# Role of type of fibre on intestinal microbiota and performance in rabbits

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**ABSTRACT** – In this work is reviewed the new approaches and methods to the dietary fibre concept and its relation with the intestinal microbiota. Fibre is the largest dietary fraction in rabbit diets. It influences rate of passage and constitutes an significant substrate for intestinal microbiota that might affect rabbit health and performance. However, the definition of fibre, its separation in different fractions and the way to quantify and characterize it is still under discussion. Besides, new molecular techniques have been developed to improve the study of intestinal microbiota that might help to understand better its relationship with the level and type of fibre. Recent data seems to indicate the important role of dietary soluble fibre level, as well as its balance with insoluble fibre, in minimizing the effects of epizootic rabbit enteropathy.

Key words: Soluble fibre, Insoluble fibre, Intestinal microbiota, Rabbit performance.

## INTRODUCTION

Fibre is the major fraction in rabbit diets and accounts for 40-50% of them. The importance of fibre in rabbit is due to its influence on rate of passage, and its function as substrate for microbiota, both related to rabbit health and performance. The concept of fibre, its quantification and characterization either of the total fraction or the different constituents are still under revision and discussion. The difficulty to find an agreement on the dietary fibre concept lay down on its complex physical structure and chemical composition of cell wall, and by the wide diversity of cells types, and accordingly of cell walls, that constitute the different plant tissues, and in the wide and different physiological effects of the different constituents. It implies that the quantitative analysis of the whole components of this fraction cannot be obtained by any analytical method or combination of methods.

Usually in animal and especially in rabbit nutrition, the insoluble fraction of fibre has been considered more important, and this is reflected in the nutritional recommendations that usually included only this fraction. Insoluble fibre accounts for around 77% of the total fibre in rabbit diets, it can be quantified by using relatively easy methodology, and it is the best dietary fraction to predict the dietary digestible energy value. Furthermore, many studies have demonstrated that the rabbit require at least around 30 and 33% NDF. Lower NDF level delay the rate of passage, reduce performance and increase the risk of suffering digestive pathologies (De Blas and Mateos, 1998; Gidenne and García, 2007). However, not only the level of insoluble fibre is important for the animal, but also its chemical (degree of lignifications) and physical characteristics (particle size) as they influence rate of passage and its fermentability (Nicodemus *et al.*, 1999; 2006). Other fibre characteristics as water

holding and buffer capacity also influence rabbit digestive physiology (García *et al.*, 2000).

On the opposite, there are few studies dealing with the effect exerted by soluble fibre on rabbit traits, in spite of it might have a major influence on intestinal microbiota than insoluble fibre, due to its higher fermentability. The reason is that soluble fibre is a minority fraction inside total fibre (around 23% of total fibre in rabbit diets), as well to its heterogeneous composition and the methodology troubles to quantify and characterize it. One of the most important properties of soluble fibre might be its capacity of gel forming and increase viscosity in the digestive tract, but is has been scarcely investigated in rabbits.

Both, soluble and insoluble fibres are components of digestible fibre which is an important substrate for microbial growth. Faecal digestibility of insoluble fibre (NDF) is around 30% (García *et al.*, 2002) whereas that of soluble fibre might be around 75%, and 30 percentage units of this value might be digested before the caecum (Gidenne, 1992; Carabaño *et al.*, 2001). However, it is not possible to separate the effects of digestible fibre coming from soluble or insoluble fibre with the available information, as the feedstuffs rich in soluble fibre are also usually rich in digestible insoluble fibre (i.e. sugar beet pulp). Besides, the nutritional and physiological importance of the site of fibre fermentation (that might depend on the proportions of soluble and insoluble digestible fibre) is still not clarified.

Most of the effects exerted by fibre on the rabbit depend on the effect of fibre on intestinal microbiota. However, it is really difficult to study the influence of any dietary component on microbiota, as the traditional cultivate techniques only allow to work with around one fourth of the intestinal microbiota. For this reason, in order to study the intestinal microbial activity other indirect techniques have been used as the volatile fatty acid concentration, fibrolytic activity or the microbial nitrogen synthesized. But in many circumstances they do not seem to reflect adequately the changes produced in the microbiota population (Abecia, 2006).

It suggests that at least an important part of the effects that might exert the fibre fraction on microbiota could not be even identified by using the traditional techniques. The development of new molecular tools to characterize intestinal microbiota allow to study more properly what happen with microbiota, and consequently identify new effects of the different dietary fractions.

The objective of this review is to expose the last news related to the quantification and characterization of dietary fibre fraction, and to the effect of fibre on intestinal microbiota and performance of growing rabbit.

