

NUTRITION AND FEEDING STRATEGY: INTERACTIONS WITH PATHOLOGY (Chapter 10)

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Nutrition and feeding strategies are a key point for rabbit breeding, not only to optimise the production itself (meat, milk, fur, etc.), but also to prevent various pathologies by two main ways: the presence of toxic compounds in the feeds or utilization of unbalanced diets, the presence of pathogenic agents (viruses, bacteria, parasites, ...) in the feeds or in drinking water. This last aspect will not be treated here since it's not a true question of nutrition, only a question of feeding management and hygiene. Similarly, presence of undesirable pesticides in feed ingredients can impair rabbit health. Very few specific data are available for rabbits in production conditions, thus this aspect will not be treated here and the readers can consult with benefit more specialized books devoted to this aspect of animal feeding.

In this chapter, it is assumed that some efforts were made to provide the daily minimum requirements of the organism for the main individual nutrients such as energy, proteins and amino acids, minerals or vitamins as recommended in the previous chapters. Nevertheless it is generally difficult to provide all nutrients exactly at the optimum and as a consequence of available raw materials composition, it is necessary to accept an excess of some nutrients or an imbalance, to be certain that the minimum of some others nutrients is met.

By itself, an imbalance should be only responsible for low performance, not for health troubles if the breeding conditions are quite perfect. For example, in controlled experimental conditions, a diet containing only 6% fibre (ADF as %DM) has not induced any digestive trouble (Davidson and Spreadbury, 1975). A similar situation was observed with diets containing up to 28-30% crude proteins (Lebas, 1973). Such imbalances only induce a higher susceptibility of rabbits to troubles, mainly digestive disorders, and the above extreme levels must never be recommended for practical

feeding. One of the objects of this chapter is to indicate the rules, when known, able to minimize the risk of disorders and some ideas on "acceptable" imbalances in the every day feeding practice. In addition to the imbalance problems, absolute excess of different ingredients such as some minerals or vitamins like phosphorus or vitamin D, can be toxic independently of the sanitary status of rabbits. The only question is « *When such nutrient supplied above the recommended minimum or optimum, becomes toxic?* ».

Thus the present chapter will treat of health troubles (mainly digestive troubles) linked to diet's components balance and to presence of different nutrients excess, mainly in relation with initial feed's ingredients composition. One part will treat of the feeding strategies, particularly the control of the feed intake for the young rabbit to reduce the post-weaning digestive troubles. Another part is devoted to the health consequences of non nutritional components frequently associated to feed ingredients, such as mycotoxins. At the end of the chapter the last part will be devoted to water quality, since water is also able to induce nutritional disorders when some soluble components are too concentrated.

1 Methods to estimate the health status and to measure the risk of digestive troubles

A common indicator to evaluate the impact of a disease in breeding is the mortality rate. More recently, morbidity indicator was developed for the growing rabbit to assess more precisely the incidence of the clinical symptoms (Gidenne, 1995), and it could be combined with mortality to obtain the health risk index ("HRI"= morbidity + mortality rate). This approach allows a more precise assessment of the health status. But, these traits show large variations according to many factors. For instance, mortality rate of rabbits fed the same diet could range from 0 up to 70% according to various factors, such as litter effect, preventive medication, age at weaning, sanitary and immune status of the animals.... Thus it means that a large number of animals are required to detect a significant difference between two treatments in mortality. For instance, to detect a 5% deviation among two mortality rates, more than 300 animals are required in each group (table 10.1).

When the clinical symptoms (diarrhoea, caecal impaction, borborismus, ...) are clear, the morbidity rate is relatively easy to measure. However, when only a reduction of growth rate is detectable, a threshold must be defined to class the animal as morbid or not, such as the average minus 2 × standard deviation (signifying the 2.5% of the animals with lower growth rate), or up to 3 SD. But it needs to use a large set of rabbits within a group to define precisely the mean and its range of variation. Moreover, it must be outlined that adequate statistical methods are necessary to treat discrete data (such as mortality or morbidity). For instance, when analysing models with more than one factor or including more than two levels (within a factor) or to test interaction among two factors, specific categorical analysis based on a weighted least square analysis must be used instead of a simple Chi² test.

2 Troubles related with major nutrients imbalance

Among the various health troubles related to feeding, the intestinal pathology along with respiratory diseases are the predominant causes of morbidity and mortality in commercial rabbit husbandry. The first one mainly occurs in young rabbits, after weaning (4 to 10 weeks of age), while the second one preferentially affects the adults. In France, enteritis in growing rabbit induced a mortality rate of 11-12% before the appearance of the Epizootic Rabbit Enteropathy (ERE) (Koehl, 1997). However, with the generalization of the cycled production, mortality is currently around 8.5% (Lebas, 2008), but with a frequent use of preventive antibiotherapy. Nevertheless it may frequently exceed 15% and even reach up to 50%. Moreover, digestive disorders are responsible for important morbidity characterized by growth depression and bad feed conversion.

These economic losses, not so obvious than the mortality, are often underestimated by the rabbit breeders.

Diagnosis of intestinal diseases is difficult because, whatever the cause (nutritional trouble or true specific illness), symptoms and lesions are generally similar. The difficulty to recognize the aetiology for the rabbit intestinal disorders is reinforced by the fact that, as for most diseases in man or animals, several factors are involved in the development of enteritis and must be considered. The first one is the animal status itself (age, genetic, immunity...). The second one concerns the pathogenic agents involved (parasites, bacteria, viruses...). The third one is represented by environmental factors including nutritional factors, breeding conditions such as hygiene, stress, etc. Although many causes are able to provoke enteritis, the main and constant clinical sign observed is the diarrhoea which occurs in about 90% of the cases of enteritis (Licois *et al.*, 1992). This may be related to the characteristics of the rabbit intestinal tract and its complex physiology.

The composition of the caecal content as well as caecal function and caecal bacterial community and activity (see chapter 1) are deeply affected in case of enteritis (figures 10.1, 10.2). The motility of the caecum is stimulated whereas that of ileum and jejunum is inhibited in experimentally induced diarrhoea with *coccidia* (Fioramonti *et al.*, 1981). Furthermore, Hodgson (1974) observed, in rabbits fed a low fibre diet, an increased motility of the proximal colon that appeared contracted and thickened, and a higher retention of digesta in the total tract that should be related with a lower feed intake. This probably reflects a higher antiperistaltic activity of the proximal colon (see chapter 1) induced by the high proportion of fine particles in a low fibre diet. It is thus difficult to postulate that rabbit diarrhoea is characterized by a hypomotility of the caeco-colic segment. In parallel, the caecal fermentative activity is disturbed (figure 10.2): for a 6 weeks old rabbit, the caecal volatile fatty acids (VFA) concentration falls to under 50 mM/l, the butyrate is particularly affected (leading to a C3/C4 quotient in the range between 1.5 and up to 8 instead of 0.5 to 0.8), and larger inter-individual variations of the fermentation pattern are observed. Higher pH (+0.5) and ammonia level could also be observed. The caeco-colic flora could also be affected in composition, but few results are available and they are not consistent showing sometimes a decrease or an increase in *Escherichia coli* and/or *Clostridia*.

