DIETARY FIBRES: THEIR ANALYSIS IN ANIMAL FEEDING, AND THEIR ROLE IN RABBIT NUTRITION AND HEALTH

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ABSTRACT

Two centuries ago Heinrich Einhof developed the so-called Weende method (crude fibre), to first deals with the fibre content of the feeds for ruminants, and proposes to isolate a residue called the "crude fibre". Then, dietary fibre concepts evolve and differ in animal feeding compared to human nutrition and health. Animal nutritionists deal with various fibre sources, often from whole plants (forages, by products of seeds processing), and recover a larger range of polysaccharidic components, including other polymers, such polyphenolic (lignins, tannins) or polylipidic compounds (cutins). Dietary fibres are generally defined as polysaccharides and associated substances resistant to mammal enzyme digestion and absorption that can be partially or totally fermented in the gut. However, today this topic is still subjected to very active research, because of the complexity of the physical structure and chemical composition of the plant cell walls, and in the wide and different physiological effects of these different constituents. The importance of dietary fibre in animal feeding is due to its influence on rate of passage, mucosa functionality and its role as substrate for gut microbes performances and digestive health. This review will describe the definition and different structure of fibres and cell wall constituents and their analytical methods.

Key words: Dietary fibre, analysis, rabbit, nutrition, health

ABSTRAK

SERAT PAKAN: ANALISIS PAKAN TERNAK, DAN PERANAN DALAM NUTRISI DAN KESEHATAN KELINCI

Dua abad yang lalu Heinrich Einhof mengembangkan metode Weende (serat kasar), pertama kali berhubungan dengan kandungan serat dalam pakan untuk ruminansia, dan mengusulkan untuk mengisolasi residu yang disebut "serat kasar". Kemudian, konsep serat makanan berkembang yang dibedakan antara pakan hewan, nutrisi dan kesehatan manusia. Ahli nutrisi hewan meneliti berbagai sumber serat, yaitu dari seluruh tanaman (hijauan, produk samping dari pengolahan biji), dan berbagai komponen polisakarida, termasuk polimer lainnya, seperti polifenol (lignin, tanin) atau senyawa polilipida (*cutins*). Serat pakan umumnya didefinisikan sebagai polisakarida dan zat terkait yang tahan terhadap enzim pencernaan dan penyerapan mamalia yang sebagian atau seluruhnya difermentasi dalam usus. Topik ini masih dalam penelitian, karena kompleksitas struktur fisik dan komposisi kimia dinding sel tumbuhan dan efek fisiologis yang berbeda dari konstituen yang berbeda juga. Pentingnya serat dalam pakan hewan berpengaruh terhadap laju aliran pakan dan fungsi mukosa sebagai substrat untuk performans mikroba dan kesehatan pencernaan. Kajian ini menguraikan secara singkat definisi dan struktur kimiawi serat dan komponen dinding sel, serta beberapa metode analisis yang digunakan.

Kata kunci: Serat pakan, analisis, nutrisi, kelinci, kesehatan

INTRODUCTION

In animal feeding the dietary fibre concepts historically differ from those developed in human nutrition. For the latter, this is a rather modern concept, mainly developed in the sixties (Hipsley 1953) to deal with several pathologies (colo-rectal cancer obesity), regularly revisited (Trowell 1978; De Vries et al. 1999; Elleuch et al. 2011) and often restricted to the polysaccharides of the plant cell wall of the fruit and legumes. In contrast, animal nutritionists deal with other "less refined" fibre sources, often from whole plants (forages, by products of seeds processing, etc.) and recover a larger range of chemical components, including other polymers, such polyphenolic (lignins, tannins) or polylipidic compounds (cutins). Thus, two centuries ago Heinrich Einhof developed the so-called Weende method (in fact set up at Möglin in 1806, Germany, and not at Weende agronomy station) to isolate a "crude fibre" residue (Van Soest and McQueen 1973) to assess the nutritional value of ruminant feeds (forages and grasses). Over the years many systems of analysis have been proposed for the replacement of crude fibre,

but none have been successful in dislodging it as the official method, and is still used in animal feeding, e.g. for quality checking of fibre sources.

Now, these two conceptions are converging, and dietary fibres are generally defined as the polysaccharides and associated substances resistant to mammal enzyme digestion and absorption that can be partially or totally fermented in the gut. Champ et al. (2003) provide a concise synopsis of various views regarding the classification of dietary fibres. The overall tendency is towards an extension of the definition by including resistant starches as well as non-digestible oligosaccharides, and it was recently revisited by De Vries (2010) to reach an official enzymatic-gravimetric method that recover all the fibre components of the feed. Today this topic is still subjected to very active research, because of the complexity of the physical structure and chemical composition of the plant cell walls, and in the wide and different physiological effects of the different constituents. The importance of dietary fibres in animal feeding is due to its influence on rate of passage, mucosa functionality and its role as substrate for gut microbiota that relate to performances and digestive health (Montagne et al. 2003).

Our review will considere briefly the definition and structure of the different classes of fibres and of cell wall constituents, followed by a description of analytical methods routinely used for animal feeds. Secondly, the nutritional role and impact of fibres intake on digestive health will be described for the growing rabbit, since the adjustment of dietary fibres requirements are essential to reduce the risk of digestive trouble after weaning.

PLANT CELL WALL POLYMERS IN FEEDS: DEFINITION, ANALYSIS AND PHYSICOCHEMICAL PROPERTIES

Dietary fibres: a complex and evolving concept for one century

First, let us recall that the terms "cell wall" and "dietary fibres" refer to a common plant structure, and are often imprecisely used. The term "plant cell walls" should be employed when describing the structure of the plant cell, which is extremely complex, and not uniform: the type, size and shape of the wall are closely linked to the function of the cell within the plant (skeletal tissue, seeds, etc.). The plant cell walls consist of a series of polymers often associated and/or substituted with glycoproteins (extensin), phenolic compounds and acetic acid, together with, in some cells, the phenolic polymer (lignins). Cutin and silica are also found in the walls and/or in the middle lamella. A growing plant cell is gradually enveloped by

a primary wall that contains few cellulosic microfibrils and some non-cellulosic components such as pectic substances. When the plant matures, some cells develop a thick secondary cell wall consisting of cellulose embedded in a polysaccharide + lignin matrix (McDougall et al. 1996). Globally, the wall is formed of cellulose microfibrils (the backbone) embedded in a matrix of lignins, hemicelluloses, pectins and proteins (Figure 1).
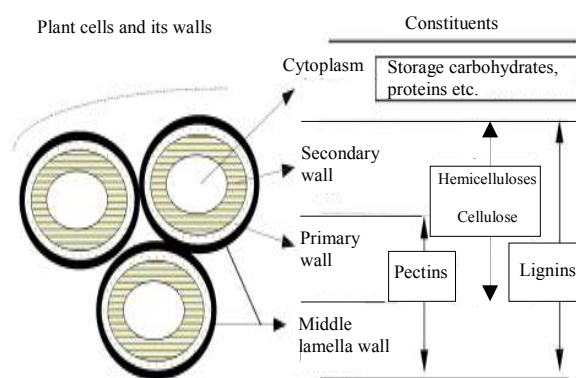
Figure 1. Organisation of the plant cell walls and their main constituents