## METHODOLOGY TO QUANTIFY AND CHARACTERIZE FIBRE FRACTION

The definition of fibre is essential to develop a valid methodology for its analysis. The heterogeneous tridimensional matrix of plant cell walls make that there is no available substances that can be used as standards to validate of the different proposed methodologies for fibre analysis. The concept of dietary fibre used in human nutrition, and extended to all mammals, has been periodically reviewed and clarified (Hispley, 1953; Burkitt *et al.*, 1972; Trowell, 1974; De Vries and Rader, 2005), and it is defined as the feed components resistant to mammal enzyme digestion and absorption, and that can be partially or total fermented in the gut. More specifically, presently the Codex Committee on Nutrition and Foods for Special Dietary Purposes of the European Union

is still discussing the definition of dietary fibre (but focused only in human nutrition), as there is no official/legal definition of dietary fibre at the EU. The definition that is being discussed is: *Dietary fibre means carbohydrate polymers with a degree of polymerization (DP) not lower than 3 which are neither digested nor absorbed in the small intestine. A degree of polymerization not lower than 3 is intended to exclude mono- and disaccharides. It is not intended to reflect the average DP of a mixture. Dietary fibre consists of one or more of: i) edible carbohydrate polymers naturally occurring in the food as consumed, ii) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means, iii) synthetic carbohydrate polymers.* It implies that dietary fibre can only be trully measured by the digestive process of the animal.

The indirect estimation of dietary fibre can be performed by using different methodologies (reviewed by Bach Knudsen, 2001, and Mertens, 2003), where the no fibrous components are extracted by solubilization, by enzymatic hydrolysis or by combining both procedures. Once isolated, fibre residue can be quantified gravimetrically (weighing the residue) or chemically (hydrolyzing the residue and determining its single constituents: sugars and lignin). According to these procedures there are three types of methodologies: chemical-gravimetric, enzymatic-gravimetric and enzymatic-chemical. By this way total dietary fibre can be quantified (non starch polysaccharides and lignin) and separated into insoluble and soluble fibre (in aqueous solution), and obtain its monosaccharide composition. The combination of the monosaccharide composition of fibre with additional chemical information may allow describing better fibre structure that influence its physic-chemical properties, and accordingly, the effect exerted in the animal on the digestive physiology and digestibility. However, these methodologies are complex, expensive, with a relatively low reproducibility (especially for monomers determination) and difficult to implement as routine analysis.

There are alternative definitions that try to approach to the animal physiology. Mertens (2003) propose to define insoluble dietary insoluble fibre for herbivores, considering their special digestive features, as it affects their digestibility and rate of passage. Insoluble dietary fibre is defined as the indigestible (lignin) or slowly digesting organic matter of feeds (mostly hemicelluloses and cellulose) that occupies space in the gastrointestinal tract. This definition excludes soluble and rapidly fermenting polysaccharides of plant cell walls (fructans, gums and pectins) that do not occupy space in a liquid environment, and show similar digestibility than starch or protein contained in the cellular cytoplasm (and their effects on health and performance are rarely studied). The quantification of insoluble dietary fibre is performed by the NDF procedure of Van Soest (Mertens, 2002), that is a method more simple, rapid, economical and reproducible than the methods derived from the dietary fibre definition, and show very close results compared to the later (Table 1). In spite of these advantages, NDF procedure is criticized due to its variability among laboratories, especially when it is compared with the results obtained in the analysis of other feed constituents (Xiccato *et al.*, 1996). This is partially explained to the different procedures to perform this analyses (Van Soest and Wine, 1967; Robertson and Van Soest, 1980; Mertens, 2002) and the different adaptations of these methods used in each laboratory. For this reason, Uden *et al.* (2005) ask for a better description of the methodology used to determine NDF (Table 2) and recommend to follow the procedure described by Mertens (2002) (where NDF is expressed ash free, and it is used amylase and sodium sulfite –that improve filtration and protein extraction, but also may extract lignin and

phenolic complexes-). The European Group on Rabbit Nutrition has also proposed some recommendations for determining feed chemical composition to improve the reproducibility among laboratories (Gidenne *et al.*, 2001).

Acid detergent fibre (ADF) and crude fibre (CF) are alternative methods to quantify insoluble fibre (both determined by AOAC procedures), but neither of them fit with total or insoluble dietary fibre definitions mentioned. ADF do not quantify all the insoluble fibre as it dissolves hemicelluloses. Besides its residue contains pectins, except if it has been previously extracted with neutral detergent solution. The main drawback of CF lies in the high variability in the chemical composition of its residue, as depending on the feed, it can dissolve up to 60% cellulose, 80% hemicelluloses and 95% lignin. As a consequence, CF digestibility was higher than than of nitrogen free extract in 25% of the feeds studied by Morrison (1956). For these reasons, these determinations are not very useful to explain the effects exerted by fibre on the animal, but both have demonstrated to be very useful to predict dietary energy value (Wiseman *et al.*, 1992), and also show a similar or even higher reproducibility than NDF.

