

Review. New trends in rabbit feeding: influence of nutrition on intestinal health

R. Carabaño¹, I. Badiola², S. Chamorro¹, J. García¹, A. I. García-Ruiz³,
P. García-Rebollar¹, M. S. Gómez-Conde^{1,4}, I. Gutiérrez^{1,5}, N. Nicodemus¹,
M. J. Villamide¹ and J. C. de Blas^{1*}

¹ Departamento de Producción Animal. ETSIA. Universidad Politécnica de Madrid. 28040 Madrid. Spain

² CReSA (UAB-IRTA). Bellaterra. 08193 Barcelona. Spain

³ Nutreco Poultry and Rabbit Research Centre. Casarrubios del Monte. 45950 Toledo. Spain

⁴ Present address: Ibérica de Nutrición Animal, S. L. 28016 Madrid. Spain

⁵ Present address: Camposotillos, S. L. Lucillos. 45684 Toledo. Spain

Abstract

The role of gut barrier against pathogens and its interaction with dietary factors in weaned rabbits affected with digestive disorders, especially epizootic rabbit enteropathy is reviewed. This interaction was studied identifying nutritive factors that influence gut barrier function of mucosa. It was examined the morphology, and enzyme activity of mucosa and the gut associated immune system. Besides, it was characterized the substrate that reaches the caecum and its capacity to favour pathogen bacteria growth, by reviewing the effect of diet on ileal digestibility of nutrients, transit time and microbiota population. The nutritional factors which affect health of early weaned rabbits are level and type of both fibre and protein. The optimal dietary level of insoluble fibre to minimise mortality is 30-32%. Furthermore, a moderate inclusion of soluble fibre (12%) improves mucosa integrity and decreases mortality and the frequency of detection of *Clostridium perfringens* and *Campylobacter* spp in the caecum. The reduction of ileal nitrogen flow had a positive effect on pups viability and frequency of detection of *C. perfringens*. Furthermore, dietary supplementation with 1% of glutamine reduced fattening mortality, the frequency of detection of *C. perfringens*, and *Helicobacter* spp, and diminished the counts of *Eimeria* spp at the jejunum. In conclusion, a correct diet formulation may help to limit the epizootic rabbit enteropathy incidence in weaned rabbits.

Additional key words: fibre, gut barrier function, protein, weaning, young rabbits.

Resumen

Revisión. Nuevas tendencias en alimentación de conejos. Efecto de la nutrición sobre la salud intestinal

En este trabajo se revisa el papel de la barrera intestinal frente a patógenos y su interacción con factores de la dieta en gazapos destetados afectados por enteropatía epizootica. Para ello, se identificaron los factores nutritivos que influyen en la funcionalidad de la barrera intestinal de la mucosa digestiva (morfología, actividad enzimática y actividad del sistema inmune asociado a la mucosa). Además, se ha caracterizado el sustrato alimenticio que alcanza el ciego y su capacidad para favorecer el crecimiento de bacterias patógenas, revisando el efecto de la dieta sobre la digestibilidad ileal de nutrientes, el tránsito digestivo y el tipo de flora intestinal. Los factores nutricionales involucrados incluyen nivel y tipo de fibra y proteína. El contenido óptimo de fibra insoluble en el pienso para minimizar la mortalidad es un 30-32%. Además, una inclusión moderada de fibra soluble (12%) mejoró la integridad de la mucosa y redujo la mortalidad y la frecuencia de detección de *Clostridium perfringens* y de *Campylobacter* spp en el ciego. Un descenso del flujo ileal de nitrógeno tuvo también un efecto positivo sobre la viabilidad de los gazapos y la frecuencia de detección de *C. perfringens*. La suplementación del pienso con un 1% de glutamina redujo la mortalidad en cebo y la frecuencia de detección de *C. perfringens* y *Helicobacter* spp, y disminuyó los conteos de *Eimeria* spp en el yeyuno. En conclusión, una formulación adecuada de los piensos puede ayudar a limitar la incidencia de enteropatía epizootica en conejos jóvenes.

Palabras clave adicionales: conejos jóvenes, destete, fibra, funcionalidad de la barrera intestinal, proteína.

* Corresponding author: c.deblas@upm.es

Received: 28-06-07; Accepted: 30-11-07.

Introduction¹

Infectious diseases of the digestive system currently account for 70% of all rabbit diseases. That percentage has always been quite high, but in recent years it has increased further due to epizootic rabbit enteropathy (ERE), which can cause up to 60% mortality, increase morbidity and delay the end of the fattening period by one to two weeks. Antibiotics have been widely used to control mortality but the corresponding veterinary treatments have increased by 2.5 fold, reducing the benefit margin for many farms. Apart from ERE, several other digestive diseases remain, mostly caused by different strains of *Escherichia coli*.

The European ban on the use of antimicrobials to prevent digestive diseases and the narrow range of molecules allowed as treatment in rabbit farming have stimulated the search for alternative solutions. Among those, nutritional management has become a priority, especially given the importance of digestive diseases. Nonetheless, according to data from commercial farms, the mortality by ERE in rabbits consuming the same feed, can range from 0 to 70%, depending on factors such as season, farm, and type of medical treatment. These results suggest that non-dietary factors are also required to begin the process.

The main cause of enteric disease is the presence of the pathogen. However, factors such as intestinal saprophyte microbiota and the animal's own defence mechanisms will tend to impede or reduce the growth of pathogens and the development of the disease. This explains why some animals survive controlled infections caused by pathogenic strains, even though they present symptoms (morbidity). Defence mechanisms are not unique to rabbits. Indeed, the role of feed has been studied in other species (e.g., pig and chicken) and also in humans, to develop strategies that favour the so-called «competitive exclusion» between bacteria and the development of mechanisms for «intestinal borders».

The mechanisms by which a non-pathogenic species predominates over a pathogenic one (competitive exclusion) are complex. According to Hampson *et al.* (2001), there are several routes by which that competition can be produced, including differences in growth on a specific substrate, efficiency of mucosa colonization,

and production of inhibitory substances of pathogen development (short chain fatty acids, deconjugated bile salts and bactericides).

The role of nutrition in this field can be focused on controlling substrates that favour the growth of microbiota, on promoting competitive exclusion mechanisms or on direct inhibition of pathogen growth. For that it is necessary: a) understand the flow of nutrients in the areas with the greatest microbial population (at the end of the small intestine and caecum), b) characterize the saprophyte or beneficial microbiota and the pathogen, c) characterize the mechanisms of competitive exclusion among pathogenic and non-pathogenic populations.

The objective of this paper is to review the role of nutrition in rabbits, exploring and discussing its different effects on the digestive health.