The concept of dietary fibres is larger than the cell wall botanical definition, since in animal nutrition it includes not only the polysaccharides (cellulose, hemicelluloses, pectic substances, etc.) but also other components that are only fermented by the microbiota, such: oligosaccharides, gums, resistant starch, inulin, etc. According to their botanical origin, they may be associated with lignins and other non-carbohydrate components (e.g., polyphenols, waxes, saponins, cutin, phytates, resistant protein). Dietary fibres are often defined by nutritionists as the feed components resistant to mammal enzyme digestion and absorption, and that can be partially or totally fermented in the gut. This 'catch-all' definition thus includes resistant starch, oligosaccharides, fructans, or protein linked to cell wall, etc. (De Vries and Rader 2005). Another approximation is the dietary fibres for polygastric animals defined by Mertens (2003) as the indigestible or slowly digesting organic matter of feed that occupy space in the gastrointestinal tract, mainly insoluble fibres. It excludes rapidly fermenting and soluble carbohydrates (oligosaccharides, fructans), and thus seems not convenient for monogastric animals. Accordingly, depending of the feed classically used for one animal species or feeding system, the dietary fibres concept differs largely. An even broader definition may include synthetic non-digestible oligosaccharides (DP>3, fructo-oligosacharides, polydextrose). Each definition is convenient for its own paradigm, sourcing from the botanical origin of fibres that differed totally according to the final target for their physiological

effects: human (legumes, cereals, fruits), ruminant (forages, straws) or monogastric animal (brans or by-products of cereals or seeds). For the latter, we will detail the biochemical characteristics of the main sources of dietary fibre in the following section.

Biochemical characteristics of dietary fibres

Biochemical features of dietary fibres are one of the main factors responsible for variations in their physiological effects (digestion, etc.) and thus it is of importance to give a short description of them. However, the biochemical features of dietary fibres are highly variable, depending on many factors such as molecular weight, nature of monomers and types of linkages. With the exception of lignins, the cell wall constituents are predominantly polysaccharides composed of neutral and/or acidic sugars.

Giving their location in the plant cell, there are two main groups of dietary fibres components, (Figure 2): 1) The cell wall components with water-soluble non-starch polysaccharides (part of β-glucans, arabinoxylans, part of pectic substances) and the water-insoluble polymers including lignins, cellulose, hemicelluloses, and pectic substances. 2) The cytoplasm of the plant cell with water soluble and insoluble components, such as oligosaccharides (DP<15), fructans, resistant starch and mannans. The water solubility of polysaccharide is generally defined from solubility in hot water (80°C).

Water-soluble polysaccharides and oligo saccharides include several classes of molecules with a degree of polymerization ranging from about 15 to more than 2000 (β-glucans). Most of them are precipitate in ethanol solution (80% v/v), since they have a low degree of polymerisation (lower than 40). Examples include soluble hemicelluloses such as arabinoxylans (in wheat, oat and barley≈ 20-40 g/kg DM) and β-glucans (in barley or oat≈ 10-30 g/kg DM), oligosaccharides such as α-galactosides (in lupin, pea or soya seeds, 50-80 g/kg DM), and soluble pectic substances (pulps of fruits or beets, from 100 to 400 g/kg DM). Fructans are usually found in cereals such levans and some of them can be even considered as oligosaccharides. They have a high water solubility. Galactomannans occur specially in legume seeds. They are constituted of a backbone of mannose.

Linked in β[1→4] with side chains of galactose. The number of branching point is frequently high making them readily water soluble. Therefore, the analysis of water-soluble polysaccharides remains difficult, because of their highly variable structures, and no satisfactory method is at present available to determine precisely and routinely these compounds in animal feeds.
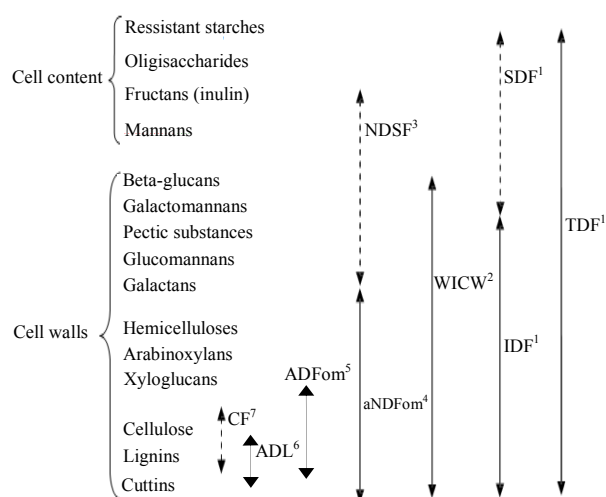
[1]Total dietary fibre (McCleary et al. 2010, AOAC 985.29 and 991.43, including resistant starch), soluble dietary fibre is determined by TDF - IDF or with AOAC 993.19 + 991.42 for IDF; [2]Water insoluble cell-wall (Carré and Brillouet 1989); [3]Neutral detergent soluble fibre (Hall et al. 1997); [4]Neutral detergent fibre assayed with a heat stable amylase and expressed free of ash; [5]Acid detergent fibre expressed free of ash; [6]Acid detergent lignin; [7]Crude fibre (AOAC 962.10)

Figure 2. Cell wall polysaccharides and their quantification by some gravimetric methods used in animal feed analysis

Pectic substances are a group of polysaccharides present in the middle lamellae and closely associated with the primary cell wall, especially in the primary cells (young tissues) of dicotyledonous plants, such as in legume seeds (40-140 g/kg DM in soybean, pea, faba bean, white lupin), and also in fruits and pulps. Pectic substances correspond to several classes of polymers, including pectins (rhamnogalacturonan backbone and side chains of arabinose and galactose or xylose) and neutral polysaccharides (arabinans, galactans, arabinogalactans) frequently associated with pectins. Their extraction requires the use of a chelating agent such as ammonium oxalate or ethylene diamine tetraacetic acid (EDTA). The latter is present in the solution for determining neutral detergent fibre (NDF) so pectins are not recovered in NDF analysis, as described below. Pectins of the middle lamellae serve as an adhesive in plant tissue, cementing plant cells together.

In the cell wall, the cellulose is the major structural polysaccharide and the most widespread polymer on earth. In contrast to hemicelluloses and pectins, cellulose is a homopolymer, formed from linear chains of β[1→4] linked D-glucopyranosyl units (whereas starch is formed of α[1→4] linked D-glucopyranosyl chains). The degree of polymerization (DP) is usually around 8,000-10,000. Individual glucan chains aggregate (hydrogen bonding) to form

micro-fibrils, and could serve as the backbone of the plant. Thus, cellulose is only soluble in strong acid solutions (i.e. 72% sulphuric acid) where it is hydrolysed. Quantitatively, cellulose represents 400-500 g/kg DM in hulls of legume and oilseeds, 100-300 g/kg DM in forages and beet pulps, 30-150 g/kg DM in oilseeds or legume seeds. Most cereal grains contain small quantities of cellulose (10-50 g/kg DM) except in oat (100 g/kg DM).