**Table 1** – Comparison of insoluble and soluble fibre obtained by different methods (adapted from Bach Knudsen, 1997; Hall, 1997 and Mertens, 2003).

| | Insoluble | | Soluble | |
|---|---|---|---|---|
| | Dietary fibre | NDF | NSP[1] | NDSF[2] |
| Maize | 9.9 | 9.5 | 0.9 | — |
| Soybean meal | 17.0 | 14.9 | 6.3 | — |
| Wheat bran | 43.4 | 42.5 | 1.5 | — |
| Sugar beet pulp | 40.7 | 45.8 | 40.7 | 37.3 |
| Grass hay | 52.7 | 57.7 | 3.1 | 3.4 |
| Alfalfa hay | 38.0 | 41.6 | 7.7 | 17.7 |

[1] Non-starch polysaccharides. [2] Neutral detergent soluble fibre.

The most important characteristics of insoluble fibre are its degree of lignification and particle size. Lignin content is determined by using the acid detergent method – using sulfuric acid- (ADL) described by Robertson and Van Soest (1981). This method requires the previous extraction with acid detergent solution, and it is more laborious and variable than NDF (Xiccato *et al.*, 1996). Another important feature of insoluble fibre is particle size, especially the proportion of particles larger than 0.3 mm. Its quantification is not easy, due to the difficulty to separate fibre particles from other particles. It must be considered that the big particles rich in starch or protein will be extensively digested at the small intestine, and accordingly do not exert the same function on rate of passage than fibre particles. The only method used to determine particle size is by wet sieving as described by García *et al.* (1999) and Lebas and Lamboley (1999). Later the residue can be ground and NDF analyzed as performed by Nicodemus *et al.* (2009).

It is more complex to asses and characterize soluble than insoluble fibre. First, the term soluble raise the question: soluble in what solution and in what conditions? Many of the methods mentioned allow quantifying soluble fibre dissolving it with an aqueous phosphate buffered solution, precipitated with diluted ethylic alcohol, weighted the residue and corrected by its protein and ash content. However, Theander *et al.* (1994) demonstrated that this method not always recovered all insoluble fibre. Furthermore, the complexity methodology of these methods difficult their routine uses. An alternative is to assess the fibre dissolved by neutral detergent extraction, the neutral detergent

soluble fibre (NDSF), proposed by Hall (1997). This is determined by difference among total dietary fibre and NDF. However, these methods have the disadvantage of using solvents quite different to those found in the animal gut. Accordingly, it is not sure if the values obtained will reflect the real ones and help to explain the physiological effects observed in the animals. Finally, another possibility is to calculate the soluble fiber content by difference: organic matter – (protein + fat + sugars + starch + NDF).

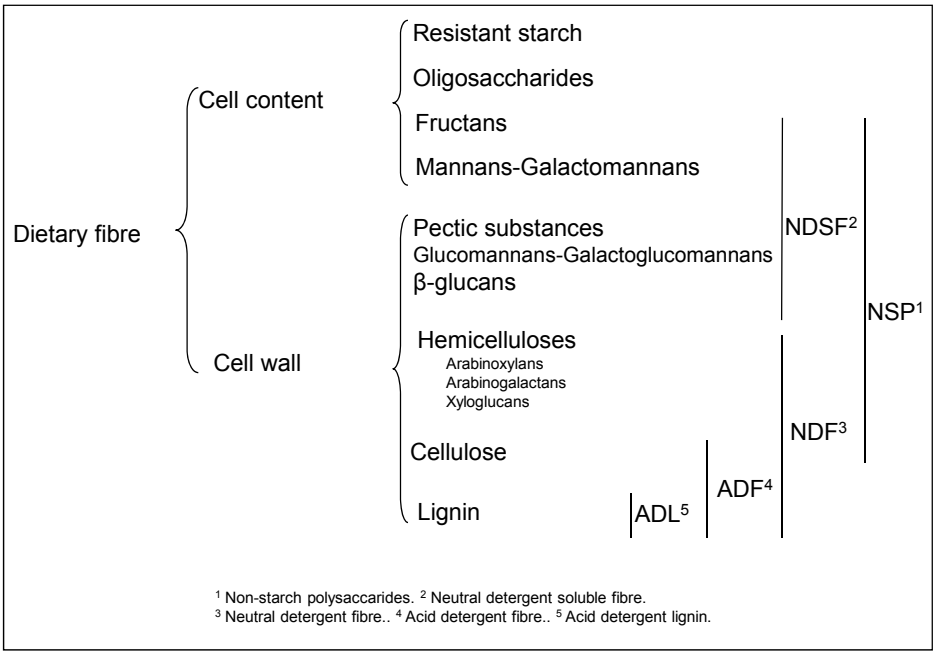
**Table 2** – Proposes nomenclature by Uden *et al.* (2005) for fibre determinations derived from sequential method of Van Soest.