Intestinal barrier

Once the pathogen is implanted, the intestinal barrier plays a fundamental role in the development of the pathogenic mechanisms of bacteria, impeding the colonization of mucosa and the transport of bacteria and toxins. This action involves several mechanisms which are developed in a cascade and turned on when required.

The barrier effect begins in the intestinal lumen via mechanisms of acidification and protection of the epithelium by a layer of mucosa secreted by the Goblet cells. That mucosa protects the epithelium from mechanical, chemical and enzymatic damage and from bacterial adhesion. Once that protection is lost, and bacteria and toxins are in contact with the epithelium, the immune system kicks in, first in an unspecific manner and then by developing tolerance mechanisms. In addition, it has been found that maintenance of the structure and functionality of the intestinal mucosa does not allow colonization by pathogenic bacteria and assures the function of the digestive tract, such as digestion, absorption, secretion and metabolism of nutrients. This explains why enteric diseases are more frequent in younger animals (immature mucosa) and during the post-weaning period, since there can be structural damage at the level of the intestinal mucosa.

Nutritional strategies have been directed towards avoiding structural damage of the mucosa and favouring

¹ Abbreviations used: AA (amino acid), ANF (anti-nutritional factors), CP (crude protein), ERE (epizootic rabbit enteropathy), GALT (gut associated lymphoid tissue), Ig (immunoglobulin), IL2 (interleukin 2), LP (lamina propria), MHCI (class I immunohistocompatibility complex), NDF (neutral detergent fiber), NS (not significant), SEM (standard error of the mean), Th (cooperating T lymphocytes), TLR (toll-like receptors).

repair mechanisms by supplying the necessary nutrients. Adequate characteristics of the mucosal barrier not only improve the immune system, but also the digestive and absorptive efficiency, decreasing the flow of nutrients to the caecum that can favour growth of pathogens. Those strategies are not easy to develop since there are many interactions among the factors involved, but, as our knowledge increases, it appears that they are a useful way to control digestive diseases.

Intestinal mucosa and intestinal microbiota

The interaction of mucosa with intestinal microbiota seems to play a key role both in the development of defence mechanisms as well as pathogenicity (colonization and translocation of bacteria and toxins). The main pathogens of the gastrointestinal tract need to be in contact with the mucosa to develop properly. Many studies have shown that virulent strains of *E. coli* need to adhere to the mucosa to be pathogenic. Other pathogenic bacteria in the gastrointestinal tract of the rabbit include the genera *Clostridium*, which tend not to adhere but produce toxins instead (*C. spiroforme*, *C. perfringens*, *C. difficile*, *C. sporogenes*, etc). The death of the animal due to epizootic rabbit enteropathy is produced by a toxin (still unclassified) or a strain of *C. perfringens* that damages the liver and kidney (Pérez de Rozas *et al.*, 2005). Nonetheless, for these toxins to arrive to the host organs they have to pass through the mucosal layer. The adhesion of *C. perfringens* toxins to the membrane, as well as the initial damage that it causes, seem to be necessary for translocation to occur (McClane, 2001).

Similarly, saprophyte bacteria in humans and monogastric animals are involved in the competitive exclusion (*Bacteroides*, *Lactobacilli*, *Salmonella*), and seem to have approximation mechanisms to the mucosa and recognition (Toll-like receptors, TLR) that allows to modulate the immune response towards its tolerance in the gastrointestinal tract (Kelly *et al.*, 2005).

The mucosal layer

For contact with the mucosa, the first intestinal barriers in the lumen must first be passed. As mentioned previously, the epithelium of the mucosa is protected by a mucosal layer. The mucins are glycoconjugates made up of a protein core joined with sugar chains of variable length. The end chains of the sugars can be sulphated

or sialylated, giving rise to acid mucins that are more resistant to microbial degradation than neutral mucins (that do not have those terminations; Van Dijk *et al.*, 2002). Mantle and Thakore (1988) purified the small intestine and colon mucins of rabbit. Colon mucins are less acidic and less resistant to proteolysis. The amount and composition of these mucins is dependent on bacterial colonisation, but little is known about the quantitative and qualitative balance compatible with an improvement in intestinal health. At the beginning of infection, and as a protection mechanism, there is an increase in the production of mucin and a change in its composition, from neutral to acidic.

Controlled infection with *Yersinia enterocolitica* in rabbits increases the production of mucins, especially in the terminal end of the ileum and the proximal colon where this intestinal disease is more severe (Mantle *et al.*, 1989). Nonetheless, variation in the composition and degradation of these mucins can alter the bacterial adhesion to the mucosa (Mantle and Husar, 1994). Hezcko *et al.* (2000) observed an increase in the production of mucus in the first moment of infection with *E. coli* O103, suggesting that the mucosal layer is fundamental for bacterial proliferation and adhesion to the mucosa and thus, for its pathogenic effects to be shown.

Saprophyte bacteria also have the ability to regulate the synthesis, composition and use of mucins, but the mechanisms are less studied than in the case of pathogens. *Bacteroides*, a significant genus in humans and rabbits, seems to have mucinolytic properties (Hill, 1986; Marounek *et al.*, 2000; Sirotek *et al.*, 2003). In humans, *B. thetaiotaomicron* also has mucinolytic capacity. This would allow to saprophyte bacteria a better contact with the mucosa, explaining the immunosuppressive effect of these bacteria (Kelly *et al.*, 2005). Pérez de Rozas *et al.* (2005) also showed that bacteria from the genera *Bacteroides* can act as a probiotic in the case of ERE.

In the intestinal lumen, a biological film of IgA is also formed, produced by the immune system of the mucosa. Besides, specific cells in the crypts produce antimicrobial peptides (Paneth cells; Kelly *et al.*, 2005). The IgA film allows the survival of saprophyte bacteria and limits the growth of pathogens. Antimicrobial peptides limit the growth of both, pathogens and saprophyte bacteria. In humans, the density of cells producing these peptides decreases from the proximal intestine until the colon, which can explain the greater microbial density in the ileum and the large intestine.

Competitive exclusion made by *B. thetaiotaomicron* can be related with the induction of a specific peptide against pathogenic bacteria.

The gut associated lymphoid tissue (GALT)

Once the luminal barriers are overcome, bacteria are then attached to the intestinal mucosa by membrane receptors. These receptors trigger a signal to the local immune system responsible for the protection from pathogens and the regulation of the inflammatory response. The immune system is particularly complicated since not only is in charge of defending from infectious agents, but also it should be able to differentiate antigens from the diet and the intestinal saprophyte flora and to develop an effective tolerance mechanism against them to avoid the development of allergies to certain feeds and inflammations. Tolerance is a preferable response to defence mechanism for the animal survival.