2.1 Fibre and starch intake

The fibre intake should be expressed in term of quantity or quality (type) of cell-wall constituents (see definition in the chapter 5), and similarly the effect of starch intake could differ according to the origin of the starch (see definition in the chapter 2).

2.1.1 Consequences of a reduction in the fibre intake

An increased dietary starch/fibre ratio (<30% NDF, <15% ADF, >20% starch), without major changes in the proportions of the cell wall constituents (hemicelluloses or lignins etc.), could lead to both a lower ileal flow of dry matter and bacterial biomass production in the caecum of the young rabbit (figures 10.1 & 10.2). In healthy growing animals, when the fibre intake is too low (<8-11 g ADF/kg LW/day), the caecal fibre level decreased, while the starch concentration remains low (around 1.5 to 4%), and there are no consistent changes in the concentration of the fermentation end-products (ammonia, VFA) and caecal pH (figure 10.3). Some authors described a lower fermentative activity (Bellier and Gidenne, 1996; Gidenne *et al.* 2000, 2002, 2004a; Nicodemus *et al.*, 2003a, 2004), but most of studies did not. However, the VFA molar proportion is affected by the fibre level, since the proportion of butyrate generally rose significantly when the fibre/starch ratio decreased.

It still remain difficult to explain how these changes in the caecal ecosystem function determine the greater incidence of digestive troubles (mainly diarrhoea, and also caecal impaction, mucus excretion, low intake) observed with low fibre diets. Probably the microbial community is largely

affected, such the archaeal community who was twofold higher with standard diet than with fibre-deficient (Bennegadi *et al.*, 2003). Furthermore, when dietary NDF reduced from 30 to 25% microbiota biodiversity increased at ileum but was reduced at the caecum (Nicodemus *et al.*, 2004). Besides, the favourable effect of a high fibre intake on rabbit digestive health was also shown using experimental infection model reproducing a colibacillosis (Gidenne and Licois, 2005) or ERE (Gidenne *et al.*, 2001b).

Several hypotheses have been suggested to explain how the dietary supply of starch and fibre affects the digestive physiology, but none was completely validated by experimental results. Prohaszka (1980) put forward the antibacterial effect of caecal VFA originating from fibre fermentation, particularly in the case of *E. coli* infection. But numerous studies have not observed a close relationship between the concentration of caecal VFA and the pH or between *E. coli* flora and caecal pH. In addition, Padilha *et al.* (1995) showed that between 29 and 49 days of age, the caecal pH is decreasing whereas *E. coli* flora remains steady.

The favourable effect on rabbit's health of a high level of low-digestibility fibre (lignocellulose or ADF) could correspond to a control of the digesta rate of passage, particularly in the caeco-colic segment. Moreover, most of the results indicate that all the factors contributing to an increase in retention time (lowering the fibre level, reducing the particle size of the feed, feed restriction) contribute to destabilising the caecal microbial activity and favour enteritis. It could be supposed that a low caecal turnover of digesta would lead to an insufficient supply of substrates available for the fibrolytic flora (figure 10.4).

Many experiments have been performed to elicit the respective effects of fibre and starch on the incidence of diarrhoea in the growing rabbit, particularly just after the weaning (Colin *et al.*, 1976; De Blas *et al.*, 1986; Blas *et al.*, 1994; Bennegadi *et al.*, 2001). This period is critical since there is a large incidence of digestive troubles, and also because an active digestive maturation is occurring and feed intake is increasing sharply. Experiments that dealt with this question compared diets having varying level of fibre and simultaneously an inverse variation of the starch level (since rabbits are fed with a complete feeds = closed 100% formula). Consequently, when a study reported a positive effect of an increased dietary fibre intake on digestive health, it was in fact difficult to exclude that there was also an effect of a reduced starch intake. We thus have to deal with two opposite hypothesis: are digestive troubles linked to a carbohydrate overload in the caecum? or linked to a fibre deficiency ? (or both ?). Recently, this question was elicited by studying the ileal flow of starch and fibre in the growing rabbit (5-9 wks old). With high starch diets ($\geq 30\%$ starch mainly from wheat) the ileal starch digestibility was very high ($>97\%$), the flow of starch remained under 2g/d (intake ≈ 30 g/d) at ileum, while that of fibre was at least 10 times higher (≈ 20 g NDF/d) (Gidenne *et al.*, 2000; Nicodemus *et al.*, 2004; Garcia *et al.*, 2004a). Thus an overload of starch appears very unlikely since starch digestion was very efficient already at 5 wks old. Moreover, a large-scale study using a network of 6 experimental breeding unit (GEC French group) demonstrated through a 2X2 factorial design (two level of starch "12 vs 19%" combined with two ADF levels "15 vs 19%") that only the fibre level play a role in digestive trouble occurrence, and not the starch level (Gidenne *et al.*, 2004b).

Furthermore, by comparing iso-fibre diets but with several starch sources varying in their intestinal digestion (maize, wheat, barley) Gidenne *et al.* (2005a, b) observed no effect of starch ileal flow on diarrhoea incidence in the weaned rabbit. These results support the minor influence of starch on the health status of the animal when fibre requirements are supplied. The positive effect of enzymes supplementation (cocktail of β -glucanases, β -xylanases, α -amilases and pectinases) on mortality (Gutiérrez *et al.*, 2002b; Cachaldora *et al.*, 2004) might be thus related with the partial hydrolysis of non-starch polysaccharides that produce complex oligomers, which might modulate gut microbiota and lead to a better digestive health. Besides, since starch digestion is incomplete in the young rabbit, replacement of some starch by lactose has been studied, as occurs in piglets diets. However, lactose ileal digestibility was much lower than that recorded for starch (74 vs 92%), which might be due to the severe reduction of lactase activity after weaning. This result led to a

higher ileal flux of lactose and a higher mortality (Gutiérrez *et al.*, 2002a), possibly explained by a microbiota unbalance in the caecum.

Fibre intake plays thus a major role in the determinism of digestive trouble in the classically weaned rabbit (28-35d old). With rabbit weaned more early (at 25d of age), Gutiérrez *et al.* (2002a) observed that mortality remained low and similar with diets having 36 vs 30% of NDF, but mortality rate tended to increase ($P=0.06$) after a feed shift (at 39d of age, from experimental to commercial diet) for those previously fed with 36% NDF diet.

Accordingly, several large-scale studies aimed to validate clearly the relationship among dietary fibre/starch levels and diarrhoea incidence for the "classically" weaned rabbit, using experimental design with a high number of animals per treatment. The relationship between low fibre diets (<14% ADF) and a higher incidence of diarrhoea was clearly established in two studies where the quality of fibre, e.g. the proportions of fibre fraction as analysed through the Van-Soest procedure, has been controlled (Blas *et al.*, 1994; Bennegadi *et al.*, 2001). In France, the GEC group performed several large-scale studies (using at least 300 animals per treatment and 5 sites) to precise the fibre recommendations for prevention of digestive troubles in the growing rabbit (weaned). The relevance of Van-Soest criteria was studied, since crude fibre method was too imprecise for this purpose. A review of these studies and of new fibre recommendations was recently published (Gidenne, 2003). We here proposed a summary of the fibre requirement (table 10.2) for post-weaned and growing rabbits from French (INRA) and Spanish (Univ. Madrid) research groups.