| | |
|---|---|
| aNDFom | NDF assayed with a heat stable amylase and expressed exclusive of residual ash. |
| NDFom | NDF not assayed with a heat stable amylase and expressed exclusive of residual ash. |
| aNDF | NDF assayed with a heat stable amylase and expressed inclusive of residual ash. |
| NDF | NDF assayed without a heat stable amylase and expressed inclusive of residual ash. |
| ADFom | ADF expressed exclusive of residual ash. |
| ADF | ADF expressed inclusive of residual ash. |
| Lignin (sa) | Lignin determined by solubilization of cellulose with sulphuric acid. |
| Lignin (pm) | Lignin determined by oxidation of lignin with permanganate. |

Figure 1 shows a diagram including the different fibre components and the way to asses them derived from Van Soest procedure that is the most feasible alternative in practical conditions.

Dietary insoluble fibre can also be estimated by using NIR technology, that have already demonstrated its usefulness for predicting dietary dry matter, protein, fat, starch, and even the dietary digestible energy value. However, ADF is the only fibre fraction that can be adequately estimated by this technique, whereas both NDF and ADL are estimated with lower precision (Xiccato *et al.*, 2003).

**Figure 1** – Major constituents of dietary fibre (adapted from Hall, 2003).



## EFFECT OF TYPE OF FIBRE ON INTESTINAL MICROBIOTA

Fibrolytic activity of rabbit microbiota take place in the small intestine and in the caecum, dominating in both segments pectinolytic activity compared to xilanolytic and cellulolytic (Marounek *et al.*, 1995). The dietary factors affecting the variability of fibrolytic activity have been scarcely studied, but it seems that low fibre diets might reduce it at the caecum (Gidenne *et al.*, 2000 and 2002). Type of fibre also influence fibrolytic activity, and sugar beet pulp and wheat bran based diets increased caecal pectinolytic + cellulolytic and xilanolytic activities, respectively (Falcao-e-Cunha *et al.*, 2004). This activity is evident when ileal and caecal fibre digestibility is determined. The monomers best digested at ileum are uronic acids, an important constituent of pectins. If fact, around 75-80% of the fibre fermented at ileum are pectins that are the more soluble fibre fraction (Gidenne, 1992; Carabaño *et al.*, 2001). On the opposite, glucose and xylose, the major monomers in most fibre sources, showed a much lower ileal digestibility even with values close to zero. The result of this microbial activity is the release in the gut of volatile fatty acids, which concentration tend to increase in the caecum with NDF level and to reduce with its degree of lignification (García *et al.*, 2002).

From all this information is difficult to know what implications may have these observations on the animal if they are not complemented with the study of the effect of fibre on the microbiota, as the later will exert on the animal most of the effects derived from the fibre fraction of the diet. In this way, the development of new molecular tools as RFLP (Restriction fragment lenght polymorphism) allows to improve our knowledge of intestinal microbiota.

The utility of RLFP on the study of the effect of fibre on intestinal microbiota intestinal has been showed in two experiments carried out in collaboration among microbiologist and nutritionist (CReSA-Barcelona and UPM-Madrid). The nutritional factors studied were the level of fibre –insoluble and soluble- and the type of fibre –particle size- (Nicodemus *et al.*, 2004, Gómez-Conde *et al.*, 2007, Gómez-Conde *et al.*, 2009). These experiments were done in a farm affected by epizootic rabbit enteropathy in rabbits weaned at 25 d, medicated or not. In Table 3 is shown a summary of the results obtained. Mortality was minimized with a dietary insoluble and soluble fibre content of 30% (NDF) and 12% (NDSF), respectively.

