digestible fibres, such as hemicellulose (digestibility coefficients : 25 to 35 %) and pectins (digestibility coefficients : 70 to 76 %).

The positive influence of low-digested fibres on digestive health of weaned rabbits was first observed many years ago (MAITRE *et al.*, 1990) and was confirmed therefater in several studies (PEREZ *et al.*, 1994; 1996; GIDENNE *et al.*, 2001a; Figure 10). Recent studies also demonstrated the interest of more digestible fibres both to reduce digestive troubles and as energy source (PEREZ *et al.*, 2000; GIDENNE *et al.*, 2001b; GIDENNE *et al.*, 2004). The whole of these data made it possible to propose dietary fibres recommendations for digestive troubles prevention in growing rabbits, including level and quality of lignocellulose as well as level of digestible fibre (GIDENNE, 2003).
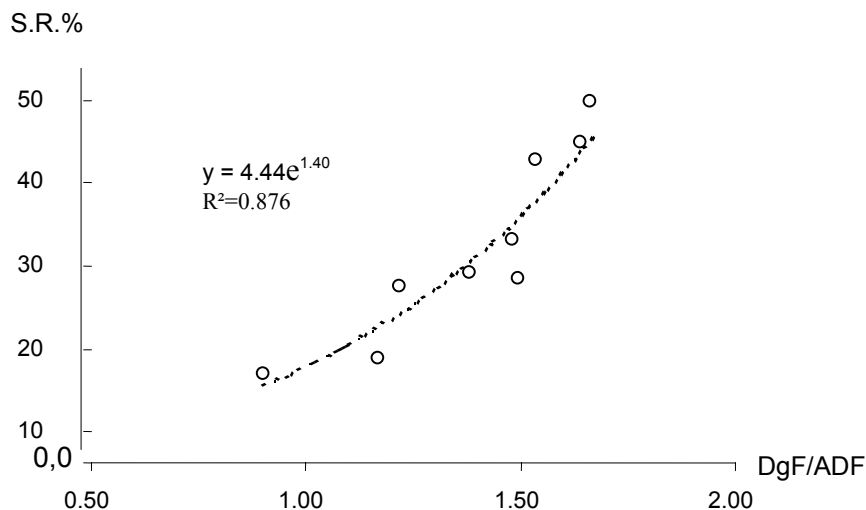

Figure 10 : Sanitary risk according to the ratio digestible fibres / lignocellulose when lignocellulose is over 15% (From Gidenne, 2003).
SR% = sanitary risk from digestive troubles (mortality + morbidity rate by diarrhoea). DgF : digestible fibre = water insoluble pectins + hemicelluloses. ADF = lignocellulose (Van-Soest sequential procedure).

Mechanisms underlying the positive effects of low-digested and more digestible dietary fibre in digestive health of weaned rabbits are not elucidated. A shorter digestive transit time when dietary fiber level decreased (GIDENNE *et al.*, 2000, GIDENNE *et al.*, 2004) could limit the possibility of attachement and/or development of pathogen microorganisms. However, feed restriction in growing rabbits (20 to 40 % of ad libitum feed intake) significantly reduced the incidence of digestive troubles (GIDENNE *et al.*, 2003a) while it lowers the transit time (GIDENNE, personal communication). An increase of dietary fibres also favour the activity of fibrolytic bacteria as suggested by higher volatile fatty acids content and bacterial fibrolytic activity in the caecum (GIDENNE *et al.*, 2000; GIDENNE *et al.*, 2004). However, the influence of dietary fibres level on microbial ecosystem, particularly cellulolytic community, leads to contradictory results. BOULHAROUF *et al.* (1991) observed a higher level of fibrolytic bacteria when the percentage of crude fibre in the diet increase from 11 to 17%. In the study of BENNEGADI *et al.* (2003), a decrease of dietary fibre level (19% to 9% ADF) decreased the percentage of archea but increased the *Flexibacter-Cytophaga-Bacteroides* community and percentage of *Ruminococcus albus*. Finally, the botanic source of fibres could also influence microbial population (BELENGUER *et al.*, 2000).

Several data also suggests that the nutritional composition of the diet given to the young before weaning influence the health status after weaning. A high level of fibre in the diet given to the rabbit before weaning could improve their health status after weaning (MORISSE *et al.*, 1989; FORTUN-LAMOTHE *et al.*, 2001; DEBRAY *et al.*, 2002) and increase their resistance to experimental inoculation of a pathogenic agent (LICOIS and GIDENNE, 1999). A favourable effect of fibres in the establishement of a balanced microbial ecosystem in young rabbits could be hypothesized (GIDENNE and FORTUN-LAMOTHE, 2002) but has not yet been demonstrated.