The lymphoid tissue of the intestine is distributed in two different ways:

- In an organised manner: in lymphoid follicles, distributed in the so called Peyer's patches, the *vermiform appendix* and *sacculus rotundus*.

- In diffused form: both in the lamina propria (lymphocytes of the lamina propria) as well as in between the epithelial cells of the intestinal mucosa (intra-epithelial lymphocytes).

The organized lymphoid tissue contains many follicles, where part of the cells of the intestinal immune system is made and where the immune response begins. The antigens are transported to those follicles by specialised cells called M cells, and are presented to the lymphocytes in the germinal centres of the lymphoid follicles by the M cells and by other specialised cells called dendritic cells. After maturation and proliferation of the specialised lymphocytes for a specific antigen, they migrate mainly towards the intestinal mucosa (in the lamina propria and intra-epithelium) to develop immunocompetence against subsequent contacts with antigens.

The lamina propria (LP) is where more antibodies are produced (immunoglobulins) for the whole mucosa. In humans, close to 80% of the B lymphocytes (cells used to synthesize immunoglobulins) are present in the LP. The most common immunoglobulin in the intestine is IgA, where its main role is to maintain the integrity of the mucosa against possible infections and toxic

agents. Another important immunoglobulin that is important in the intestine is IgE, which tends to be made in during allergic reactions.

The intraepithelial lymphocytes are the first line of defence from mucosal infections and tend to have cytotoxic and regulatory activity. The T cytotoxic lymphocytes play an important role in the defence from viral and toxic agents and in recovery after infections that are common in intestinal mucosa.

After recognition of the antigens, the macrophages and dendritic cells process them and present the antigenic proteins to the cooperating T lymphocytes (Th) or CD4+. The Th lymphocytes modulate the response by segregating cytokines (soluble proteins) that will activate the humoral response (Th2 complex) or a cellular type response (Th1 complex). The humoral response activates the B lymphocytes, which will secrete the immunoglobulins (IgA, IgM, IgG) against the specific antigens and will lead to a tolerance response. The cell response is mediated by the Th1 cytokine complex and will lead to the activation (IL2: Interleukin 2) of the T cytotoxic lymphocytes or CD8+ and to the death of cells that contain the antigen. In addition, the cytotoxic lymphocytes can also be activated by the mucosal cells (enterocytes) via the class I immuno-histocompatibility complex (MHC I). This response is possible when there is damage to the mucosa and it facilitates the penetration of bacteria, virus or toxins.

The equilibrium between the tolerance (Th2) and the cytotoxic response (Th1) of the immune system is not completely understood, but it seems that the saprophyte bacteria, especially some genera, can mediate towards the activation of the Th2 response (Kelly *et al.*, 2005).

The development of the immune system

According to Knight and Crane (1994), the development of the immune system in the rabbit, and especially of the B cells, can be divided into three stages. The first stage, foetal and neonatal, consists in a lymphopoiesis that will create the neonatal lymphocyte repertory and will be carried out in the liver and the medulla, mostly. The second phase consists in the creation of a primary repertory of antibodies between weeks 3 and 8 of life by the proliferation and diversification of the GALT lymphocytes. The last stage corresponds to the formation of a second repertory of antibodies in adults,

which will be mainly centred on the proliferation of B cells in the secondary lymph organs.

The repertory that is created in the foetus depends on genetic factors and placental transfer during gestation. Nonetheless, it appears that the primary repertory development depends on intestinal microbiota. Several authors have found an abnormal development of the GALT and a reduction in the number of lymphocytes in animals raised free of bacteria (Stepankova and Kovaru, 1978; Tlaskalova and Stepankova, 1980). This lack of development has also been observed in pigs. Rothkötter *et al.* (1994) found, in germ free pigs, that the maturity of the immune system at 45-d was similar to that of normal 5-d old pigs, concluding that the stimulation of bacterial flora, as well as the presence of food antigens, is necessary for a normal development of the GALT. A number of reviews, both in rabbit (Knight and Winstead, 1997; Lanning *et al.*, 2000) and in humans (Kelly *et al.*, 2005), indicate that the presence of saprophyte flora and, possibly of some specific genera, can be crucial for the development of the primary repertoire.

In rabbits, the flora begins to develop during the lactation stage, but the fermentative area only begins to grow after feed consumption. According to Lebas and Laplace (1972) the weight of the caecal contents (relative to the live weight of the animal) increases twofold between the 3rd and the 5th week of life, and remains that high until the 7th week. Dasso *et al.* (2000) reported that the follicular area in the vermiform appendix increased between the 3rd and the 6th week of life, and maintained the same relative importance until the adult stage. The proliferative area of the follicles also reaches a maximum at six weeks. Similar results have been obtained in our department for the vermiform appendix and the Peyer's patches. The change due to

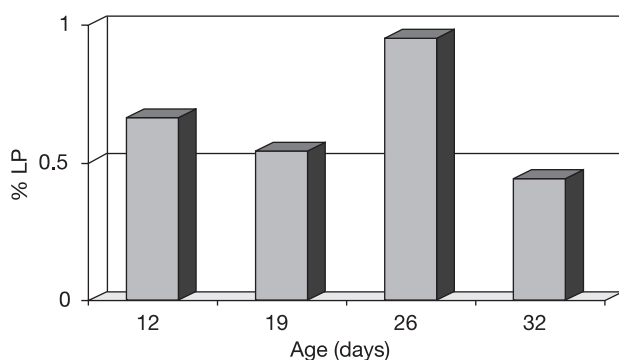


Figure 1. Evolution with age of the proportion of lymphocytes over the total cells in the lamina propria (LP) and the jejunum in lactating young rabbits (Campín *et al.*, 2003).

the development of the immune system affects the centres of proliferation of the lymphocytes, as well as their profile in the LP, and thus, the capacity and diversity of their response. As shown in Figures 1 and 2 the proportion of total lymphocytes and the proportion of B cells increased in rabbits between 19 and 26 d of age (Campín *et al.*, 2003). Different immune indicators suggest that the immune system in the kit, around the time of weaning, is more sensitive to pathogens than in adult animals.

Consequences of weaning

A study on the development of the follicular tissue of the vermiform appendix and the Peyer's patches (Campín *et al.*, 2003) in rabbits 32 d old has not shown an effect of weaning (at 25 d) on the number of follicles. However, weaned rabbits presented larger follicular area in the vermiform appendix and an increase in lymphocytes number without changes in their profile at the level of the LP (Table 1). Nonetheless, the weaned animals had a larger proportion of undifferentiated T cells that responded to the double marking CD4⁺/CD8⁺. These results suggest that weaning can accelerate the maturation of the immune system but, as indicated above, symptoms of immaturity are still present.