The reduction of mortality when NDSF increased was also related to a reduction of the frequency of detection of *C. perfringens* in the caecum. A strain of this bacteria produces a toxin that might be responsible of the mortality due to ERE (Pérez de Rozas *et al.*, 2005; Marlier *et al.*, 2006). Furthermore, it has been also observed that some opportunistic potentially pathogen bacteria, usually linked to mucosa, as *Campylobacter, Yersinia* o *Helicobacter*, seems to be more sensible at ileum to dietary changes than at the caecum. Soluble fibre did not influence the presence of fibrolytic bacteria with potentially probiotic properties as *Bacteroides fragilis*, *Butirivibrio fibrosolvens*, *Propionibacterium* spp. and *Ruminococus* spp, being some of them affected by the level of insoluble fibre and particle size. However, soluble fibre affected similarity rate especially at the caecum compared to the ileum, as caecal microbiota of rabbits fed the same diet grouped together in the same clusters (Figure 2; Gómez-Conde *et al.*, 2009).

**Table 3** – Effect of type of diet on biodiversity, the proportion of animals where potential pathogenic bacteria were detected and on the mortality in rabbits. (Nicodemus *et al.*, 2004; Gómez-Conde *et al.*, 2007, 2009; adapted from Carabaño *et al.*, 2007).

| | | Decrease in the level of fibre (30 *vs*. 25% NDF) | Increase of particle size (normal *vs*. large) | Increase of soluble fibre |
|---|---|---|---|---|
| Biodiversiy | Ileon | Increase | Decrease | No effect |
| | Caecum | Decrease | Decrease with 25% NDF | No effect |
| *C. perfringens* | Ileon | No effect | No effect | No effect |
| | Caecum | No effect | No effect | Decrease |
| Other bacteria | Ileon | Increment of *Bacteroides* | Decrease of *E. Coli, Helicobacter* and *Yersinia.* Increase of *Ruminococus.* | Decrease *Campylobacter*. |
| | Caecum | Decrease *Bacteroides and Ruminococus* | Decrease *Helicobacter* and *Propionibacterium* | Decrease *Campylobacter* |
| Mortality | | Increase | No effect | Decrease |

In relation with the intestinal ecology of microbiota, in piglets have been observed that a intestinal microbial population with low biodiversity favor the proliferation of *C. perfringens*. Pigs with parenteral feeding showed a higher presence of *C. perfringens* respect to those with solid feed. However, in rabbit experiments no relation was detected between the presence of *C.perfringens* and biodiversity, but mortality seems to increase with biodiversity. The later is not in agreement with other authors (Zoetendal *et al*., 2004) that related a high biodiversity with a more stable intestinal microbial ecosystem (and probably healthier for the animal). Anyway, biodiversity might be used as intestinal health indicator if these results were confirmed.

These results indicate that dietary changes influence intestinal microbiota composition, but not always animal health (as dietary particle size). Consequently, we can modify the intestinal microbiota profile through the diet. Besides, RFLP has demonstrated to be very useful to get a better approach to intestinal microbiota. However, at present is still difficult to perform an adequate interpretation of the results due to the interactions among different microorganisms, and between them and different dietary components.

In this way, a big experiment was performed in four different farms using two diets containing 31% NDF and 21% starch, and a moderate or low level of soluble fibre (by including pulps or straw, respectively), and a third one in which starch decreased (11%) and was substituted by insoluble (37% NDF) and soluble fibre (Margüenda *et al.*, 2006). The effect of diet changed according to the farm. In no medicated farms the diet with 31% NDF and moderate soluble fibre level reduced mortality, whereas the more fibrous diet (low starch, high soluble fibre) reduced mortality in the farm with the worst health status and where animals were medicated. In another two farms, also with medication, there was no effect of diets. The diet with the highest NDF and lowest starch contents impaired both feed efficiency and dressing out performance, except in the farm in which it reduced mortality where it also improved feed efficiency (but not the dressing out performance due to the higher digestive tract weight).

**Figure 2** – Effect of fibre source and neutral detergent soluble fibre level on similarity rate of the intestinal microbiota (SR, expressed in percentage) of 35 d old rabbits (OH: oat hulls diet, DA: dehydrated alfalfa diet, B-AP: Beet-apple pulp diet. The first number of each animal is its identification and the second one the mother's identification (M); Gómez-Conde *et al.*, 2009).