The hemicelluloses are a group of several polysaccharides, with a lower degree of polymerization than cellulose. They have a β[1→4] linked backbone of xylose, mannose or glucose residues that can form extensive hydrogen bonds with cellulose. Xyloglucans are the major hemicelluloses of primary cell wall in dicotyledonous plants (in vegetables, in seeds), whereas mixed linked glucans (β[1→3,4]) and arabinoxylans are the predominant hemicelluloses in cereals seeds (the latter two include partly water-soluble and water-insoluble polymers, described above). Hemicelluloses include other branched heteropolymers (units linked β[1→3], β[1→6], α[1→4] α[1→3]) such as highly branched arabinogalactans (in soybean), galactomannans (seeds of legumes), or glucomannans. Polymers formed of linear chains of pentose (linked β[1→4]) such as xylans (in secondary walls), or hexose such as mannans (in palm kernel meal) are also considered as hemicelluloses. Pentosans such as xylans and arabinoxylans are soluble in weak basic solutions (5-10%), or in hot dilute acids (5% sulphuric acid). Hexosans such as mannans, glucomannans or galactans can only be dissolved in strong basic solutions (17-24%). Quantitatively, hemicelluloses (estimated by difference between NDF and ADF) constitute 100-250 g/kg of the DM in forages and agro-industrial by-products (brans, oilseeds and legume seeds, hulls and pulps) and about 20-120 g/kg DM of grains and roots.

Lignins are polyphenolic compounds of the cell wall. It can be described as very branched and complex three-dimensional network (high molecular weight), built up from three phenylpropane units (conyferilic, coumarilic and sinapyilic acid). Lignins network tend to fix the other polymers in place, exclude water and make the cell wall more rigid and resistant to various agents, such as bacterial enzymes. Most concentrate feeds and young forages contain less than 50 g lignin/kg. The degree of lignification of the plant cell wall may reach 120 g/kg with ageing in forages, or up to 590 g/kg in grape seed meal.

Other constituents are also present in cell walls, but frequently in smaller quantities. Minerals, such as silica, are essentially in graminaceous leaves. Phenolic acids are chemically linked to hemicelluloses and lignins in graminaceous plants. Some proteins are linked to cell walls through intermolecular bonds from amino acids such as tyrosine, and thus resist standard extractions. In addition, plant epidermal cells may be covered by a complex lipid (cutin for aerial parts, suberin for underground structures) which could encrust and embed the cell walls, making them impermeable to water. Other phenolic compounds can also be mentioned, i.e. condensed tannins, which may exist in higher plants. They form cross-linkages with protein and other molecules. They could be included in the sum of indigestible polysaccharides+lignins. However, condensed tannins, lignins and indigestible proteins are closely related because indigestible complexes of these substances are common in plants (Van Soest 1994).

Analysis of the fibre fractions in animal feeds

Because of the wide diversity of plant cells types, and accordingly of cell walls, that constitutes the different plant tissues, it implies that the analysis of the different fibres fractions could be only approached by a combination of procedures. In animal feeding, these procedures are essentially based on gravimetric method, i.e. weighing a residue after hydrolysis of specific cell components. The fractionation procedures thus varied and were developed according to the material tested. There is no global method used, and the choice of the method to investigate fibres in feed depends on the composition of each particular dietary fibre fraction. Detailed reviews have been published on this subject (Hall 2003; Mertens 2003; De Vries and Rader 2005). The methods mentioned in the Figure 2 describe techniques of fractionation that are sufficiently precise and pertinent in a "routine" laboratory in charge to control the quality of the feed sources and to give values of fibre parameters for implementing the databases for feed formulation.

Crude fibre and fibre fractionation with the Van-Soest procedures

Initially, the crude fibre method (AOAC, 2000: official method 962.10) must be mentioned because it is highly reproducible, quick, simple, cheap and frequently used all over the world. This technique extracts one fibrous residue after an acidic followed by a basic hydrolysis. The main drawback of crude fibre 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% pentosans and 95% lignins. For these reasons, this criterion is not able to explain the physiological effects exerted by most of the fibres sources on the animal digestive physiology.

But, within a raw material this criteria is very useful to verify the fibre content compared to tables.

The main alternative to crude fibre is the sequential procedure of Van Soest developed in 1967 and successively updated (Mertens 2003). The neutral detergent fibre (NDF) method was designed to isolate insoluble dietary fibre components in plant cell walls by using a hot neutral detergent solution: cellulose, hemicelluloses, and lignins (Mertens 2003), as pectin substances are partially solubilised. This method is criticized due to its variability among laboratories, especially when it is compared with the results obtained with other feed constituents. It is partially due to the different procedures that can be used to perform it (with heat-stable amylase and/or sodium sulphite or not, ash free or not), but usually described with the same reference (Uden et al. 2005). The acid detergent fibre (ADF; AOAC, official method 973.18) method isolates cellulose and lignins, the worst digested fibrous fractions, by a hot acid detergent solution. For complex feed (such for monogastrics), it is designed to be done after NDF analysis, as when it is performed directly also retain pectins. As crude fibre, it was used to predict dietary energy value for some species, such pigs or rabbits (Wiseman et al. 1992). Finally, it can be obtained acid detergent lignin (ADL; Robertson and Van Soest 1981) which isolate lignin fraction by using a strong acid solution at room temperature. The main advantages of this sequential methodology are that is possible to obtain an approximate estimation of lignin (ADL), cellulose (ADF-ADL) and hemicelluloses (NDF-ADF) content, and that it is relatively quick, simple, economical, have an acceptable reproducibility when used a standardized methodology (Egran 2001) and improves the fractionation of the cell wall.

These methods have been complemented by the estimation of the fibres dissolved by the neutral detergent solution (NDSF: neutral detergent soluble fibre; Hall et al. (1997) that mainly includes fructans, galactans, β-glucans, and pectic substances. The NDSF is obtained gravimetrically as the difference between ethanol/water insoluble residue and starch and NDF after correction for protein and ash. Therefore, the NDSF measurement may be affected by the accumulation of errors in the measurement of the different components, as well as the error linked to the value used for protein correction (Hall 2003). Now, the determination of NDSF is not adapted for routine analysis in animal feeding.

The concepts of water-insoluble cell wall, TDF and soluble dietary fibres

In parallel to the difficulties to estimate the water-soluble polysaccharides, the concept of dietary fibres has emerged, first in human nutrition, and now is extended to other mammals (Trowell 1978; De Vries 2010), and assayed in the feeding of monogastric animals such the rabbits, because of high dietary fibres contents (>50%). For instance, in poultry feeding, the concept of water insoluble cell wall "WICW" (Figure 2) was developed to predict simply (with one single criterion) and precisely the metabolisable energy content of a feed (Carré 1990). WICW is a criterion obtained through a simple enzymatic-gravimetric procedure. It corresponds to lignins and polysaccharides that are water- insoluble (Carré and Brillouet 1989) and not digested by poultry.