The favourable effect of dietary fibre was also recently analysed in the young **during the weaning** period (3 to 5 wks old) by Fortun-Lamothe *et al.* (2005) with a large-scale study (6 sites + 3 reproductive cycle). They reported a lower mortality rate for litters fed a diet rich in fibre or when fibre+lipids replaced starch. However, in the suckling rabbit (or before 5 weeks old) it can be supposed that the feed intake regulation is not completely established and neither is the pancreatic enzymatic activity (see chapter 1). The combination of these two factors would lead to high flow of starch into the caecum (Gidenne *et al.*, 2005a), that may then favour digestive disturbances.

The substitution of starch for fibre was also studied for rabbit does diets, using five isoenergetic diets (10.6 MJ DE/kg) with increasing levels of NDF (from 27.8 to 37.1%) and fat (from 2.0 to 5.1%) at the expenses of the level of starch that decreased from 23.7 to 11.7% (De Blas *et al.*, 1995). Some impairment in does performances were observed in rabbits does fed the highest levels of fibre, which might be explained by higher fermentation losses in the caecum, together with an insufficient uptake of glucose from the gut to meet the requirements for pregnancy and milk lactose synthesis. Conversely, negative effects of high dietary starch concentrations were also mentioned and related to an increase in diarrhoea mortality for the does.

2.1.2 *Effect of the type of cell wall constituents.*

A part from the important role of fibre intake, the quality of fibre (see chapter 5) also interferes with diarrhoea incidence in the growing rabbit (30-70d old). The favourable effect of lignocellulose on the digestive disorders and mortality in fattening rabbits was evidenced by several studies. For example, the health risk index (HRi = mortality + morbidity rate) decreased from 28 to 18% when the dietary ADF content increased from 15 to 19% (Gidenne *et al.*, 2004b). But, within lignocellulosic components, the favourable effect of the lignin fraction (criterion ADL = Acid Detergent Lignin) was also demonstrated with several studies, and a strong negative relationship was found with the HRi (figure 10.5, Perez *et al.*, 1994; Nicodemus *et al.* 1999; Gidenne *et al.*, 2001). The cellulose (ADF-ADL), also favours the digestive health (Perez *et al.*, 1996). However, lignins play a specific role since an increase of the ratio lignins/cellulose (L/C) is associated with a lower HRi (Gidenne *et al.*, 2001a). Globally, the lignin requirement (ADL) for the growing rabbit, can be assumed as to 5 to 7g/d, and that of cellulose from approximately 11 to 12 g/d. Besides, Nicodemus *et al.* (2006) reported better performances (milk production, etc.) for does fed a high lignin diet (59 g/kg ADL). However, to date, no correct and quick analytical method for lignins is available. Consequently, estimating the amount of lignins in a raw material remains difficult,

particularly in tannin-rich ingredients (grape marc, etc.), and caution must be taken to fit requirements.

Although digestive health of the classically weaned rabbit depends of the level and quality of lignocellulose, it also vary greatly for the same ADF level (figure 10.6), because the level of more digestible fibre fractions "DgF", i.e. [hemicelluloses (NDF-ADF) + water-insoluble pectins], could also vary independently of lignin and cellulose levels. Thus, a dietary recommendation for lignocellulose only appears to be insufficient to prevent digestive disturbances in the rabbit. For instance the ratio DgF/ADF ranged from 0.9 to 1.7 in the figure 4.3.2. The DgF fraction would play a key role for the digestive efficiency and for the digestive health, since it is rapidly fermented (compared to ADF), in a delay compatible with the retention time of the caeco-colic segment (9-13h, see chapter 1). Without changes in ADF dietary level, digestive troubles are rather reduced when DgF replaces starch (Perez et al., 2000) or protein (Gidenne et al., 2001b). This could originate from the favourable effect of DgF (compare to starch or protein) on caecal fermentative activity (Jehl and Gidenne, 1996, Garcia et al., 2002), and possibly from their moderate effect on the rate of passage (Gidenne et al., 2004b). However, a too high incorporation of DgF with respect to lignins and cellulose should be avoided to minimise the Health risk (morbidity + mortality) during fattening. It is thus recommended that the ratio DgF/ADF remain under 1.3 (when dietary ADF level is over 15%, see table 10.2).

Another way to analyse the role of cell-wall polysaccharides that are rapidly fermented is to determine the NDSF residue (Hall et al., 1997), which corresponds to the cell wall polysaccharides soluble in neutral detergent solution (= sum of water soluble and insoluble pectins + β -glucans + fructans + oligosaccharides[DP>15]). Although the level of NDSF is moderate in rabbit feeds, a reduction of its level (12% vs 8%) could be unfavourable on digestive health of the early-weaned rabbit (Gómez-Conde et al. 2004b and 2005; Table 4.3.3). Reversely, a higher level of NDSF improved the mucosal morphology and functionality and its immune response. Besides, soluble fibre reduced the proportion of animals with *Clostridium perfringens* in the caecum and other pathogens as *Campylobacter* both in the ileum and in the caecum. Accordingly, mortality due to REE was reduced with a diet with 12% soluble fibre (Table 10.3; Gómez-Conde et al., 2007, 2009).

Dietary fibre quality could also be completed by the determination of the particle size pattern. It is acknowledged that the particle size distribution of a feed could affect the digestive motility and more particularly the caeco-colic rate of passage. Fibrous raw materials with a small proportion of large particles (> 0.3 mm) due to grinding (screen size 0.5 to 1mm) or to a previous processing leads to longer retention time (Laplace and Lebas, 1977; Gidenne et al., 1991; García et al., 1999), but are not associated with a negative effect on the digestive health status (Lebas et al., 1986; Gidenne et al., 1991, Nicodemus et al., 2006). Only a very low rate of large particles (< 21% particles lower than 0.3 mm) would have a negative impact on the performances. Nevertheless, a rate of coarse particles lower than 25 % is unusual in practice, since on a series of 77 commercial French feeds the average proportion of coarse particles is 38.8% (minimum = 22.7%, mean minus 2 S.D. = 27%; Lebas and Lamboley, 1999).

In conclusion, one criteria is not sufficient for fibre recommendation, since the risk of digestive trouble in the growing rabbit is **jointly** dependent of low-digested "ADF" and digestible fibre "DgF". As for other nutrients, there is a minimum under which it can be spoken of "fibre deficiency" (see table 10.2). In perspectives fibre role for the young rabbit should be precised, particularly the effects of NDSF fibre fraction.