**Figure 2a** – Similarity rate of ileal icrobiota.

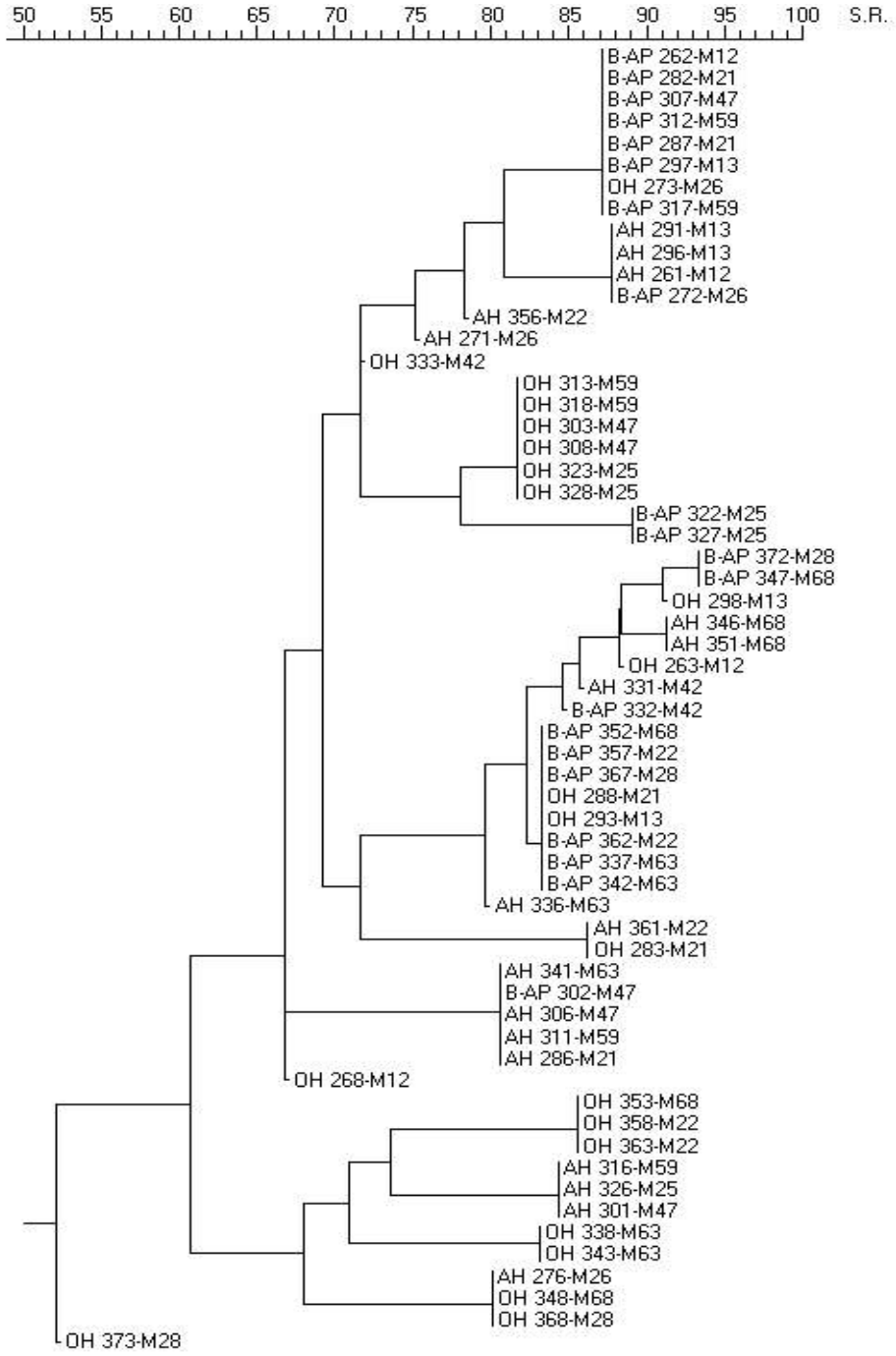
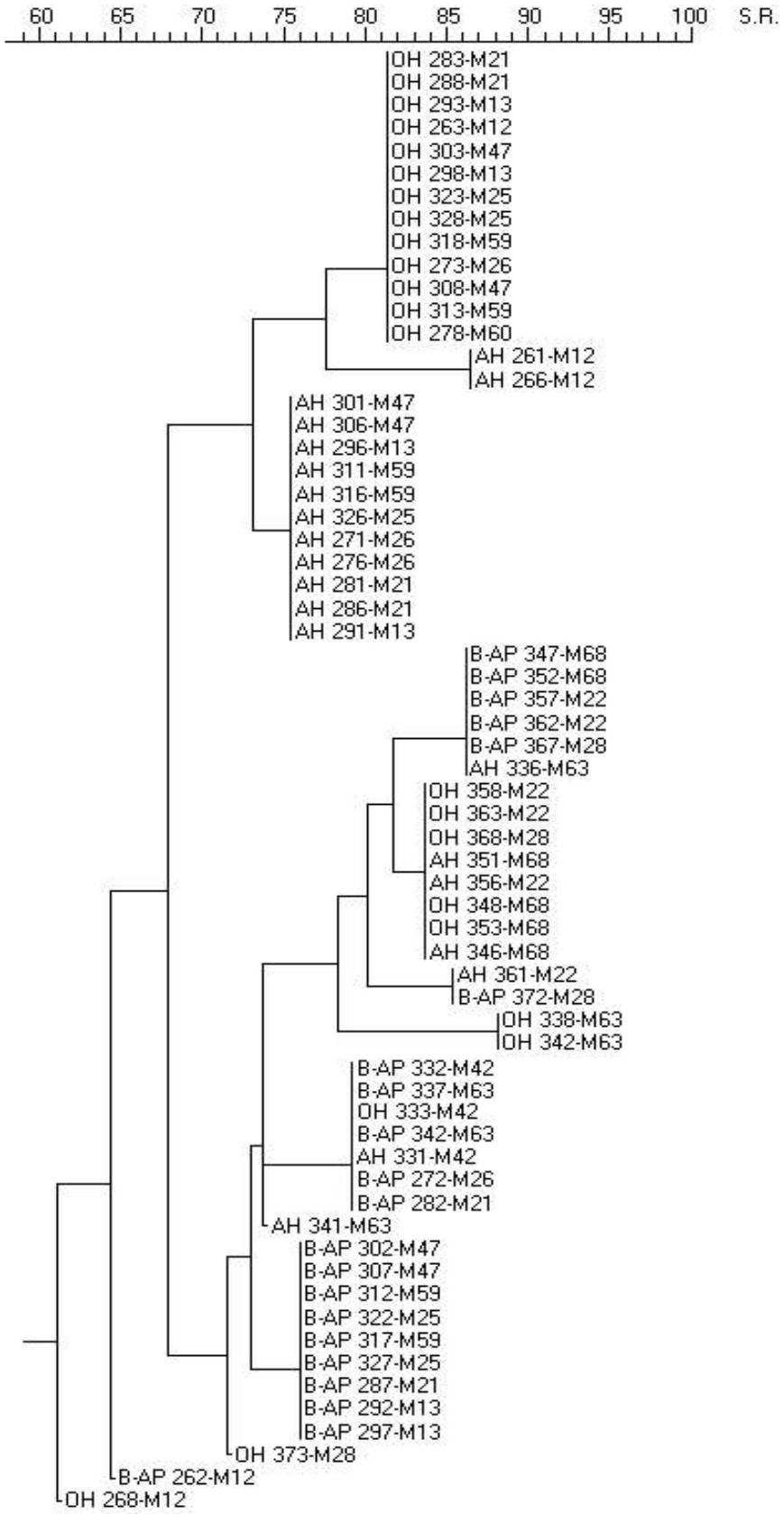