As the important nutritional distinctions between insoluble and soluble dietary fibre emerged, AOAC official Method 985.29 was modified to allow the isolation and quantification of the insoluble and soluble dietary fibre fractions. The distinction between the two fibre fractions is somewhat arbitrary, and based on the solubility of the soluble fraction in a pH-controlled enzyme solution (as in the human alimentary system). The *de facto* defining method depends on the soluble fibre being precipitated in a mixture of one volume of aqueous enzyme solution, and four volumes of 95% ethanol, a solution long used by analytical chemists to separate complex (high DP) from simple molecules (DP<15). While this is the case in the method, the dietary definition *per se* does not imply insolubility or precipitation in aqueous ethanol as a requirement. The modified methodology was validated by collaborative study and adopted as Official Method 991.42 (Insoluble Dietary Fibber in Food and Food Products). Later, in 1993, Official Method 993.19 was adopted to determine Soluble Dietary Fibber (Figure 2). This occurred after practical experience and improvements in techniques allowed the quantification of soluble fibre directly (see details further) as opposed to determining soluble dietary fibre as the difference between total dietary fibre (985.29) and insoluble dietary fibre (991.42). Method 993.19 treats the filtrate of 991.42 with 4 parts alcohol to precipitate the soluble dietary fibre, which is then isolated and quantified gravimetrically.

Currently, total dietary fibre (TDF) is primarily analysed by enzymatic-gravimetric methods (Table 1) based on AOAC procedures 985.29 and 991.43 that solubilise the different fibres fractions with enzymes and solvents and measure the weight of residues after these treatments (as reviewed by Bach Knudsen 2001; De Vries 2010; Elleuch et al. 2011). Recently, these procedures (Figure 2) have been updated to include also non-digestible oligosaccharides and resistant starch (McCleary et al. 2010). Insoluble dietary fibre (IDF) could be quantified by the above mentioned AOAC method for TDF, by avoiding the recovery of water-soluble structural polysaccharides (AOAC

991.42). IDF should correspond to polysaccharides that are slowly hydrolysed ad fermented in the gut: i.e. mostly lignins (indigestible) hemicelluloses and cellulose. Reversely, IDF should not include "soluble" polysaccharides which are rapidly fermented (e.g. pectins, beta-glucans), and highly digested (at similar levels compare to starch or proteins).

When calculating the difference between the residue TDF and any measurement of "insoluble fibre" (NDF, WICW) you can estimate this "soluble" fibre fraction content (SDF). According to Van Soest et al. (1991), "soluble fibre" may be obtained by subtracting the content of NDF (after corrections for ash and protein) from the TDF value, thus including non-starch polysaccharides, i.e. fructans, galactans, β-glucans and pectins. One of the problem for calculating a difference between two methods (e.g. TDF and NDF) is that for some raw materials we obtained negative values (such for sunflower meals, Table 1). Soluble fibre content may also be calculated by difference as: organic matter - (protein + fat + soluble sugars + starch + NDF).

As mentioned above, soluble fibre content of a feed can be determined directly according to the AOAC Prosky enzymatic-gravimetric procedure (Prosky et al. 1992; AOAC 2000; Megazyme Ltd 2005; AOAC 993.19, used in conjunction with 991.42 for insoluble dietary fibre), the carbohydrates are solubilised in phosphate buffer or MES (4-morpholine-ethanoesulfonic acid)/TRIS buffer, α-glucans are hydrolyzed by amyloglucosidase, insoluble fibre is separated by filtration, solubilised dietary fibre is precipitated with ethanol solution from the solvent extract and measured gravimetrically after correction for protein and ash contents. Inaccuracies in the SDF determination may arise by the partial degradation of carbohydrates, the incomplete extraction and/or precipitation with the addition of ethanol, the interference by other substances, and differences in the nature of the analysed feed (Theander 1995; Hall et al. 1997).

Besides, let us recall that for a biochemist the solubility of polysaccharide is related to its structure; they can be set regularly (insoluble) or irregularly (soluble) on the backbone or as side chains. For example, the presence of a substitution group such as COOH increases solubility. But since, the soluble and insoluble nature of dietary fibres involves differences in their technological functionality and physiological effects the terms "soluble" is frequently indifferently used for biochemical or physiological properties, and this provide some confusion for non advertised readers.

Other approaches for cell wall polysaccharides analysis

Another approach to estimate dietary fibre is to analyse the non starch polysaccharides (NSP) and lignins. There are several methods available to estimate total, soluble and insoluble NSP (Bach Knudsen 2001; De Vries and Rader 2005), where the no fibrous components are extracted by solubilisation, by enzymatic hydrolysis or by combining both procedures. Once isolated, fibre residue can be quantified gravimetrically or chemically (hydrolyzing the residue and determining its single constituents: sugars and lignins). 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 lignins) and separated into insoluble and soluble fibre (in aqueous solution), and obtain its monosaccharide composition. The combination of the monosaccharide composition of fibres with additional chemical information may allow describing better fibres 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.

Conclusions about fibres analysis in animal feeds

The determination of the fibres content of a compound feed or a raw material is highly variable, depending on the analytical method of estimation. The choice of which definition is to be used by the nutritionist thus depends on the type of information required (to relate to digestive processes, to predict the nutritive value). They can be determined using sophisticated extraction techniques, and examples of their concentration in some feedstuffs are given in the Table 1.

Finally, the enzymatic-gravimetric determination using the Van-Soest procedures is still (NDF, ADF, ADL) the simplest, low-cost, rapid and reproducible method, for analysing the fibres fractions that are slowly digested in the gut. Now, to examine the effectsof the highly digested fractions of the dietary fibres (water insoluble pectins, β-glucans, water soluble pectins, oligosaccharides) new criteria are assayed. One

Table 1. Cell-wall constituents (% DM) according to several methods of analysis in some raw materials used in rabbit feeds

Ingredients	Wheat straw	Wheat bran	Dehydrated alfalfa	Sugar-beet pulp	Sunflower meal	Soybean hulls	Grape pomace
aNDFom[a]	80.0	45.0	46.0	47	48	62	64
ADFom[b]	54.0	11.0	34.0	22	32	44	54
ADL[c]	16.0	3.0	8.0	2	11	2	34
NDSF[d]	-	3.0	18.0	30	-	22	-
Crude fibre[e]	40.0	10.0	27.0	19	26	36	26
WICW[f]	84.0	45.0	47.0	58	39	72	69
WIP[g]	2.2	2.9	7.6	27	8	10	8
SNSP[h]	1.0	3.0	3.0	10	1	2	1
INSP[i]	55.0	36.0	33.0	64	26	55	36
TDF[μ]	85.0	46.0	48.0	68	41	-	72
IDF[μ]	82.0	45.0	42.0	55	37	68	
Rhamnose	<1.0	<1.0	<1.0	11	<1	11	<1
Arabinose	2.0	8.0	2.0	18	3	4	<1
Xylose	18.0	16.0	6.0	2	5	7	8
Mannose	<1.0	<1.0	<1.0	1	1	6	2
Galactose	<1.0	<1.0	<1.0	4	1	2	2
Glucose	33.0	9.0	19.0	19	11	29	19
Uronic acids	2.0	2.0	7.0	18	5	6	5
Crude protein	3.0	15.0	16.0	9	34	11	13

[a]Neutral detergent fibre assayed with a heat stable amylase and expressed free of ash; [b]Acid detergent fibre expressed free of ash; [c]Acid detergent lignin (Van Soest et al. 1991); [d]Neutral detergent soluble fibre (Hall et al. 1997; Hall 2003); [e]According to the Weende method (AOAC 2000: official method 962.10); [f]Water-insoluble cell wall, including lignins (Carré and Brillouet 1989); [g]Water-insoluble pectins (uronic acids + neutral sugars of pectins insoluble in hot water); [h]Water-soluble non-starch polysaccharides (Brillouet et al. 1988; Englyst 1989); [i]Insoluble non-starch polysaccharides, not including the lignins, determined by direct monomeric analysis of cell wall polysaccharides (Englyst 1989; Barry et al. 1990); [μ]McCleary et al. (2010).

approach is to estimate this "soluble" fraction by difference, from TDF and another criterion for insoluble fibre (NDF). Although these "soluble" fibres fractions remain hard to analyse in feed, their effects on the digestive physiology of the animal are presently subjected to many researches, and results are summarised for the rabbit in the following section.