In this context of immune immaturity, weaning promotes substantial changes that can modify the capacity of response against pathogens. Weaning implies a

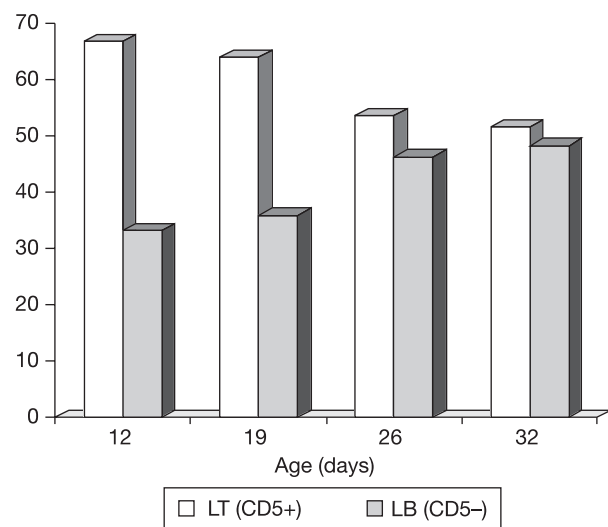


Figure 2. Evolution with age of the proportion of T and B lymphocytes over the total of lymphocytes in the lamina propria of the jejunum in lactating kits (Campín *et al.*, 2003).

Table 1. Effect of weaning (25 d of age) on the lymphoid organs and the lymphocytes of the lamina propria of kits at 32 d of age

	Lactating	Weaned Soya diet	Weaned Sunflower diet	SEM	P
<i>Vermiform appendix</i>					
Follicles number	32.3	34.4	33.4	1.16	NS
Follicular area (mm ²)	3.01 ^b	3.98 ^a	3.92 ^a	0.33	0.05
<i>Peyer's patches</i>					
Follicles number	7.32	7.46	6.86	0.43	NS
Follicular area (mm ²)	3.62	3.91	3.41	0.31	NS
<i>Lamina propria</i>					
Lymphocytes (% total)	0.44 ^b	0.94 ^a	1.18 ^a	0.13	0.05
Lymphocytes CD8+ (%)	21.5	24.7	28.5	4.2	NS
Lymphocytes CD4+ (%)	19.9	14.0	18.9	2.85	NS
Lymphocytes CD8+/CD4+	4.6 b	13.0 ^a	12.1 ^a	1.82	0.05

Source: Campín *et al.* (2003), Gutiérrez *et al.* (2003).

stressful situation for the animal due to separation from the mother, and in many cases, differences in housing and social group. In addition, there is a transition from milk to solid feed that decreases the intake of milk immunoglobulins and several bactericide nutrients, as peptides or short-chain fatty acids (Skrivanova *et al.*, 2005). As a result, suckling rabbits excrete less pathogen coli bacilli (Gallois *et al.*, 2005b) and contain less *C. perfringens* in the caecum (Romero *et al.*, 2007) than weaned animals of the same age. Accordingly, milk intake seems to confer a transitory protection against several important pathogens.

These results explain the significant increase in viability of weanling rabbits observed in several studies when weaning age was delayed (Feugier *et al.*, 2006; Sánchez, 2006; Romero *et al.*, 2007). The effect was higher and more significant when the comparison was made with weaning at very early age (as 23-d) or when sanitary conditions of the farm were poor. However, more studies are needed to confirm this trend, as contradictory results have been reported (Garrido *et al.*, 2006). Early weaning also leads to a lower postweaning weight gain in comparison to suckling rabbits of the same age, although these differences are compensated during the whole fattening period (Méndez *et al.*, 1986).

An insufficient feed intake after weaning can also imply that animal requirements for growth or intestinal development and immunity capacity are not met. Furthermore, consumption of fibre and other nutrients can result in an impairment of villi morphology. These changes in the mucosal structure and also in its functional

capacity can facilitate the translocation of bacteria. Accordingly, dietary changes around weaning can impair the characteristics of the intestinal barrier in rabbits. In 25-d-weaned rabbits, Gutiérrez *et al.* (2002) observed intestinal atrophy, accompanied by reduction in the activity of the enzymes bound to the intestinal mucosa, in comparison with unweaned rabbits of the same age (35-d). However, in other studies (Gallois *et al.*, 2005a; Gómez-Conde *et al.*, 2007), the use of starter diets containing noticeable amounts of soluble fibre did not impair or even improved villi length in weaned compared with suckling rabbits.

Substitution of solid feed for milk in young animals also leads to a decrease of nutrient digestibility and then to a higher flow of undigested substrate at the distal ileum. Depending on the type of diet, the microbiota can be then altered with variable effects whatever the pathogenic or the saprophytic flora is favoured. As opposed to what happens in the small intestine, the development of the caecum and large intestine seems to be improved by weaning (Gutiérrez *et al.*, 2002; Xiccato *et al.*, 2003; Gallois *et al.*, 2005a), so that bacterial colonization might favour the development and diversification of the immune system.

Effect of diet on the intestinal barrier and gut health

According to previously discussed, it is apparent that the mechanisms for survival to infection are quite

complex. This is especially important in the case of the ERE, where the pathogenic flora involved has not been completely identified up to now. In this context, the best criteria to characterize the effects of diet on the mucosal barrier in other species (Van Dijk *et al.*, 2002; Montagne *et al.*, 2007) were mucosal morphology, the activity of mucosal enzymes (as an indicator of the functionality and maturity complementary to previous information) and the study of the lymphocyte profile of the LP (as an indicator of the immune response). In the case of microbiota, the use of molecular techniques has the advantage of identifying the species that cannot be cultured (70% of total).

Regarding nutrition, the existing information is scarce and contradictory (de Blas *et al.*, 1999a). Design of starter feeds should take into account both minimizing the weaning stress (by providing starter diets containing highly digestible nutrients, richer in fat and protein, and lower in fibre content than traditional commercial fattening feeds) and the limited capacity for hydrolysis of several components at early ages (especially for fat and starch). Dietary factors most related with the appearance of digestive diseases were the level and type of fibre (de Blas *et al.*, 1999b; Gidenne, 2000) and the level and type of protein (Carabaño *et al.*, 2006).