2.2 Protein level and quality

The protein requirements are high in the young animals (see chapter 12 and 3), not only for body growth but also for intestinal mucosa development and renewing. Reversibly, an excessive protein supply did not affect the growth itself, but would favour the incidence of diarrhoea. For instance, De Blas et al. (1981) observed an increased mortality during the fattening period with high protein

diets. A level of 1.8 to 1.9 g crude protein per Mega Joule of digestible energy seems optimum, even if with an increase up to 2.6 g CP MJ⁻¹ DE, Kjaer and Jensen (1997) observed only a slight non significant increase of mortality. In the same way, Catala and Bonnafous (1979) have shown that a higher ileal flow of protein (obtained through a reduction of protein digestion by a ligation of the pancreatic duct) leads to an increase in microbial proliferation in the hindgut. Similarly, an excess of dietary protein could also favour the proliferation of *Clostridia* in the rabbit and could also slightly increase the prevalence of *E. coli* (Cortez *et al.*, 1992; Haffar *et al.*, 1988) and thus could lead to an increase of enteritis mortality. For instance, in a large scale study Gidenne *et al.* (2001b) showed that the replacement of protein by digestible fibre reduced the Health risk for diarrhoea (figure 10.7). A hypothesis to explain it could be a higher availability of substrates for microbial growth, with prevalence of pathogenic species, when animals are fed with high protein diets. In the same way, a higher ileal flow of protein is associated with a lower caecal pH in the young rabbit (Gutiérrez *et al.*, 2003; Nicodemus *et al.*, 2003b, 2004), that may affect the commensal microbiota.

The weaning also implies a switch from milk to vegetal proteins. The digestion of the latter is worse, and sometime contains antinutritive factors, such lectins, antitryptic or antigenic factors. This could impair the apparent ileal digestion or induce changes in the morphology of intestinal mucosa as occurs in other species. In rabbits, Scheele and Bolder (1987) observed an increase of mortality before weaning (35 days old) in rabbits fed diets containing a high proportion of soybean meal (20%) with respect to diets based on animal protein (31 vs 10%, respectively). In this sense, Gutiérrez *et al.* (2000), observed that the substitution of soybean meal with animal plasma had a positive effect on the morphology of intestinal mucosa, feed intake, growth and mortality. In other study, Gutiérrez *et al.* (2003) compared four protein concentrates (sunflower meal, soybean meal 48, soybean concentrate and potato protein) in isonutritive starter diets. Animals fed diets with the protein sources with lesser content in antinutritive factors (sunflower meal and soybean concentrate) showed higher apparent ileal protein digestibility and growth performance and lower mortality rate than the other diets. However, the gastric acidity, villus morphology and faecal digestibility were similar among diets, and no differences on phenotypic distribution of lymphocytes in the duodenal lamina propria were detected, which might be related by the development of a tolerance mechanism by the animals. The importance of the reduction of the ileal flow of protein (by using digestible sources or reducing protein level) in reducing the mortality rate have been also supported in recent experiments (García-Ruiz *et al.*, 2006; Chamorro *et al.*, 2007).

Thus, most of the feed manufacturers limit dietary protein level in fattening diets because of the increased mortality rate observed in rabbit farms when protein levels exceed by 2 points or more the minimum levels recommended for maximum growth rate. Moreover, an excessive protein supply will probably become more and more scarce in Europe, because of an increased dietary cost and mostly because the European breeding policy favour the reduction of the nitrogen excretion in the environment, through the use of low protein diets (Maertens *et al.*, 1999).

2.3 Lipids

Few studies dealt with the role of dietary lipids on digestive health of the growing rabbit, since lipids dietary levels are usually under 3% and are well digested in the small intestine. Furthermore, it is difficult to separate the effect of lipids itself from that of DE intake. However, it has been recently found that some medium-chain fatty acids (MCFAs), such caprylic and capric acid (as triacylglycerol form), exhibit antimicrobial activity for some bacteria of the caecal digestive flora (Marounek *et al.*, 2002). Moreover, the maternal milk, rich medium-chain fatty acids, protects the young rabbit against a colibacillosis (Gallois *et al.*, 2007), and addition of MCFAs in the feed would have a favourable impact on the digestive health of the growing rabbit (Skrivanova and Marounek,

2006). However, contrasting results are obtained when rabbits are experimentally infected with a pathogenic *E. coli* (Skrivanova *et al.*, 2008; Gallois *et al.*, 2008).

Some fatty acids, such as omega3 class, would also be implicated in the development of immune response (Fortun Lamothe and Boullier, 2007), and Maertens *et al.* (2005) reported a higher post-weaning viability for young fed a diet having a low n-3/n-6 ratio (1.0 vs 4.4). Besides, fat addition to starter diets would increase the energy intake of kits and contribute to maintain a good body nutritional condition. Thus, it would favour a harmonious digestive maturation and immune system development, thus reducing weaning risk and improving resistance to digestive troubles.

In return, the incorporation of fat in breeding does diets could be of interest to increase their digestible energy intake. However, contrasted results have been obtained indicating either a higher kits mortality (Lebas and Fortun-Lamothe, 1996) or a trend for a lower kits mortality (Fraga *et al.*, 1989, Fernandez-Carmona *et al.*, 1996). Besides, neither average weight of breeding does nor fertility or prolificacy were significantly affected by dietary fat incorporation (Fortun-Lamothe L., 2006).

3 Feed intake strategy and digestive pathology of the growing rabbit

Usually, studies on intake regulation aim to analyse the effects on the carcass quality of the growing rabbit, or to analyse the digestive efficiency. But, more recently some studies dealt with the relationship between intake level and digestive trouble incidence, including a study with an experimental ERE infection. The effect of a quantitative linear reduction of the feed intake level (100 to 60%) on digestive health and growth of the rabbit was measured through a large-scale study (6 experimental units, 2000 rabbits per treatment, Gidenne *et al.*, 2009a). During feed restriction, the mortality and morbidity rates were significantly reduced (resp. from 12 to 3.5%, and from 12 to 6% for ad-lib+90% feeding level vs 70+60%). The feed restriction during 20d after weaning reduced proportionally the growth rate. Thereafter, returning to an ad-libitum feed intake led to a compensatory growth and to a higher feed efficiency. On the whole fattening period, the live weight loss of the more restricted rabbits (60%) was 7.7%, compared to control rabbits fed ad-libitum since weaning. The favourable effect of limiting the intake on the digestive health of the young rabbit was recently confirmed by another large-scale study (Gidenne *et al.*, 2009b), whereas there was no major effect of the mode of feed distribution (one or two times a day). Moreover, Boisot *et al.* (2003) also demonstrated a similar positive effect of feed restriction when rabbits were challenged with ERE inoculum. Physiological mechanisms explaining such a favourable effect of reducing the intake level on diarrhoea incidence remained to be elicited.

In addition, similar results were also obtained by reducing the intake level through a time restriction for water consumption (Boisot *et al.*, 2004; Verdelhan *et al.*, 2004). Consequently, strategies for controlling the intake of the young after weaning are now widespread in French professional breeders, in parallel to the development of new automatic feeding equipments.