DIETARY FIBRES FOR THE GROWING RABBIT: ROLE IN FEEDING, NUTRITION AND DIGESTIVE HEALTH

Plant polymers are the major fraction in rabbit diets and accounts classically for at least 40-50% (Table 2). The importance of fibres is due to its influence on intake, rate of passage and its role as substrate for microbiota. But, for the growing rabbit one of the main challenges is to provide fibre recommendation for digestive troubles prevention without large impairment of performances (growth, feed efficiency).

The concepts of dietary fibres, fibres quantification and characterisation of the different fractions are still largely discussed. This promoted changes in fibres recommendations for the growing rabbit that may differ among the research teams and according to the fibre criteria taken into account (De Blas et al. 1981; Gidenne 2003; Gidenne et al. 2010a; Trocino et al. 2013).

Dietary fibres level and intake regulation of the growing rabbit

One of the main dietary components implicated in feed intake regulation, after weaning, is the digestible energy (DE) concentration. The domestic rabbit (fed a pelleted balanced diet) is able to regulate its DE intake (and thus its growth) when the dietary DE

concentration is between 9 and 11.5 MJ/kg (Figure 3) Gidenne et al. (2010b). But a higher correlation is obtained with the lignocellulose level of the diet, and when the dietary fibres level is between 10 and 25% ADF (Acid Detergent Fibre). However, the incorporation of fat in the diets, while maintaining the dietary fibres level, increases the dietary DE level, but leads to a slight reduction of the intake.

Table 2. Fibres levels and other main constituents in commercial pelleted feeds used for the growing rabbit in conventional breeding

Chemical criteria	Mean range (g/kg as fed)
Total dietary fibre[2] (TDF)	450-600
Neutral detergent fibre (aNDFom)	280-460
Acid detergent fibre (ADFom)	150-230
Acid detergent lignin (ADL)	35-65
Crude fibre	120-180
Soluble fibre[1]	35-120
Starch	80-130
Sugars	30-60
Crude protein	140-190
Ether extract	20-40

[1]Calculated as: OM-CP-EE-aNDFom-Starch-Sugars;
[2]McCleary et al. (2010)

Finally, the voluntary feed intake is more related to the dietary ADF level because of the low digestion of this fraction, and probably because the ADF level also corresponds to a "ballast" value that limits the intake. For instance, the replacement of starch" by digestible fibres fractions (hemicelluloses or pectins), without changing the ADF level, did not greatly affect the intake (Perez et al. 2000; Gidenne et al. 2004b). Further researches are required to assay the effects of other fibres fractions such the most "soluble" ones, on the intake behaviour.

In return, when the dietary fibre level is very high (>25% ADF), the animal cannot increase its intake sufficiently to meet its energetic needs, thus leading to a lower growth rate, but without digestive problems.

The digestion of fibres in the rabbit: a main source of energy from the microbiota activity

Polysaccharides of the cell wall are hydrolysed and then fermented only by bacterial enzymes. Accordingly in monogastric mammals, the fibres became energy source from the activity of the microbiota that takes place mainly in the large intestine: caecum and proximal colon for the rabbit. However, the extent of the fibre digestion is rather different according to the fraction (Table 3), ranging

from 10% for cellulose to 90% for the most soluble fibres fractions (TDF-NDF, Trocino et al. 2013). Obviously, the fibre digestion is lower than that of protein or starch, and increasing the dietary fibres level led to reduction in the digestive efficiency.

For the adult rabbit, fed a high fibre diet, the energy provided by the caecal VFA absorption could represent up to 50% of the maintenance energy (Marty and Vernay 1984; Gidenne 1994). But, increasing the fibres intake (and lowering that of starch) either increase or has no effect on the fibrolytic activity and caecal VFA concentration (ranging from 80 to 100 mM), while a lower butyrate molar proportion is generally observed. Since the fibre digestibility is frequently not affected by the dietary fibre concentration, it may be assumed that the quantity of fibre entering the caecum is not a limiting factor for the fermentation processes, as the digesta retention time in the caecum is relatively short, allowing, predominantly, degradation of the more easily digestible fibre fractions such as pectins or hemicelluloses.

DFI: daily feed intake measured between weaning (4 weeks) and 11 weeks of age
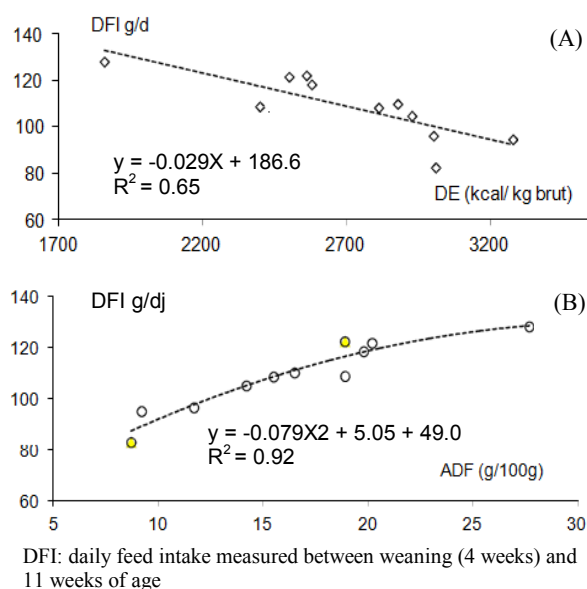
Figure 3. Voluntary feed intake of the rabbit, after weaning, according to the digestible energy (DE) (A) or to the ADF concentration (B) of a pelleted feed

Table 3. Digestibility coefficients (%) for some dietary fibres fractions, in the growing rabbit

Dietary fibre criteria	Mean range (%)
Neutral detergent fibre (aNDFom)	10-60
Cellulose (ADFom-ADL)	5-25
Hemicelluloses (aNDFom-ADF)	20-60
Water insoluble Pectins	30-80
Uronic acids	30-85
Soluble fibre (TDF-aNDFom)	70-90
Lignin (ADL)	-15-15

The quality of fibres, particularly their fermentability, modulates the microbial activity. For instance, increasing the levels of pectins through the incorporation of beet pulps in a diet increases the VFA concentration in the caecum. In a collaborative study, Garcia et al. (2002) reported that caecal VFA level decreases with the degree of lignification of NDF, and that dietary uronic acids concentration (provided mainly by pulps) is positively correlated to the caecal VFA and pH. In association to changes in microbial activity, it is suspected that dietary fibres supply would be able to modulate the microbiota balance and diversity, as suggested by Combes et al. (2013).