- ***Starch***
For a long time, starch was supposed to be a factor predisposing to the development of undesirable flora in rabbits (CHEEKE and Patton, 1980). But data concerning the relation between starch intake and digestive troubles are controversial. Intake of a diet rich in starch resistant to ileal digestion (and thus increasing levels entering the caecum) did not affect the sanitary status of animals (PINHEIRO and GIDENNE, 2000; GIDENNE *et al.*, 2003b). However, GIDENNE *et al.* (2004) observed higher starch concentrations at the terminal ileum and higher occurrence of digestive troubles in fattening rabbits (28 to 70 days of age) when the starch level of the diet increase (2,5 to 37,4 %) in relation with a decrease in fibres level.

The digestive capacities of starch is very low in young rabbit before 25 days of life and sharply increase thereafter (DEBRAY *et al.*, 2003). Consequently, the starch content in the ileum is quite high in 4-week old rabbits (about 7-9%, BLAS and GIDENNE, 1998) and very low 2 weeks later. But, young rabbits eat before the weaning, the same diet than their mother, often formulated to meet the high energy requirements of reproductive females. Several authors hypothesized that the intake of diet rich in starch could exceed the intestinal enzymatic capacities of young rabbits, and the overflow of starch entered the caecum could impair the establishment of a balanced microbial ecosystem. However, such assumptions remain to be demonstrated.

- ***Fat***

The fat level is generally low in rabbit feed (2 to 6%) and fat digestibility coefficients are high (Xiccato, 1998). Digestion of fat mainly occurs in small intestine because a high lipase activity have been found even in young rabbit (Debray *et al.*, 2003). Data concerning effect of fat level in the diet on caecal microbial activity leads to contradictory results. Falcao-e-Cunha *et al.* (1998) observed a lower butyric acid proportion while proportion of other volatile fatty acids was higher (propionate) or tended to be higher (acetate) in caecal content of rabbit given fat-enriched diet. Falcao-e-Cunha *et al.* (2000) found opposite results and several studies observed no effects of fat inclusion in the diet on fermentation or fibrolytic activity in the caecum (Xiccato *et al.*, 1998 and 2001, Falcao-e-Cunha, 2001). Interactions between fat inclusion and bacterial fibrolytic development, appreciated by crude fiber digestibility, lead also to contradictory results (Fernandez-Carmona *et al.*, 2000).

- ***Proteins***

Recent data were obtained concerning the ileal digestibility coefficient of dietary crude proteins and main amino acids in rabbits (Carabano *et al.*, 2000). However, few studies assess the caecal microbial activity according to the dietary supply of protein or ileal flow of protein. Cortez *et al.* (1992) reported that an excess of dietary protein could favour the development of pathogens such as *Clostridia* and *Escherishia coli*. More recently, Gidenne *et al.* (2001b) observed a better health status in growing rabbits when the digestible fibres / crude proteins ratio increased over 1.3 (DF>20% and CP<16%).

- ***Prebiotics***

An alternative approach to the manipulation of the gut microflora is the use of prebiotics. A prebiotic has been defined as a non-digestible food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gut (Gibson and Roberfroid, 1995). Prebiotics generally refer to carbohydrates, mainly oligosaccharides, which target the beneficial bacteria of caeco-colic microflora. Oligosaccharides can be found naturally in certain fruits and vegetables but can also results from *in vitro* enzymatic process. In human, dietary supplementation of oligosaccharides increased the level of beneficial gut flora (bifidobacteria) and seemed to diminish the inflammatory process as suggested by the decreased expression of IL-6 mRNA in blood monocytes (Guigoz *et al.*, 2002).

In rabbits, Peeters *et al.* (1992) observed increased levels of volatile fatty acids and lower pH of the caecal content with galacto-oligosaccharides and Gidenne (1995) found no influence of gluco-oligosaccharides on this paramaters, but observe a higher mortality rate. Studies with fructo-oligosaccharides on caecal fermentation activity lead to contradictory results : levels of volatile fatty acids were higher (Morisse *et al.*, 1990,; Morisse *et al.*, 1993) or similar (Lebas, 1993; Luick *et al.*, 1992) when rabbits were fed supplemented diets. Fructo-oligosaccharides showed some protective effects against experimental inoculation of E. coli (Peeters *et al.*, 1992; Morisse *et al.*, 1993) but in field conditions their positive effects on health have not been strongly demonstrated. However, some authors reported increased growth rate or lower feed conversion rate (Lebas *et al.*, 1996; Aguilar *et al.*, 1996).