4 Troubles associated with dietary compounds present at toxic level

4.1 Minerals and vitamins.

Despite recommendations for optimum and maximum level of mineral and vitamins were exposed more extensively in chapter 7, it seemed necessary to have a small part of this chapter 10 devoted to maximum acceptable levels in diets. Effectively at the occasion of a diet formula validation it is highly recommended to control that the calculated nutrients levels, even if the analysis are not available, are far enough from the toxic levels.

The main available information is summarized in table 10.4. The values are those from Lebas *et al.* (1996) amended according to the most recent data obtained mainly during the last World Rabbit Congresses: Bernardini *et al.* (1996) and Virag *et al.* (2008) for vitamin E in growing rabbit; Abdel

Khalek *et al* (2008) for vitamin E and C in breeding does, Abd El-Rahim *et al.* (1996) for iron, Guimarães and Motta (2000) and Ayyat and Marai (2000) for zinc.

It must be pointed out that the maximum acceptable level is in general widely higher than the recommended level, but with some noticeable exceptions such as potassium, phosphorus or vitamin D.

4.2 Mycotoxins

Mycotoxins are metabolites produced by certain fungi in the field on standing crops or during the harvesting of feedstuffs. The mould growth could also occur on stored grains or other raw materials because of non hygienic storage-conditions. The toxic substances may be contained within the spore or secreted into the substrate on which the fungi are growing. Most of these substances have a high degree of animal toxicity. Feeding rabbits on naturally moulded diets (mixed toxins contamination) is responsible for many troubles such as decreased feed intake, functional alteration of liver and genital tract, changes in blood constituents (Abdelhamid, 1990). Mycotoxicoses appear in chronic or acute forms. The acute form is caused by the rapid ingestion of large amounts of toxin over a short period. For more details see the review of Mézes (2008).

Aflatoxins are naturally-occurring toxins produced in grains and other feedstuffs both before and after harvest by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 (AFB₁) is of primary concern since it is the most abundant and the most toxic. Acute or chronic aflatoxicosis may occur depending on dietary concentration of toxins. Rabbits are extremely sensitive to aflatoxin. The acute oral single-dose LD₅₀ is about 0.3 mg kg⁻¹ body weight (Newberne and Butler, 1969), among the lowest of any animal species. Moderate-to-severe death losses can be encountered with diets containing even low concentration of toxin (<100 ppb) (Krishna *et al.*, 1991). Signs of toxicity included hepatic lesions (Abdelhamid *et al.*, 2002), anorexia, weight loss, emaciation followed by icterus in terminal stages (Morisse *et al.*, 1981). Acute aflatoxin poisoning (AFB₁ daily doses > 0.04 mg kg⁻¹ body wt) causes prolonged blood-clotting time, extensive liver damage and death from liver failure (Clark *et al.*, 1980; 1982; 1986).

Zearalenone (F-2 toxin) is an estrogenic substance frequently recovered in maize and other grains contaminated by *Fusarium graminearum* (Perez and Leuillet, 1986). Zearalenone causes hypertrophy development of the genital tract of the female rabbit (Pompa *et al.*, 1986; Abdelhamid *et al.*, 1992). This substance can also affect components of uterine tubal fluid known to be of critical importance during the early pre-implantation period (Osborn *et al.*, 1988). Zearalenone induces changes in blood serum enzymes activities. Low doses (10 µg/Kg⁻¹) resulted in significant increase in ALP activity, while higher doses (100 µg/Kg⁻¹) led to significant increases in activities of AST, ALT, AP, GGT, and LDH, indicating possible liver toxicity due to chronic effects of the toxin (Čonková *et al.*, 2001). Levels of zearalenone in feed as low as 1-2 ppm can interfere with normal reproductive activity of rabbit when fed for only a few days (1-2 weeks). This high sensitivity of rabbits to this mycotoxin could be related to the slow hepatic transformation of zearalenone mainly into α -zearalenol, a more uterotrophic metabolite (Pompa *et al.*, 1986).

Another group of toxins produced by *Fusarium* species are the trichothecenes: T-2 toxin and vomitoxin.

T-2 toxin is produced by some strains of the fungus *Fusarium tricinctum*. It is relatively frequent in fibrous raw materials harvested or stored in bad conditions. In affected rabbits, T-2 causes marked feed refusal, lesions of the digestive tract and impairment of blood-clotting mechanisms. (Gentry *et al.*, 1982; Fekete *et al.*, 1989). Long-term (4-7 weeks) feeding of sublethal quantities of T-2 (0.19 ppm) altered ovarian activity of sexually mature female rabbits (Fekete and Huszenicza, 1993). Administration *per os* of 4 mg kg⁻¹ body weight of T-2 toxin causes death within 24 h (Vanyi *et al.*, 1989).

Vomitoxin (4-deoxynivalenol) may occur on cereal grains. Contamination of rabbit feeds with this toxin results in feed refusal and vomiting. Adverse effects on foetal development were also

encountered in does. Thus, Khera *et al.*, (1986) observed that a level of 0.024% vomitoxin in the diet caused a 100% incidence of fetal resorption.

The nephrotoxins (ochratoxin and citrinin) have been also implicated in rabbit mycotoxicosis.

Ochratoxin is produced by toxigenic strains of *Aspergillus ochraceus*. Galtier *et al.* (1977) examined the excretion of ochratoxin A in rabbit female after a single intravenous administration (1-4 mg kg⁻¹ body wt) and demonstrated the passage of the toxin in the milk: the level in milk reached 1 ppm for the highest dose of administration. The real toxicity for rabbits is unknown but it can be pointed out that in the above mentioned experiment, the lactating does have supported a single dose of 4 mg kg⁻¹ body wt.

Citrinin is found in mouldy cereals contaminated by various fungal species of *Aspergillus* and *Penicillium*. Ingestion of this toxin induces acute erosive gastritis, fluid diarrhoea, with some rabbits dying less than 24 h after oral administration of a single 100-130 mg kg⁻¹ body weight oral dose (Hanika *et al.*, 1983). In the rabbit, it causes also renal damage with tubular dysfunction and necrosis similar to that found in other animal species (Hanika *et al.*, 1984).

5 Water quality and pathology

In most of the treatises on animal nutrition, the part devoted to water quality is very short. It's generally written: « water employed for animals watering must be "drinkable" » and the recommended values given are those for human consumption without comments.

If these values are effectively obtained at the watering point available for the animals, there is effectively no health problem linked to water quality. Nevertheless bacterial and chemical composition of water available for animal watering does not always respect all the recommended criteria.

In no way water polluted with bacteria can be recommended for rabbit nutrition even if we know that animals are more tolerant than humans. As very simple low-cost systems are available, the solution is disinfection. The classical criteria for bacterial quality of drinkable water are reminded in the table 10.5.