But, the extent of the fibre digestion is ultimately determined by the time necessary for microbiota to hydrolyse and ferment the polysaccharides. Because the retention time in the caeco-colic segment of the rabbit is relatively short (8-12h, Gidenne 1997), only the most rapidly fermentable cell-wall polysaccharides are highly digested (pectins, soluble fibres fractions), whereas lignocellulose is degraded at a smaller extent. For instance, when wheat bran and beet pulp replaced starch (with constant level of ADF), the whole tract digestibility of the diet was not reduced (Gidenne and Bellier 2000; Gidenne and Perez 2000). The utilisation for growth of these fibres fractions is particularly high and comparable to that of starch, since the replacement in a complete diet of 10 points of starch by hemicelluloses (NDF-ADF) and pectins do not affect the feed efficiency in the growing rabbit (Gidenne and Perez 2000).

However, it must be stated that for some diets, the level of digestible cellulose is higher than that of digestible hemicelluloses. Lignins and cutin are considered almost totally undegradable, although positive values for lignins digestibility have been obtained, which might indicate a solubilisation rather than digestion. In rabbit feeding, the two main raw materials that increase the digestible hemicelluloses level in the diet are sugar beet pulp (low lignified and with a high hemicelluloses/cellulose ratio, 1.1 compared to alfalfa, 0.4) and wheat bran (with the highest hemicelluloses/cellulose ratio, 3.2). Uronic acids, an important constituent of pectins are more soluble than other cell wall components and are the substrate more easily fermented. It would suggest that other components of soluble fibres (pentosans, mannans, galactans, etc.) might have a similar or even higher degradability than uronic acids.

While the fibres are mainly degraded in the large intestine, there are some evidences that some components of structural carbohydrates are degraded prior to entering the caecum of rabbits. This has also been observed in other non-ruminant species such as pigs and poultry. The extent of precaecal fibres digestion in rabbits varies from 5 to 43% for NDF (Gidenne and Ruckebusch 1989; Merino and Carabaño 1992) and from 0 to 37% for NSP (Gidenne 1992; Carabaño et al. 2001). It must be pointed out that the values obtained using NDF respect to those obtained with NSP might be overestimated due to solubilisation and filtration of cell wall components which would be considered digested. When NSP were analyzed, arabinose and uronic acids, typical monomers of pectic substances, were largely digested before the ileum (from 0.2 to 0.5). On the opposite, glucose and xylose, the major monomers in most fibres sources, showed a much lower ileal digestibility (0-0.2). These results imply that around 0.4 (from 0.2 to 0.8) of total digestible fibre (including water-soluble NSP) is degraded before the caecum, which is similar to that observed in pigs (Bach Knudsen 2001). It could be explained from the caecotrophy practice of the rabbit: soft faeces very rich in live microbiota are daily ingested and thus would provide fibrolytic enzymes, that have been observed in the stomach and small intestine (Marounek et al. 1995).

Dietary fibres impact on hindgut ecosystem of the rabbit

Most of the effects exerted by fibres on the rabbit digestive physiology depend on their hydrolysis and fermentation by the digestive microbiota. However, it is difficult to study the influence of any dietary component on microbiota, as the traditional cultivation techniques allow to work with around one fourth of the intestinal microbiota. For this reason, other indirect techniques have been used, as the volatile fatty acid concentration, the microbial nitrogen synthesized or the fibrolytic activity. Caecal microbial population secretes enzymes capable of hydrolyzing the main components of dietary fibres. Greater enzymatic activity for degrading pectins and hemicelluloses than for degrading cellulose has been detected in several studies (Marounek et al. 1995; Jehl and Gidenne 1996). These results are parallel to faecal digestibility of the corresponding dietary fibres constituents in rabbits (Table 3), and are also consistent with the smaller counts of cellulolytic bacteria in the rabbit caecum compared with xylanolytic or pectinolytic bacteria (Boulahrouf et al. 1991).

The caecal VFA profile is specific to the rabbit, with a predominance of acetate (77 mmol 100 ml-1 as average, and ranging from 65-87) followed by butyrate (17 mmol 100 ml-1 as average, and ranging from 6-28) and then by propionate (6 mmol 100 ml-1 as average, and ranging from 3-11). These molar proportions are affected by fibres levels. For instance, the proportion of acetate increases and that of butyrate generally decreases significantly when fibres levels increase, whereas propionic acid proportion was only positively

correlated to dietary uronic acids concentration (Garcia et al. 2002).

However, these indirect methods in many circumstances do not seem to reflect adequately the changes produced in the microbiota population. The development of new molecular tools to characterize intestinal microbiota is improving our knowledge about nutrition and digestive microbiota functions in relation to the fibres intake. For instance, the caecal microbiota is able to adapt very quickly (within one weeks) to a change in the dietary fibres levels (Michelland et al. 2011). Further studies are presently conducted using high throughput sequencing of the 16S rDNA and would provide new data about the relationship between microbiota and dietary fibres (Combes et al. 2013).

Dietary fibre and digestive health of the growing rabbit

Among the various health troubles, the intestinal pathology along with respiratory diseases is 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 reproducing female. In France, digestive pathology of the growing rabbit induced a mortality rate of 8-12%, even with antibiotherapy strategies. Moreover, digestive disorders are responsible for important morbidity characterized by growth depression and bad feed conversion, and constitute a priority problem to be solved. Till the eighty's , only the crude fibre criterion was used to define the fibre requirements for the growing rabbit, and the value ranges from 6 to 18% according to the authors. Consequently, the precise assessment of the fibre requirements with more "adequate" criteria is essential to reach a low risk of digestive troubles without a too large impairment of the growth and feed efficiency.

Assessment of the digestive health of the growing rabbit

The "digestive health" is preserved when the animal maintains its intestinal "balance", in response to various factors such nutrients intake or exogenous micro-organisms. If the intestinal balance is not maintained, therefore troubles could appear, such diarrhoea in the young mammal (piglet, rabbit around the weaning period), either because of gut colonisation by an identified pathogen (e.g. *E. coli*) or from a multifactorial origin.

However, within a group of growing rabbits, animals differently developed the clinical symptoms (diarrhoea, impaction) and all sick animals do not die. Several mechanisms of defence could explain the

variability in the disease sensibility such: the gut barrier function and the competitive exclusion between saprophyte and pathogen bacteria, the immune status. Nutrition and feeding strategies also plays an important role for digestive health, in supplying the adequate nutrients quantity and quality, to improve: 1) Mucosa integrity and immune response (avoiding pathogen attachment and colonisation), 2) The growth/stability of the commensal microbiota (barrier effect).

To develop accurate nutritional strategies it is necessary to identify the specific nutrients or bioactive components in feeds (or milk) that enhance these mechanisms of defence. These nutritional strategies must be focused around the weaning period, since it is a critical phase for sensibility to digestive diseases, probably linked to the processes of digestive maturation, including the development of microbiota and immune system.

The classical indicator to evaluate the impact of a disease in groups of young domestic mammals is the mortality rate, but it appears too restrictive. Thus, morbidity indicator was developed to assess more precisely the sanitary status of the rabbit, by including the incidence of the clinical symptoms (Gidenne 1997). 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 of a group of animals.