Exogenous flora to stimulate the mucosa digestive immunity (probiotics)

The demonstration that the gut microbiota is an important constituent in the intestine's mucosal barrier has introduced the concept of probiotic therapy or prevention i.e. application of potentially beneficial microorganisms to increase the host defence. A consensus definition was issued few years ago and states that "oral probiotics are living microorganisms which, upon ingestion in specific numbers, exert health benefits" (GUARNER and SCHAAFSMA, 1998).

Probiotic consumption is reported to exert their beneficial effects through several ways including balancing of colonic microbiota and enhanced immune response (KAUR et al., 2002; Figure 11) both at local and distant mucosal sites (CLANCY, 2003). Probiotics have been reported to modulate favourably both innate and aquired immunity (DUGAS et al; 1999). Both way of aquired immunity, cellular and humoral response, could be enhanced (CHIANG et al., 2000; FANG et al., 2000). Specific use of probiotics modulates the host's immune responses to potentially harmful antigens. Oral introduction of *Bifidobacterium bifidum* was shown to enhance antibody response to ovalbumin (MOREAU et al., 1990) and *Bifidobacterium breve* was shown to stimulate IgA response to cholera toxin in mice (YASUI et al., 1992). Several studies indicate that the immunomodulating effects of probiotic bacteria may depend on the immunologic state of the host. They further suggest that form of immune signal is strongly strain-dependant (CROSS, 2002). A recent study in chicks reveals that a single inoculation dose of the *Lactobacillus johnsonii* probiotic strain ousted the harmful *Clostridium perfringens* from chicks' gut (LA RAGIONE et al., 2004). These results reinforce the strategy of the rational probiotic distribution to prevent enteric diseases.
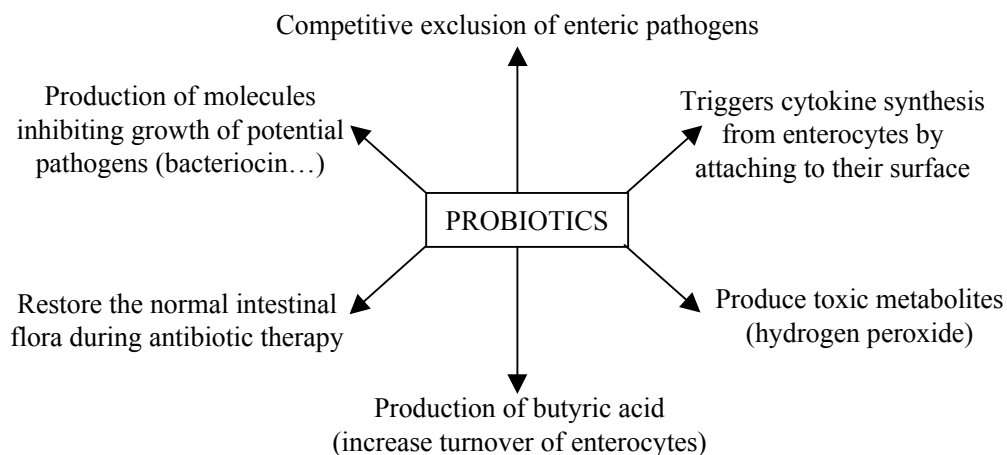
Competitive exclusion of enteric pathogens

Production of molecules inhibiting growth of potential pathogens (bacteriocin…)

Triggers cytokine synthesis from enterocytes by attaching to their surface

PROBIOTICS

Restore the normal intestinal flora during antibiotic therapy

Produce toxic metabolites (hydrogen peroxide)

Production of butyric acid (increase turnover of enterocytes)

Figure 11 : Purported mechanisms of action of probiotics (adapted from Kaur et al., 2002).

Probiotics are mainly bacteria and yeast naturally present or not in the resident microflora. In humans or breeding species, are especially used *Lactobacillus,*

Streptococcus and Bifidobacterium (lactic bacteria), *Enterococcus, Bacillus* or *Saccharomyces*. At the present time, in european union, only *Bacillus cereus* (var *toyoi* or not) and *Saccharomyces cerevisiae* are authorized for reproductive does or growing rabbits. In this species, many studies have been published on the influence of probiotics on food digestibility and performance of females or growing rabbits. Yeast addition (*Saccharomyces cerevisiae*) could improve growth performance (ONIFADE *et al.*, 1999) and viability of weaned rabbits (MAERTENS and De GROOTE, 1992). Studies containing commercial preparations (Lacto-Sacc, Acid-Pak 4-way) lead to contradictory results concerning health or performance (HOLLISTER *et al.*, 1990; EL GAAFARY *et al.*, 1992; GIPPERT *et al.*, 1992; YAMANI *et al.*, 1992; KAMRA *et al.*, 1996). These works need careful interpretation since they include several strains of microorganisms and also enzymes or acidifiers. Unfortunately, effects of probiotics on the digestive mucosa immunity were never studied in the rabbit.