Fibre

The beneficial role of fibre in preventing digestive diseases is mostly based on the control of intestinal microbiota through its effects on the digestive transit, and its availability as substrate for bacterial growth. Using common sources of fibre (alfalfa, straw and wheat bran) in weanling rabbits diets, Gutiérrez *et al.* (2002) and Feugier *et al.* (2006) showed that a reduction in fibre content from 36-38 to 30-32% NDF reduced mortality and enhanced performance and feed efficiency, in association with an improvement of mucosal structure (Álvarez *et al.*, 2007).

Otherwise, a minimal content of insoluble fibre is also required to reduce diarrhoea incidence. Several studies (Nicodemus *et al.*, 2004; Gidenne *et al.*, 2004a,b; Gidenne and Licois, 2005) have shown a significant increase of mortality in fattening rabbits when NDF levels were decreased below 30-32%. Insoluble fibre might be necessary to decrease mean retention time of digesta in the gut, dilute dietary and ileal starch and protein content and reduce total microbial growth (García *et al.*, 1995, 2000; De Blas *et al.*, 1999b).

According to Marounek *et al.* (1995) and Lavrencic (2007), the caecal microbiota of 28-d old rabbits is limited with respect to adult animals and it is specialized in the fermentation of soluble fibrous carbohydrates (such as fructans, galactans, β -glucans and pectic substances; Hall *et al.*, 1997). Consequently, the type of dietary fibre could be important to promote the growth of beneficial microbiota in order to improve the competitive exclusion. In addition, the type of fibre can also affect the mucosal structure and the intestinal barrier. Dietary inclusion of soluble fibre favours the growth of intestinal villi and the activity of the enterocytes, while the inclusion of lignified fibre produces structural atrophy, lower activity of intestinal cells and proliferation of *C. perfringens* (Chiou *et al.*, 1994; García-Ruiz *et al.*, 1997; Margüenda *et al.*, unpublished data).

Recent results obtained with starter diets (Gómez-Conde *et al.*, 2007) indicate that the level of soluble fibre can play a role in reducing the caecal population of *C. perfringens* and opportunistic pathogens, such as *Campylobacter*. These effects were concomitant to a decrease of the incidence of diarrhoea mortality (Table 2). The inclusion of moderate levels of soluble fibre (a mixture of 15% beet pulp and 5% apple pulp) with respect to diets based on highly insoluble fibre (15% oat hulls) also improved the structure of the mucosa, its functionality, and the immune response. From these and other results (Fabre *et al.*, 2006; Margüenda *et al.*, 2006) it can be concluded that besides an optimal NDF content of 30-32%, the addition of 11-12% of soluble fibre to the diet can be recommended in order to reduce ERE incidence.

Protein

Protein and amino acid (AA) requirements are relatively high in young rabbits, not only for tissue accretion but also because of the high needs for intestinal growth (Lebas and Laplace, 1972; Trocino *et al.*, 2000), and maintenance of the intestinal mucosa. Weaning can also change the needs for total protein and AAs. Due to the relatively slower daily gain after weaning, the higher weight of gut maintenance on total requirements can increase significantly the relative needs for some essential and non-essential AAs with respect to later stages of growth. In addition, the defence mechanisms of the intestinal barrier can have specific needs for AAs. Thus, threonine is a major component of mucin

Table 2. Effect of the type of fibre in isofibrous diets (30% NDF) on the integrity and activity of the intestinal barrier, pathogenic flora and mortality in early (25 d) weaned rabbits at 35 d of age

	Beet and apple pulps	Alfalfa	Oat hulls	P
<i>Intestinal barrier (Jejunum)</i>				
Villi length, μm	721 ^a	567 ^b	492 ^c	0.05
Crypts depth, μm	89 ^b	115 ^a	113 ^a	0.05
Sucrase activity (U mg^{-1})	8,500 ^a	7,100 ^b	5,400 ^c	0.05
<i>Lamina propria</i>				
Lymphocytes CD4+ (%)	35	33	26	NS
Lymphocytes CD8+ (%)	21 ^b	27 ^b	31 ^a	0.05
<i>Frequency of detection of pathogens at the caecal contents</i>				
<i>C. perfringens</i>	5.7 ^b	2.87 ^b	17.6 ^a	0.05
<i>Campylobacter</i> spp.	19.4	21.2	37.8	0.07
Fattening mortality (%)	5.3 ^b	8.5 ^{ab}	14.4 ^a	0.05

Source: Gómez-Conde *et al.* (2007).

proteins, whereas glutamate is the main AA used by enterocytes as energy source and plays an essential role in the repairing mechanisms of mucosa tissue (Le Floch and Séve, 2000; Reeds *et al.*, 2000). Recent work (Chamorro *et al.*, 2007a,b) indicates that dietary supplementation with 1% of glutamine reduced the mortality caused by ERE, modified ileal microbiota (with a decrease of the frequency of detection of several pathogens as *C. perfringens* and *Helicobacter* spp), and diminished the presence of *Eimeria* spp at the jejunum.

Weaning not only implies a change in the level but also in the main sources of protein in the feed. Easily hydrolysable milk proteins are replaced primarily with less digestible plant proteins, as animal proteins are mostly banned from animal feeds. The level and source of plant protein included in starter diets also have an influence on intestinal disorders, as a decrease of dietary protein content or an increase in ileal protein digestibility (sunflower meal vs soybean meal or potato protein concentrate) reduced the flow of protein towards the fermentative area and decreased the mortality due to ERE incidence (Gutiérrez *et al.*, 2003; García-Ruiz *et al.*, 2006; Chamorro *et al.*, 2007c; see Fig. 3). Other studies have indicated that an increase of the nitrogen flow reaching the terminal ileum incremented the populations of *Clostridia* spp. (Haffar *et al.*, 1988), *E. coli* (Cortez *et al.*, 1992), *C. perfringens* (Chamorro *et al.*, 2007c; Table 3) and total anaerobic bacteria (García-Palomares *et al.*, 2006). Nonetheless, on the

contrary to what happens in piglets, no changes were observed in the mucosal structure or in the profile of lymphocytes of the LP (Tables 1 and 3).

In this context it is relevant the information obtained in recent studies (García-Ruiz *et al.*, 2005; Llorente *et al.*, 2006, 2007), made to characterise the ileal digestibility of protein and AAs of some common ingredients of rabbit diets. These data are useful not only for determining the AAs supply, but also for estimating the amount of protein that remains available for microbial growth in the caecum.