**Figure 2b** – Similarity rate of caecal microbiota.



Similarly, Fabre *et al.* (2006) reduced starch level up to 5% by increasing soluble and insoluble fibre (41% NDF) and observed that low starch high fibre diet reduced mortality in three farms (all medicated). In this experiment, the increase of level of fibre tended again to reduce dressing out performance. On the opposite, Volek *et al.* (2005) substituted starch by sugar beet pulp observed a trend to increase mortality. However, these authors reduced mortality when sugar beet pulp was supplemented with inulin, and related this result with the prebiotic property of inulin. This mixture increases the caecal concentration of VFA and decreased caecal pH, which was also observed by Castellini *et al.* (2007). These authors did not observe any change in the immune traits controlled. Finally, when there is no mortality, an increase of level of soluble fibre (obtained including apple pulp) reduced intake and growth rate, improving slightly feed efficiency (Álvarez *et al.*, 2006). This effect is similar to that observed with the inclusion of high levels of inclusion of sugar beet pulp (García *et al.*, 1993; Motta Ferreira *et al.*, 1996). Both the accumulation of digesta in the caecum (not observed in this work) as an hypothetic increment of intestinal viscosity (not determined but observed by Volek *et al.*, 2005) might explain the reduction of intake.

All these experiments seem to indicate the important role of dietary soluble fibre level (usually not controlled in formulation) in order to minimize the effects of ERE, as well as its balance with insoluble fibre. However, it is essential to study more in depth the way of action of soluble fibre, and especially its influence on intestinal microbiota, as Peters *et al.* (1995) related it to enterotoxaemia due to by *Clostridium spiroforme*.

In this way, these results might suggest that the microbiota in the different farms might be different (not determined in any farm) which could explain the different response of rabbits to the same feed depending on the farm. If this hypothesis is confirmed, it would imply that in farms affected by digestive diseases would be needed to formulate specific diets depending on the microbiota found in the affected farm. Anyway, it will be important to clarify either what is the reason for this apparently incoherent results, or if these effect might depend on the origin of soluble fibre.

Finally, in Figures 3 and 4 is included the cost of including soluble fibre in the diet, and in Table 4 are shown the last recommendations in carbohydrates for growing rabbits proposed by INRA and UPM.