Other strategies to improve host defences

With the development of new biological tools, another fighting strategy is the use of vaccines. Compared with non-specific approaches, efficient vaccines protect almost 100% of vaccinated animals. The protection is linked to the induction of a specific immune response against a pathogen. However, the functioning of the intestinal immune system complicates the development of vaccines against enteric pathogens. Indeed, the systemic and the local immune response are almost independent. Thus, to obtain a good intestinal immune response, the oral route of inoculation is almost mandatory. The vaccine must resist the digestive attack of the upper digestive tract to reach the Peyer's Patches intact. Then, the vaccine must be recognised to stimulate a specific immune response, not associated with an uncontrolled inflammatory response or an oral tolerance. Stimulation of local immune responses by mucosal administration of most non-replicating antigens is often relatively inefficient, requiring multiple administrations of large quantities of immunogens and yielding most often tolerance. One strategy to obtain oral vaccines is the use of live attenuated strains derived from pathogenic strains. This strategy requires the knowledge of the virulence mechanisms of the target strain to avoid the development of residual inflammatory responses (CZERKINSKY *et al.*, 1999). Following this approach, BOULLIER *et al*. (2003) develop a live attenuated strain against rabbit colibacillosis by target disruption of virulence genes. These strategies can be used when the pathogen responsible for the disease is identified. For several years now, a new disease has appeared in rabbit breeding unit, leading to important economical loss, the epizootic rabbit enterocolitis. Despite intensive search, the causative agent for this disease has not been identified yet. Without the causative agent, other strategies than vaccine has to be developed.

CONCLUSION

The maintenance of gut health is complex and relies on a delicate balance between the mucosa (including the absorptive epithelium and the digestive immune system) and the commensal microbiota. Environmental factors are also very important to create a stable equilibrium, among which the diet take a place of primary importance. Young rabbits are

very susceptible to digestive troubles, more especially around weaning. Strong modification of the diet, from milk to solid feed could partly explain such a susceptibility. Indeed, this diet change leads to important evolution of both absorptive epithelium and microbial ecosystem and thus fermentative activity. Additionally, maturation of the digestive immune system is dependant of the commensal flora. More especially in the rabbit since the diversification of antibody repertoire occurs after birth in response to microflora stimulation.

In this context, feeding strategies could permit to made a beneficial synergy in the dialogue between gut mucosa and microflora. Dietary fatty acids (ω3/ω6 ratio) lead to improved immune competency and health status in several species and could be of interest in rabbits. However, the current difficulty for immunological research on rabbits lies in the limited tools still available. Nevertheless, several efforts were recently devoted to go beyond this obstacle (BOULLIER *et al.*, 2003; CAMPIN *et al.*, 2003; CANO *et al.*, 2003). The importance of dietary fibres level and quality on health status of animals have been strongly demonstrated in weaned rabbits, and strong relations between fibres supply and caecal microflora were evidenced. The development of new methodologic tools such as RNA oligonucleotides probes (BENNEGADI *et al.*, 2003) should allow us to progress in the knowledge of commensal flora in rabbits and its relation with nutrition and health. In any case, the nutritional needs of young rabbits remain to be deeply studied, in relation with health. As in weaned rabbits, some works reported an influence of fibre level in the diet given to the young before weaning on health status of rabbits after weaning. However, these works evidenced an antagonism between nutritional requirement of the suckling rabbits and their mothers (FORTUN-LAMOTHE and GIDENNE, 2002). Therefore, nutritional needs of the young and thus feeding strategy around weaning could not be thought without keep in mind such an antagonism. Exogenous flora could also be added to the diet (probiotics) to stimulate digestive immunity and increase host's defence capacities or compete with pathogens. Finally, vaccines permit to protect animals against a specific pathogen but the obtaining of a good immunisation against enteric pathogens is tricky, due to the particularities of the digestive immune system. One can imagine the development of a global approach to improve the immune response and then the health status of young rabbits by combining feeding strategies and probiotic supplementation.

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