The addition of hydrolysable tannins has also led to a decrease of mortality during fattening in an ERE environment (Maertens and Struklec, 2006). This result

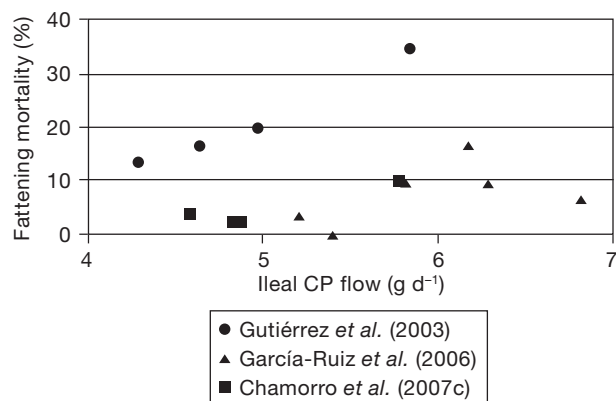


Figure 3. The effect of ileal CP flow, in the two-weeks after weaning period, on average mortality in the whole fattening period according to several experiments.

Table 3. Effect of the level of protein in isofibrous diets (30% NDF) on the integrity and activity of the intestinal barrier, pathogenic flora and mortality in early (25 d) weaned rabbits

	18.6% CP	16.1% CP	SEM	P
Villi height, μm	632	606	48	NS
Crypts depth, μm	134	134	4.3	NS
Ileal CP flow, g d^{-1}	6.0	5.0	0.25	0.05
Frequency of detection (%) of <i>C. perfringens</i> at the ileal contents	47.2	18.0	—	0.05
Fattening mortality (%)	21.2	11.0	—	0.05

CP: crude protein. Source: Chamorro *et al.* (2007c).

is explained by the formation of non degradable complexes of tannins with dietary protein (which would reduce protein substrate for microbial growth) and/or by the inhibition of microbial proteolysis.

Endogenous nitrogen (digestive enzymes, mucoproteins, desquamated cells, urea) is other relevant source of protein for the gut microorganisms, which can account in rabbits for about 40-60% of the total ileal protein flow (García-Ruiz *et al.*, 2004; Llorente *et al.*, unpublished data). This contribution is widely variable and influenced primarily by dry matter intake and secondarily by diet composition, as level of fibre or anti-nutritional factors (ANF), frequently present in legumes, might damage the intestinal mucosa and increase the flow of nitrogen towards the caecum. Thus, the replacement of low ANF protein sources as sunflower or meat meal with soy protein in weanling rabbits impairs the mucosal integrity (Gutiérrez *et al.*, 2000) and increases fattening mortality (Scheele and Bolder, 1987; Gutiérrez *et al.*, 2003).

According to the above results, feeding strategies should be developed with the aim of minimizing the use of undigested protein for microbial growth, keeping a sufficient supply of essential AAs to ensure a high animal performance.

References

- ÁLVAREZ J.L., MARGÜENDA I., GARCÍA-REBOLLAR P., CARABAÑO R., DE BLAS J.C., CORUJO A., GARCÍA-RUIZ A.I., 2007. Effects of type and level of fibre on digestive physiology and performance in reproducing and growing rabbits. *World Rabbit Sci* 15, 9-17.
- CAMPÍN J., EIRAS P., REBOLLAR P.G., CARABAÑO R., 2003. Estudio del tejido linfoide asociado a intestino en gazapos en torno al destete. *ITEA* 24, 660-662.
- CARABAÑO R., BADIOLA I., LICOIS D., GIDENNE T., 2006. The digestive ecosystem and its control through nutritional or feeding strategies. In: *Recent advances in rabbit sciences* (Maertens L., Coudert P., ed). Ilvo, Merelbeke, Belgium. pp. 211-227.
- CHAMORRO S., CARABAÑO R., BADIOLA I., GARCÍA J., DE BLAS J.C., 2007a. Effet d'une supplémentation alimentaire en glutamine et en arginine sur la croissance, quelques paramètres de la flore iléale et caecale et la santé intestinale chez le lapereau, sevré précocement et atteint par le syndrome de l'EEL. 12èmes Journées de la Recherche Cunicole, INRA, Le Mans. [In French].
- CHAMORRO S., CARABAÑO R., GRANT G., GARCÍA J., DE BLAS J.C., 2007b. Efecto de la adición de glutamina y arginina sobre la mortalidad y barrera intestinal de gazapos. II Congreso Ibérico Cunicultura, Vila Real, Portugal. pp. 91-94. [In Spanish].
- CHAMORRO S., GÓMEZ-CONDE M.S., PÉREZ DE ROZAS A.M., BADIOLA I., CARABAÑO R., DE BLAS J.C., 2007c. Effect on digestion and performance of dietary protein content and of increased substitution of lucerne hay with soya-bean protein concentrate in starter diets for young rabbits. *Animal* 1, 651-659.
- CHIOU P.W.S., YU B., LIN C., 1994. Effects of different components of dietary fibre on intestinal morphology of domestic rabbits. *Comp Biochem Physiol A* 108, 629-638.
- CORTEZ S., BRANDEBRUGER H., GREUEL E., SUNDRUM A., 1992. Investigations of the relationships between feed and health-status on the intestinal flora of rabbits. *Tierär Umschau* 47, 544-549.
- DASSO J.F., OBIAKOR H., BACH H., ANDERSON A.O., MAGE R.G., 2000. A morphological and immunohistological study of the human and rabbit appendix for comparison with the avian bursa. *Dev Comp Immunol* 24, 797-814.
- DE BLAS J.C., GUTIÉRREZ I., CARABAÑO R., 1999a. Destete precoz en gazapos. Situación actual y perspectivas. XV Curso de Especialización FEDNA, Madrid. pp. 67-81. [In Spanish].
- DE BLAS J.C., GARCÍA J., CARABAÑO R., 1999b. Role of fibre in rabbit diets. A review. *Ann Zootech* 48, 3-13.
- FABRE C., JUBERO M.A., BLAS E., FERNÁNDEZ-CARMONA J., PASCUAL J.J., 2006. Utilización de un pienso rico en fibra digestible e indigestible y pobre en almidón en conejos de engorde: ensayo en condiciones de campo. In: XXXI Symposium de Cunicultura, Lorca. pp. 67-72. [In Spanish].