But, these traits show large variations according to many factors. For instance, HRi of rabbits fed the same diet could range from 0 up to 40% according to various factors, such: litter effect, preventive medication, age at weaning. Thus it means that a large number of animals is required to detect a significant difference in mortality or HRi between two treatments. For instance, to detect a significant 5% deviation among two mortality rates, more than 300 animals are required in each group.

If the clinical symptoms are clear (diarrhoea, caecal impaction, stomachal borborigmus) the morbidity rate is relatively easy to measure, but depends of the frequency of the measurements within a time period. For instance, if the morbidity is checked daily, the measure is more precise and give a higher value compared to a weekly control (Bennegadi et al. 2001). Moreover, when only a reduction of growth rate is detectable, a threshold must be defined to class the animal as morbid or not, such the average minus 2×standard deviation (signifying the 2.5% of the animals with the lowest 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 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.

Relevance of fibre intake compared to starch: effect on rabbit digestive health

Many experiments have been performed to elicit the respective effects of fibre and starch on the incidence of diarrhoea in the growing rabbit, but most of them compared variations of the fibre: starch ratio, since in complete feed formulation, one nutrient is substituted by another one. Consequently, when a study reported a positive effect of an increased dietary fibre intake on digestive health, it was in fact difficult to exclude also an effect of a reduced starch intake.

There are two opposite hypothesis: are digestive troubles linked to a carbohydrate overload in the caecum? or linked to a fibre deficiency? (or both?). This question was elicited by studying the ileal flow of starch and fibre in the growing rabbit (5-9 weeks old). With high starch diets (30% starch mainly from wheat) the ileal starch digestibility was very high (>97%), the flow of starch remained under 2 g/d (intake β 30 g/d) at ileum, while that of fibre was at least 10 times higher (β 20 g NDF/d) (Gidenne et al. 2000). 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 units (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. 2005) observed no effect of starch ileal flow on diarrhoea incidence in the weaned rabbit. Fibre intake plays thus a major role in the determinism of digestive trouble in the classically weaned rabbit (28-35d old).

Thus in France, the GEC group has perform several large-scale studies to validate clearly the relationships among dietary fibre fractions and digestive health for the "classically" weaned rabbit, using experimental design with a high number of animals per treatment (over 300 animals per treatment and 4 to 6 experimental sites). The relevance of the Van-Soest criteria was studied, since crude fibre was too imprecise for this purpose.

Major role of the cellulose and lignins intake: impact of quantity and quality of the lignocellulose (ADF)

The favourable effect of dietary lignocellulose (ADF) supply on the frequency of the digestive disorders and mortality in fattening rabbits was first shown by (Maître et al. 1990) using a large scale experimental design (380 rab/diet, in 5 sites): from 15 to 21% d'ADF the mortality decreased linearly from 14 to 7%. The impact of ADF on mortality reduction after weaning was then confirmed by Perez et al. (1994). The relationship between low fibre diets (<14% ADF) and a higher incidence of diarrhoea was also clearly established in two studies where the quality of fibre, e.g. the proportions of fibre fractions as analysed through the Van-Soest procedure, has been controlled (Blas et al. 1994; Bennegadi et al. 2001).

However, within a classical dietary ADF range (15-22%, see the dotted circle in Figure 4) the mortality rate varied greatly, even if within one study the ADF globally reduced the risk of mortality.

Nevertheless, the measurement of the health risk index (HRi = mortality + morbidity) is more precise to determine the sanitary status of a rabbit group. A similar trend is obtained for the relationship between the HRi and dietary ADF (Figure 5), but only 41% of the variations of HRi are explained by those of ADF. This also explains why past fibre recommendations with the crude fibre criteria only, also vary greatly from 6 to 18% according to authors.

The favourable effect of supplying lignocellulose was also shown in the young during the weaning period (3 to 5 weeks old) by Fortun-Lamothe et al. (2005) with a large-scale study (six sites + three reproductive cycles). They reported a lower mortality rate for litters fed a diet rich in fibre or when fibre + lipids replacedstarch, but after weaning no favourable effect of dietary ADF concentration was found on mortality.

Thus a single criterion, such as the supply of lignocellulose is not sufficient enough to relate the fibre supply and the "level of security" of a feed for the growing rabbit. A first step, is to determine if, apart from the quantity of lignocellulose, the quality of the ADF, i.e. the respective effects of lignins and cellulose (according to the Van-Soest procedure) could have an impact on digestive health.

The nutritional role of the lignins and the cellulose were addressed in two successive studies (Figure 6). The effects of the lignins was first studied (Gidenne and Perez 1994; Perez et al. 1994). Increasing the intake of lignins (criterion ADL: Acid Detergent Lignin) involves a sharp reduction of the feed

digestibility (Figure 4, slope = -1.6), associated to a reduction of the digesta retention time in the whole tract (-20%), and with a rise of the feed conversion ratio. For the latter, the botanical origin of lignins seems to modulate the effects observed. In parallel, a linear relationship (R^2 = 0.99; Figure 5, n = 5 feeds) between ADL and mortality by diarrhoea was outlined for the first time (without major effect of the botanical origin of lignins).
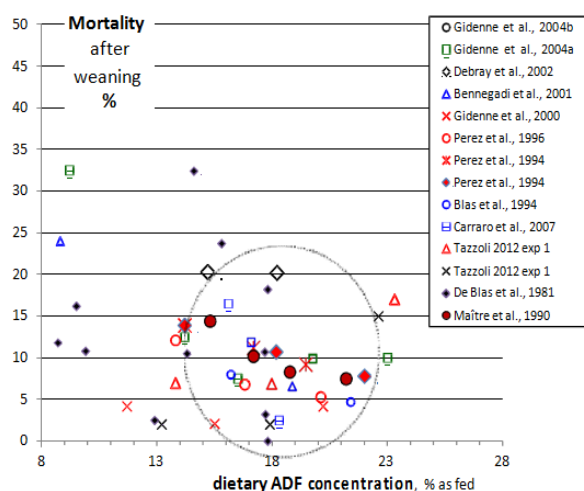
Data from 12 studies and 46 diets, without antibiotic, and varying in their ADF concentration. ADF: acid detergent fibre according to the Van-Soest sequential procedure (Egran 2001). Mortality: from digestive disorders measured from weaning (28-35d) to slaughter (63-77d of age), on at least 36 rabbits/diet.
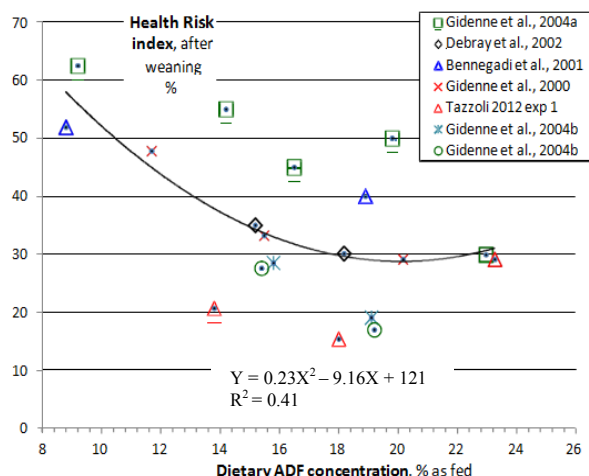
Figure 4. The rabbit post-weaning mortality decreases when the dietary lignocellulose (ADF) concentration increase, but with a variable impact within the classical dietary ADF range (15-22%)

The effects of cellulose intake are less important than for ADL, regarding the decrease of the digestibility (Figure 4: slope = -1) or that of retention time (Gidenne and Perez 1996; Perez et al. 1996). The cellulose (ADF-ADL), also favours the digestive health.