For minerals, removing the excess is technically possible in most cases, but the cost is very high, and the constant question is « *Is it necessary for rabbit's health?* ». Different experiments were conducted to establish the real tolerance of rabbits to minerals concentration in drinking water, mainly in hot sub-desertic areas or in intensive animal production areas. The results are summarized in the table 10.6 Values are indicated for each mineral, but it does not mean that a water with all criteria at the maximum should be accepted by rabbits.

It can be pointed out that when known, the tolerance limits of rabbits are very wide from the maximum "officially" acceptable values for human consumption. One of the most significant, is the tolerance of rabbits to high levels of nitrates or nitrites: 10-fold the maximum accepted for human consumption, which create great polemic in intensive animal production regions such as the Netherlands or Brittany in France. None of the maxima for rabbits are lower than those recommended for human consumption. It's why no specific chemical control is necessary if water provided for rabbits watering is that provided for human consumption by a controlled public system. On the contrary, alteration of water quality by increasing of some minerals can be illegal for human consumption, but is not necessarily injurious for rabbit health (table 10.6).

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Table 10.1 Number of rabbits per treatment required (n) to detect a significant difference (P = 0.05) for mortality rate between two treatments.

<i>Difference to be detected, percentage units</i>	Number of rabbits required (n)
5	338
10	87
15	40
20	23

Table 10.2 : Fibre and starch requirements for the young weaned rabbit to prevent digestive troubles

Unit ⁽¹⁾	INRA		Univ. Madrid	
	Post weaning (28-42d old)	Growing (42-70d old)	Post weaning (25-39d old)	Growing (39-70d old)
Neutral Detergent Fibre "NDF"	NDF≥310	NDF≥270	300≤NDF<360	320≤NDF<350
Lignocellulose "ADF"	≥190	≥170		160≤ADF<185
Lignins "ADL"	≥55	≥50		≥55
Cellulose "ADF-ADL"	≥130	≥110		
Ratio Lignins/ Cellulose	>0.40	>0.40		
Hemicelluloses "NDF-ADF"	>120	>100		
DgF ² /ADF	≤1.3	≤1.3		
Neutral detergent soluble fibre "NDSF" ³	–	–	120	–
Particles > 0.3 mm	–	–	–	> 210
Starch			<200	145<starch<175

⁽¹⁾: g.kg⁻¹ as fed basis, corrected to a dry matter content of 900 g.kg⁻¹.

⁽²⁾: Digestible fibre fraction = [hemicelluloses (NDF-ADF) + water-insoluble pectins].

⁽³⁾According to Hall et al. (1997)

Table 10.3. Effect of dietary NDSF level on mucosa morphology and functionality, immune response and frequency of detection of *C. perfringens* at 35d, and mortality of 25d weaned rabbits during fattening (Gómez-Conde *et al.*, 2007 and 2009).

Dietary NDSF ¹ level, % (as fed)	12	9	7	P
Jejunum morphology and functionality (35d)				
<i>Villi length, μm</i>	722 ^a	567 ^b	493 ^c	0.001
<i>Crypt depth, μm</i>	89 ^b	115 ^a	113 ^a	0.001
<i>Sucrase specific activity (μmol glucose/g protein)</i>	8671 ^a	6495 ^b	5202 ^c	0.019
Immune response in lamina propria (35d)				
<i>CD4+</i> , %	35.1	33.9	26.2	NS
<i>CD8+</i> , %	21.3	26.9	30.3	0.074
Frequency of detection <i>C. perfringens</i> , % ²				
Ileum	0	22.2	9.1	0.062
Caecum	5.7 ^a	2.9 ^a	17.6 ^b	0.047
Mortality 25-60 d, %	5.3 ^a	8.5 ^{ab}	14.4 ^b	0.016

¹ Neutral detergent soluble fibre according to Hall *et al.* (1997). ² Frequency of detection in the ileum and caecum (T-RFLP approach).

Table 10.4: Maximum levels of minerals or vitamins experimented without any trouble and levels known to induce signs of toxicity in the rabbit.

	Maximum level observed without any trouble	Concentration with Signs of toxicity	Period of life
Minerals			
- Calcium	25 g kg ⁻¹	40 g kg ⁻¹	growth
	19 g kg ⁻¹	25 g kg ⁻¹	reproduction
- Phosphorus	8 g kg ⁻¹	-	growth
	8 g kg ⁻¹	10 g kg ⁻¹	reproduction
- Magnesium	3.5 g kg ⁻¹	4.2 g kg ⁻¹	growth
- Sodium	6 g kg ⁻¹	7 g kg ⁻¹	growth
- Potassium	16 g kg ⁻¹	15 - 20 g kg ⁻¹	growth
	16 g kg ⁻¹	20 g kg ⁻¹	reproduction
- Chlorine	4.2 g kg ⁻¹	-	growth
- Copper	150-200 ppm	200-300 ppm	growth
- Fluorine	-	400 ppm	growth
- Iodine	10 000 ppm	-	growth
	-	100 ppm	reproduction
- Iron	400 ppm	500 ppm	growth
- Manganese	-	50 ppm	growth
- Selenium	0.32 ppm	-	growth
- Zinc	200 ppm	400	growth
Vitamins			
- Vitamin A	100 000 IU kg ⁻¹	-	growth
	40 000 IU kg ⁻¹	75 000 IU kg ⁻¹	reproduction
- Vitamin D	2 000 IU kg ⁻¹	3 000 IU kg ⁻¹	reproduction
- Vitamin E	300 mg kg ⁻¹	-	growth
	160 mg kg ⁻¹	-	reproduction
- Vitamin C	2 g kg ⁻¹	-	growth
	400 mg kg ⁻¹	-	reproduction

Table 10.5: Recommended bacteriological status of drinkable water for human consumption*

Microorganisms	Maxi count
- <i>Salmonella</i> sp.	0 in 5000 ml
- <i>Staphylococcus</i> sp.	0 in 100 ml
- Enteroviruses	0 in 10000 ml
- Faecal <i>Streptococcus</i> sp	0 in 100 ml
-Thermo-tolerant <i>Coliform</i>	0 in 100 ml
- <i>Clostridium</i> sp.	1 in 20 ml

* Official Journal of the European Communities, 1998; Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption

Table 10.6: Chemical composition of drinkable water for rabbits

<i>Physical parameter (units)</i>	Official recommendations for Human consumption*		Maximum experimented on rabbits without any trouble	
	Recommended Maxi	Maxi tolerable	Value	Authors
- pH	7 - 8.5	6.5 - 9.2	3.5 - 9.0	Porter <i>et al.</i> , 1988
<i>Chemical Parameters (in ppm)</i>				
Total soluble salts	500	1500	3000	Abdel-Samee & El-Masry, 1992
- Sodium	100	150	900	Ayyat <i>et al.</i> , 1991
- Potassium	10	12	140	Ayyat <i>et al.</i> , 1991
- Phosphorus	2	5		
- Calcium	75	200	400	Porter <i>et al.</i> , 1988
- Magnesium	30	150		
- Iron	0.2	1.0		
- Copper	0.1	1.5	60	Abo El-Ezz <i>et al.</i> , 1996
- Manganese	0.05	0.5	12	Abdel-Samee & El-Masry, 1992
- Zinc	5	15	55	Abdel-Samee & El-Masry, 1992
- Aluminium	0.2	-	250	Rémois & Rouillière, 1998
- Antimony	0.01	-		
- Arsenic	0.05	0.20		
- Cadmium	0.005	0.05		
- Chromium	0.05	1.0		
- Cobalt	-	1.0		
- Fluoride	1.5	2.0		
- Lead	0.05	0.10	0.40	Habeeb <i>et al.</i> , 1997
- Mercury	0.001	0.01		
- Nickel	0.05	1.00		
- Selenium	0.01	-		
- Silver	0.01	-		
- Vanadium	-	0.10		
- Chloride as Cl	250	600	1100	Habeeb <i>et al.</i> , 1997
- Sulphate as SO ₄	200	400	1340	Rémois & Rouillière, 1998
- Nitrate as NO ₃	45	50	600	Kammerer & Pinault, 1998
- Nitrite as NO ₂	0.05	0.10	11	Morisse <i>et al.</i> , 1989
- Ammonium as NH ₄	0.05	0.50		
- H ₂ S	0.05	0.10		
- Bicarbonate			400	Ayyat <i>et al.</i> , 1991
- Nitrogen (N from NO ₂ & NO ₃ excluded)	2	-		
- Cyanide as CN	0.05	-		

* Official Journal of the European Communities, 1998; Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption

Figure 10.1: Changes in the caecocolic ecosystem occurring in case of digestive troubles (diarrhoea) in the growing rabbit

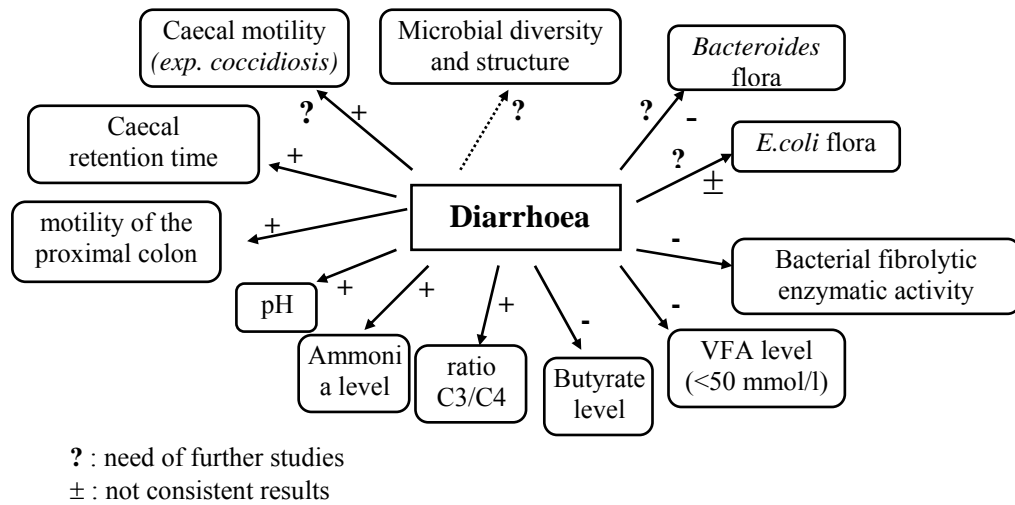


Figure 10.2: In-vivo⁽¹⁾ caecal fermentation pattern of healthy and sick⁽²⁾ growing rabbits

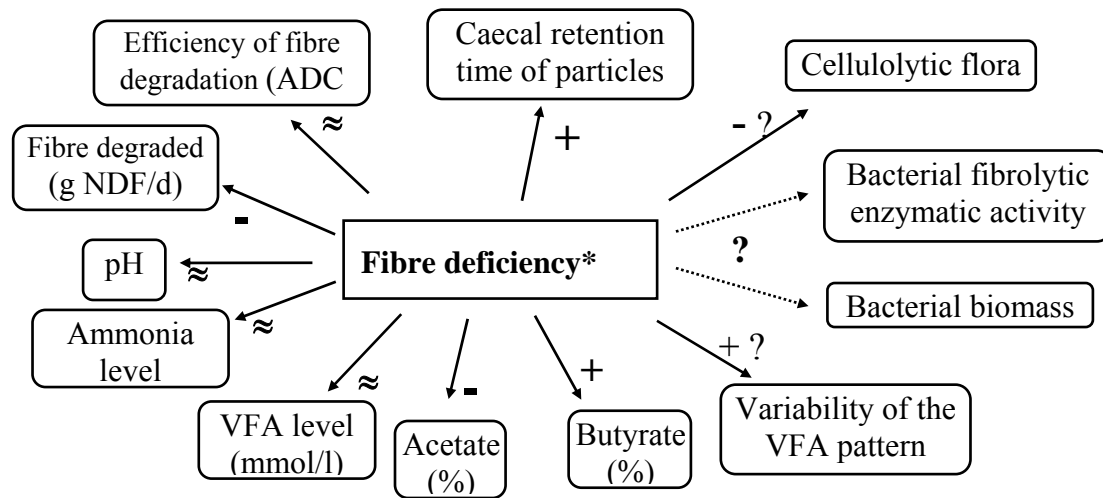
	Caecal VFA level (mmol/l)		pH	
Sick :	(14)	48±17	(103)	(5.65) 6.73±0.59 (7.89)
healthy :	(41)	58±9	(77)	(5.71) 6.45±0.3 (7.03)
	Butyrate level (mmol/l)		NH ₃ (mmol/l)	
Sick :	(0.3)	2.2±1.1	(4.2)	(1.7) 8.3±4.4 (15.2)
healthy :	(2.6)	5.0±1.7	(9.7)	(1.4) 5.6±3.0 (11.0)

Figures in parenthesis are minimum and maximum values observed from a set of 80 and 21 rabbits respectively for healthy and sick animals (Data from Bellier, 1994)

⁽¹⁾: cannulated rabbits, between 7 and 11 weeks of age.

⁽²⁾: rabbits having punctual digestive troubles, or abnormally low intake.

Figure 10.3: Effect of a fibre deficiency* on several parameters of the caecal ecosystem in the growing healthy rabbit .



* **lower than** 8-11 g ADF/kg LW/day compare more than 12g ADF/kg/day for a diet balanced in fibre quantity.
 ? : need of further studies
 ≈ : not significant effect

Figure 10.4: Relationships between feeding the growing rabbit with low fibre/high starch diets and the incidence of digestive troubles.