- FEUGIER A., SMIT M.N., FORTUN-LAMOTHE L., GIDENNE T., 2006. Fibre and protein requirements of early weaned rabbits and the interaction with weaning age: effects on digestive health and growth performance. *Anim Sci* 82, 493-500.
- GALLOIS M., GIDENNE T., FORTUN-LAMOTHE L., LE HUEROU-LURON I., LALLES J.P., 2005a. An early stimulation of solid feed intake slightly influences the morphological gut maturation in the rabbit. *Reprod Nutr Dev* 45, 109-122.
- GALLOIS M., BOULLIER S., MILON A., GIDENNE T., 2005b. Age au sevrage et sensibilité a une infection expérimentale par une souche d'*E. coli* O103. 11èmes Journées de la Recherche Cunicole, INRA, Paris. [In French].
- GARCÍA J., DE BLAS J.C., CARABAÑO R., GARCÍA P., 1995. Effect of type of lucerne hay on cecal fermentation and nitrogen contribution through caecotrophy in rabbits. *Reprod Nutr Dev* 35, 267-275.
- GARCÍA J., CARABAÑO R., PÉREZ ALBA L., DE BLAS J.C., 2000. Effect of fiber source on cecal fermentation and nitrogen recycled through caecotrophy in rabbits. *J Anim Sci* 78, 638-646.
- GARCÍA-PALOMARES J., CARABAÑO R., GARCÍA-REBOLLAR P., DE BLAS J.C., CORUJO A., GARCÍA-RUIZ A.I., 2006. Effects of a dietary protein reduction and enzyme supplementation on growth performance in the fattening period. *World Rabbit Sci* 14, 231-236.
- GARCÍA-RUIZ A.I., GARCÍA J., DE BLAS J.C., PIQUER J., CARABAÑO R., 1997. Efecto de la fuente de fibra sobre la actividad enzimática de la amilasa pancreática y las sacarosas en yeyuno e íleon. *ITEA* 18, 190-192. [In Spanish].
- GARCÍA-RUIZ A.I., DE BLAS C., CARABAÑO R., 2004. Effect of type of diet (casein-based or protein-free) and caecotrophy on ileal endogenous nitrogen and AA flow in rabbits. *Anim Sci* 79, 231-240.
- GARCÍA-RUIZ A.I., DE BLAS C., CARABAÑO R., 2005. Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. *Anim Sci* 80, 169-178.
- GARCÍA-RUIZ A.I., GARCÍA-PALOMARES J., GARCÍA-REBOLLAR P., CHAMORRO S., CARABAÑO R., DE BLAS J.C., 2006. Effect of protein source and enzyme supplementation on ileal protein digestibility and fattening performance in rabbits. *Span J Agric Res* 4, 297-303.
- GARRIDO S., NICODEMUS N., CHAMORRO S., DE BLAS J.C., 2006. Influencia de la edad al destete (25 vs 35), la nave y el periodo sobre la mortalidad y parámetros productivos de gazapos. In XXXI Symposium Cunicultura Asescu, Lorca. pp. 95-101. [In Spanish].
- GIDENNE T., 2000. Recent advances in rabbit nutrition: emphasis on fibre requirements. A review. *World Rabbit Sci* 8, 23-32.
- GIDENNE T., LICOIS D., 2005. Effect of high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of *Escherichia coli*. *Anim Sci* 80, 281-288.
- GIDENNE T., MIRABITO L., JEHL N., PÉREZ J.M., ARVEUX P., BOURDILLON A., BRIENS C., DUPERRAY J., CORRENT E., 2004a. 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., JEHL N., LAPANOUSE A., SEGURA M., 2004b. 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. *Br J Nutr* 92, 95-104.
- GÓMEZ-CONDE M.S., GARCÍA J., CHAMORRO S., EIRAS P., GARCÍA REBOLLAR P.G., PÉREZ DE ROZAS A., BADIOLA I., DE BLAS C., CARABAÑO R., 2007. Neutral detergent-soluble fiber improves gut barrier function in 25 d old weaned rabbits. *J Anim Sci* 85, 3313-3321.
- GUTIÉRREZ I., CACHALDORA P., CARABAÑO R., DE BLAS J.C., 2000. Effect of supplementation with animal plasma and antibiotics on jejunal morphology of early weaned rabbits. *World Rabbit Sci* 8, 263-268.
- GUTIÉRREZ I., ESPINOSA A., GARCÍA J., CARABAÑO R., DE BLAS J.C., 2002. Effect of levels of starch, fiber and lactose on digestion and growth performance of early-weaned rabbits. *J Anim Sci* 80, 1029-1037.
- GUTIÉRREZ I., ESPINOSA A., GARCÍA J., CARABAÑO R., DE BLAS C., 2003. Effect of source of protein on digestion and growth performance of early-weaned rabbits. *Anim Res* 52, 461-472.
- HAFFARA., LAVAL A., GUILLOU J.P., 1988. Entérotaxémie à *Clostridium spiroforme* chez des lapins adultes. *Le Point Vét* 20, 99-102. [In French].
- HALL M.B., LEWIS B.A., VAN SOEST P.J., CHASE L.E., 1997. A simple method for estimation of neutral detergent-soluble fiber. *J Sci Food Agric* 74, 441-449.
- HAMPSON D.J., PLUSKE J.R., PETHICK D.W., 2001. Dietary manipulation of enteric disease. In: *Digestive physiology of pigs* (Lindberg J.E., Ogle B., eds). Ed CABI Publishing. pp. 247-260.
- HEZCKO U., ABE A., FINLAY B.B., 2000. In vivo interactions of rabbit enteropathogenic *Escherichia coli* O103 with host: an electron microscopic and histopathologic study. *Microbes Infect* 2, 5-16.
- HILL R.R.H., 1986. Digestion of mucin polysaccharides in vitro by bacteria isolated from the rabbit cecum. *Curr Microbiol* 14, 117-120.
- KELLY D., CONWAY S., AMINOV R., 2005. Commensal gut bacteria: mechanisms of immune modulation. *Trends Immunol* 26, 326-333.
- KNIGHT K.L., CRANE M.A., 1994. Generating the antibody repertoire in rabbit. *Adv Immunol* 56, 179-218.
- KNIGHT K.L., WINSTEAD C.R., 1997. Generation of antibody in rabbits. *Curr Opin Immunol* 9, 228-232.
- LANNING D., SETHUPATHI P., RHEE K.J., ZHAI S.K., KNIGHT K.L., 2000. Intestinal microflora and diversification of the rabbit antibody repertoire. *J Immunol* 165, 2012-9.
- LAVRENCIC A., 2007. The effect of rabbit age on in vitro caecal fermentation of starch, pectin, xylan, cellulose, compound feed and its fibre. *Anim* 1, 241-248.