**Figure 3** – Cost of increasing 1 g/kg of soluble fibre in a rabbit diet.

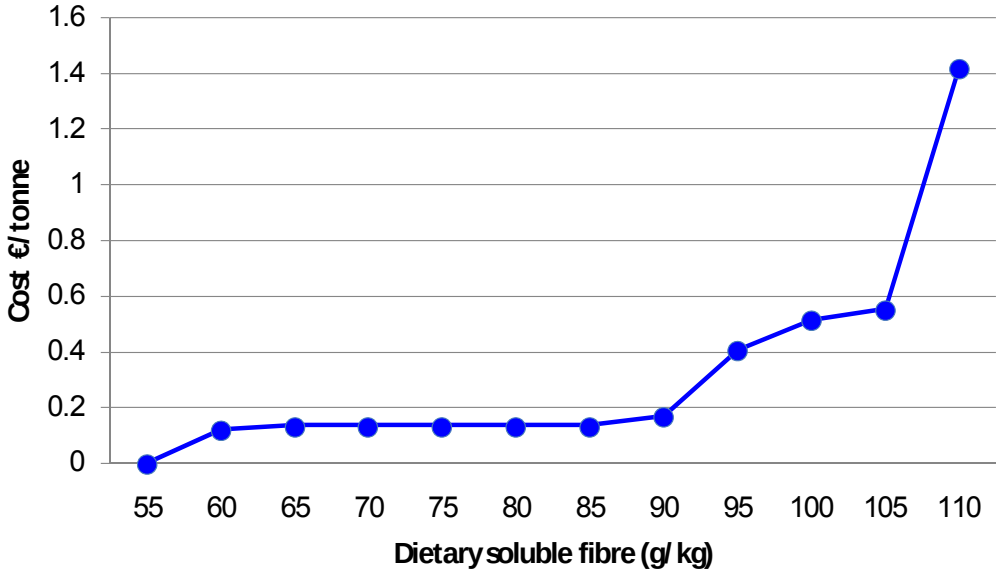


**Figure 4** – Effect of increasing 5% the most limiting nutrients in a rabbit diet formulated according to De Blas and Mateos (1998) and considering a basal level of soluble fibre of 9%.

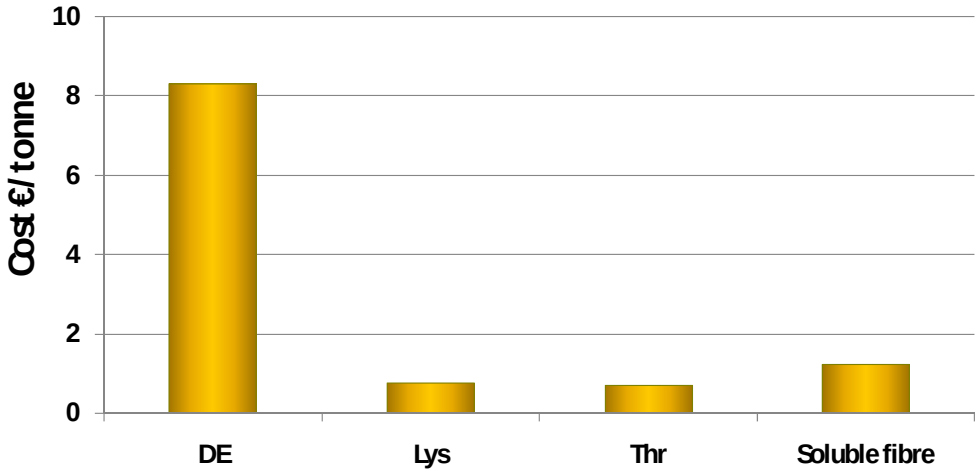



**Table 4** – Fibre and starch requirements for the young rabbit after weaning to prevent digestive troubles (Gidenne and García, 2007).

| *Unit*[1] | ***INRA*** | | ***UPM*** | |
|---|---|---|---|---|
| | Post weaning (28-42d old) | Growing (42-70d old) | Post weaning (25-39d old) | Growing (39-70d old) |
| NDF | ≥310 | ≥270 | 300≤NDF<360 | 320≤NDF<350 |
| ADF | ≥190 | ≥170 | – | 160≤NDF<185 |
| ADL | ≥55 | ≥50 | – | ≥55 |
| ADF-ADL (cellulose) | ≥130 | ≥110 | – | – |
| Lignin/cellulose | >0.40 | >0.40 | – | – |
| NDF-ADF (hemicellulose) | >120 | >100 | – | – |
| DgF[2]/ADF | ≤1.3 | ≤1.3 | – | – |
| 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)

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