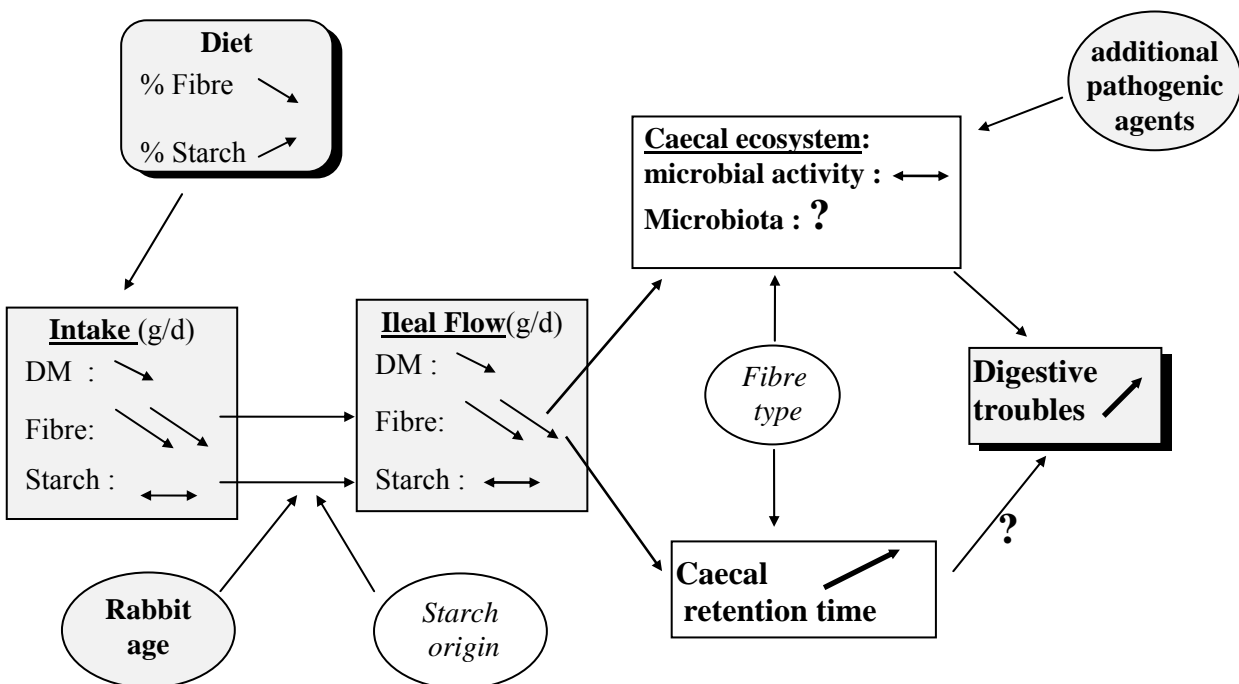
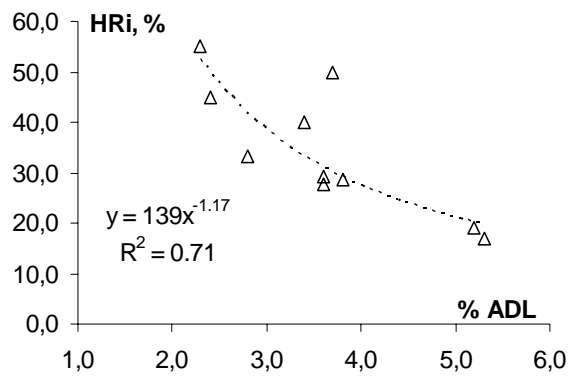
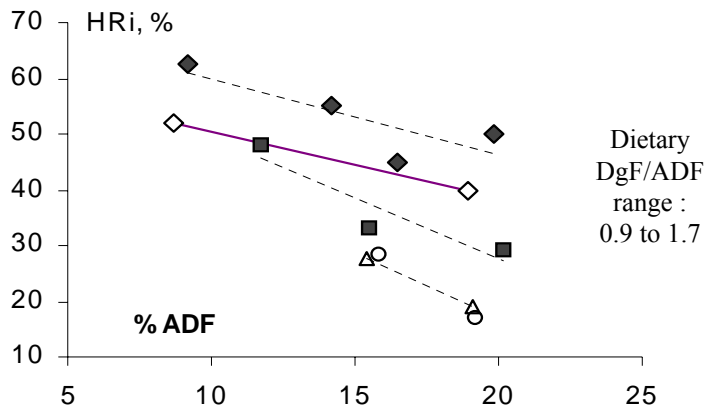


Figure 10.5: Increasing the dietary lignin concentration reduces the risk of digestive troubles (Hri) in the growing rabbit.



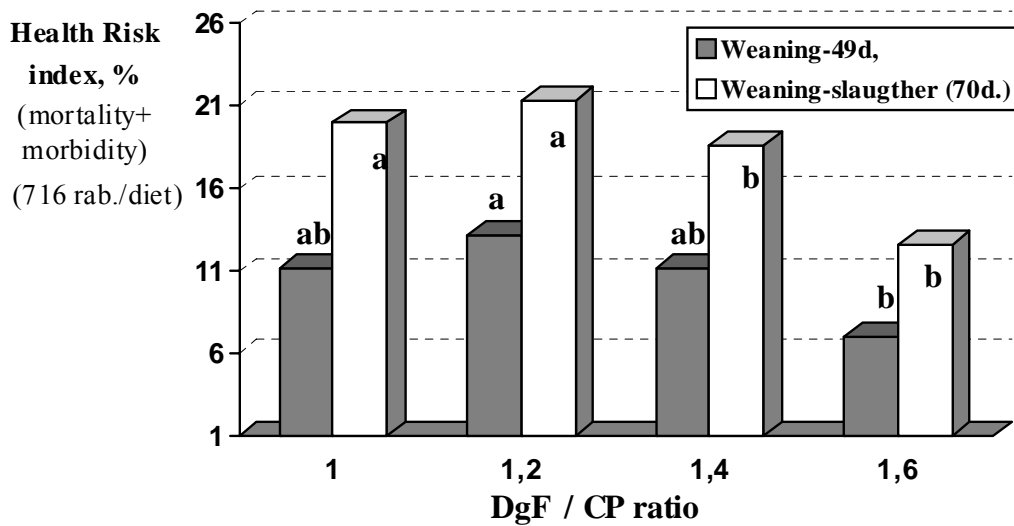
ADL = Acid detergent lignin (Van-Soest sequential procedure, EGRAN 2001).
HRI = Health risk from digestive trouble = mortality + morbidity rate by diarrhoea, measured from 28 to 70d of age, on at least 40 rabbits/diet (Data for 10 diets ranging from 14 to 20% ADF level; references : Gidenne, 2003).

Figure 10.6: The risk of digestive trouble (HRi) in the growing rabbit is jointly dependent of low-digested "ADF" and digestible fibre "DgF".



ADF = lignocellulose (Van-Soest sequential procedure, EGRAN 2001).
 DgF : digestible fibre = water insoluble pectins + hemicelluloses (NDF-ADF)
 HRi= Health Risk index from digestive trouble = Mortality + morbidity rate by diarrhoea, measured from 28 to 70d of age, on at least 40 rabbits/diet (one point = one diet, n=13; for references see Gidenne 2003).

Figure 10.7: Impact of protein (CP) replacement by digestible fibre (DgF), on the Health Risk index between weaning and slaughter.



Data from Gidenne *et al.* (2001b).