- LEBAS F., LAPLACE J.P., 1972. Mensurations viscérales chez le lapin. I. Croissance du foie, des reins et des divers segments intestinaux entre 3 et 11 semaines d'âge. *Ann Zootech* 21, 337-347. [In French].
- LE FLOC'H N., SÈVE B., 2000. Le devenir des protéines et des acides aminés dans l'intestin du porc: de la digestion à l'apparition dans la veine porte. *Prod Anim* 13, 303-314. [In French].
- LLORENTE A., GARCÍA A.I., NICODEMUS N., VILLAMIDE M.J., CARABAÑO R., 2006. Digestibilidad ileal aparente y verdadera de aminoácidos de harinas de girasol, productos de soja y guisante en conejos. XXXI Symposium Cunicultura Asescu, Lorca. pp. 117-124. [In Spanish].
- LLORENTE A., VILLAMIDE M.J., GARCÍA A.I., CARABAÑO R., 2007. Digestibilidad de la proteína de los aminoácidos de cereales y sus subproductos en conejos. II Congreso Ibérico Cunicultura. Vila Real, Portugal. pp. 87-90. [In Spanish].
- MAERTENS L., STRUKLEC M., 2006. Preliminary results with a tannin extract on the performance and mortality of growing rabbits in an enteropathy infected environment. *World Rabbit Sci* 14, 189-192.
- MANTLE M., THAKORE E., 1988. Rabbit intestinal and colonic mucins: isolation, partial characterization and measurement of secretion using an enzyme-linked immunoassay. *Biochem Cell Biol* 66, 1045-1054.
- MANTLE M., HUSAR S.D., 1994. Binding *Yersinia enterocolitica* to purified, native small intestinal mucins from rabbits and human involves interactions with the mucin carbohydrate moiety. *Infect Immun* 62, 1219-1227.
- MANTLE M., BASARABA L., PEACOCK S.C., GALL D.G., 1989. Binding *Yersinia enterocolitica* to rabbit intestinal brush border membranes, mucosa and mucin. *Infect Immun* 57, 3292-3299.
- MARGÜENDA I., CARABAÑO R., GARCÍA-REBOLLAR P., DE BLAS J.C., GARCÍA-RUIZ A., 2006. Effect of the substitution of starch sources or wheat straw with apple pulp on growth performance, mortality and carcass yield under field conditions. 3rd Rabbit Congress of the Americas, Maringá, Brazil.
- MAROUNEK M., VOVK S.J., SKRIVANOVA V., 1995. Distribution of hydrolytic enzymes in the digestive tract of rabbits. *Br J Nutr* 73, 463-469.
- MAROUNEK M., SKRIVANOVA V., DUSKOVA D., 2000. In vitro caecal fermentation of nitrogenous substrates in rabbits. *J Agric Sci Camb* 135, 437-442.
- MÉNDEZ J., DE BLAS J.C., FRAGA M.J., 1986. The effects of diet and remating interval after parturition on the reproductive performance of the commercial doe rabbit. *J Anim Sci* 62, 1624-1634.
- McCLANE B., 2001. The complex interaction between *Clostridium perfringens* enterotoxin in epithelial thigh junctions. *Toxicon* 39, 1781-1791.
- MONTAGNE L., BOUDRY G., FAVIER C., LE HUÉROU-LURON I., LALLÈS J.P., SÈVE B., 2007. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *Br J Nutr* 97, 45-57.
- NICODEMUS N., PÉREZ-ALBA L., CARABAÑO R., DE BLAS C., BADIOLA I., PÉREZ DE ROZAS A., GARCÍA J., 2004. Effect of level of fibre and level of ground of fibre sources on digestion and ileal and caecal characterization of microbiota of early weaned rabbits. 8th World Rabbit Congress. Puebla (Mexico).
- PERÉZ DE ROZAS A.M., CARABAÑO R., GARCÍA J., ROSELL J., DÍAZ J.V., BARBÉ J., PASCUAL J.J., BADIOLA I., 2005. Etiopatogenia de la enteropatía epizootica del conejo. XXX Symposium de Cunicultura. Valladolid. pp. 167-174. [In Spanish].
- REEDS P.J., BURRIN D.G., STOLL B., JAHOR F., 2000. Intestinal glutamate metabolism. *J Nutr* 130, 978S-982S.
- ROMERO C., NICODEMUS N., CORUJO A., ASTILLERO J.R., DE BLAS J.C., 2007. Effet du rythme reproductif, la teneur en fibre insoluble de l'aliment, l'âge et l'hygiène sur la composition microbienne caecale et iléale chez le lapereau. 12^{èmes} Journées de la Recherche Cunicole. INRA, Mans. [In French].
- ROTHKÖTTER H.J., KIRSHOFF T., PABST R., 1994. Lymphoid and nonlymphoid cells in the epithelium and lamina propria of intestinal mucosa of pigs. *Gut* 35, 1582-1589.
- SÁNCHEZ M., 2006. Efecto de la edad al destete sobre la composición corporal y los rendimientos de conejas gazapos. Unpublished Proyecto fin de carrera, EUITA, UPM, Madrid. [In Spanish].
- SCHEELE C.W., BOLDER N.M., 1987. Health problems and mortality of young suckling rabbits in relation to dietary composition. In: Rabbit production systems including welfare. Commission of the European Communities, Brussels. pp. 115-125.
- SIROTEK K., SANTOS E., BENDA V., MAROUNEK M., 2003. Isolation, identification and characterization of rabbit mucinolytic bacteria. *Acta Vet Brno* 72, 365-370.
- SKRIVANOVA E., MAROUNEK M., DLOUHA G., KANJA J., 2005. Susceptibility of *Clostridium perfringens* to c₂-c₁₈ fatty acids. *Letters Appl Microbiol* 41, 77-81.
- STEPANKOVA R., KOVARU F., 1978. Development of lymphatic tissues in germfree and conventionally reared rabbits. In: Lymphology (Malek P., Bartos V., Weissleder H., Witte M.H., eds). G. Thieme Publ., Stuttgart. pp. 290-294.
- TLASKALOVA-HOGENOVA H., STEPANKOVA R., 1980. Development of antibody formation in germfree and conventionally reared rabbits: the role of intestinal lymphoid tissue in antibody formation to *E. coli* antigens. *Folia Biol* 26, 81-93.
- TROCINO A., XICCATO G., QUAQUE P.I., SARTORI A., 2000. Feeding plans at different protein levels: effects on growth performance, meat quality and nitrogen excretion in rabbits. *World Rabbit Sci* 8, 467-474.
- VAN DIJK J.E., HUISMAN J., KONINKX J.F.K.G., 2002. Structural and functional aspects of healthy gastrointestinal tract. In: Nutrition and health of the gastrointestinal tract. Wageningen Academic Publisher. pp. 71-96.
- XICCATO G., TROCINO A., SARTORI A., QUAQUE P.I., 2003. Effect of weaning diet and weaning age on growth, body composition and caecal fermentation of young rabbits. *Anim Sci* 77, 101-111.