Moreover, an increase of the ratio lignins/cellulose (L/C) is associated with a lower HRi (Gidenne et al. 2001). 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), and caution must be taken to fit requirements. The favourable relationship between the dietary ADL level and the HRi was then confirmed with other experiments, as shown in Figure 7, where 77% of the variations of the HRi are explained by the variation in dietary ADL.

Globally, to reduce the risk of post-weaning digestive disorders, the lignin intake (ADL) for the

growing rabbit, can be assumed as to 5 to 7 g/d, and that of cellulose from approximately 11 to 12 g/d.
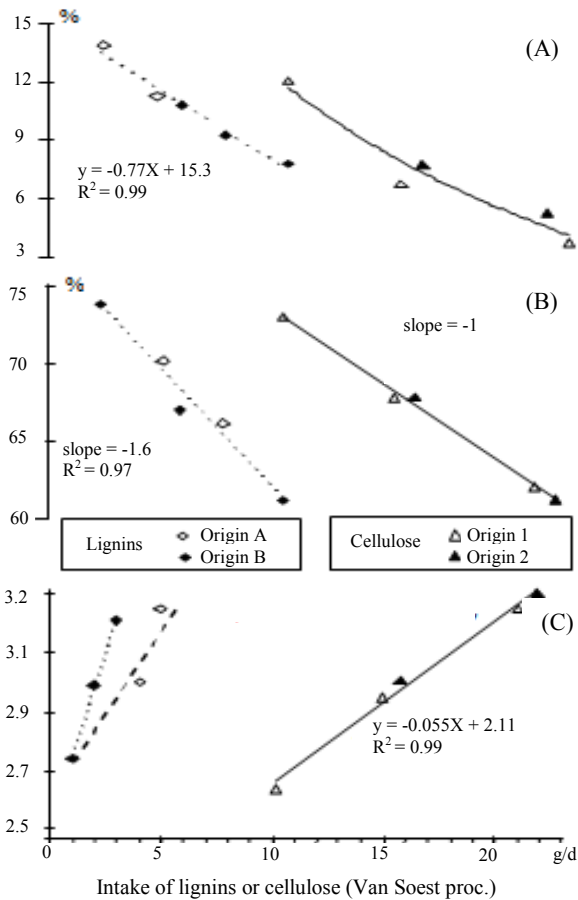

Data from six studies and 22 diets, without antibiotics, and varying in their ADF concentration. ADF: acid detergent fibre according to the Van-Soest sequential procedure (Egran 2001). HRi = mortality + morbidity from digestive disorders measured from weaning (28-35d) to slaughter (63-77d of age), on at least 40 rabbits/diet.

Figure 5. The rabbit post-weaning health risk index (HRi = mortality + morbidity) decreases when the dietary lignocellulose (ADF) concentration increases, but with a variable impact within the classical dietary ADF range (15-22%)

Effects of fibre fractions more digestible than lignocellulose

A third step in evaluating the fibre requirements for growing rabbit was to test the following hypothesis: apart from quantity and quality of ADF, is it necessary to specify the effects of more digestible fibres, such as hemicelluloses, water-insoluble pectins, or "soluble fibre"? These fractions are rather better digested than cellulose or even lignins (Table 3).

A first approach is to estimate the fibres fractions that are relatively digestible, and in a relatively high content in feeds to reduce the analytical error and to improve the prediction of HRi. Therefore, Gidenne propose in 2003 a new "combined" fibre criterion, called "digestible fibres (DgF)" that corresponded to the sum of two fractions: hemicelluloses (analytical value = NDF − ADF, according to the sequential procedure of Van-Soest) and water insoluble pectins (WIP, analysed or estimated, see Table 1). Since, the analysis of water-insoluble pectins is complex, and not practical in a routine feed laboratory, it is frequently necessary to estimate the WIP value of raw materials from literature or tables (Bach Knudsen 2001; Maertens et al. 2002). Some WIP values are given for main fibre sources in the Table 1.

Figure 6. Nutritional role of lignins and cellulose in the growing rabbit. (A) Mortality (4-10 weeks); (B) OM digestibility; (C) Feed conversion ratio.
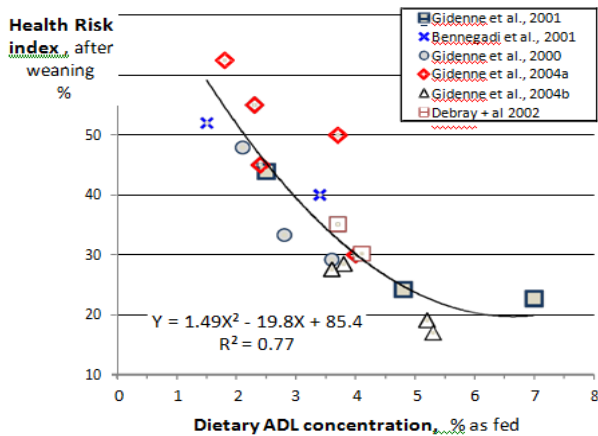
Acid detergent lignin according to the Van-Soest sequential procedure (Egran 2001). Health risk index = mortality + morbidity rate by diarrhoea, measured from 28 to 70d of age, on at least 40 rabbits/diet. Data from six studies and 19 diets varying in their ADL concentration.

Figure 7. Increasing the dietary lignin level reduced the post-weaning digestive troubles incidence in the growing rabbit

Although digestive health of the weaned rabbit depends of the level and quality of lignocellulose, it also vary greatly for the same ADF level (Figure 4 and 5). It is particularly true within the classical range of dietary ADF levels (15-22%), because the level of more digestible fibre fractions "DgF", i.e. [hemicelluloses (NDF − ADF) + water-insoluble pectins], also vary independently of lignins and cellulose levels. For instance the ratio DgF/ADF ranged from 0.9 to 1.7 in the Figure 9. Thus, 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, Gidenne 1997).
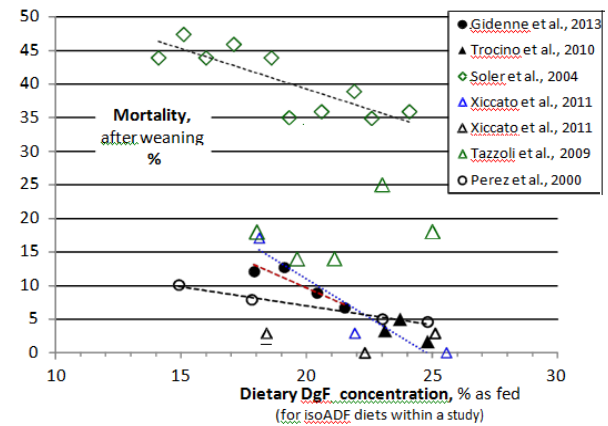
Data from six studies and 31 diets, without antibiotic, within a study the DgF level is varying but not the ADF. ADF: acid detergent fibre according to the Van-Soest sequential procedure (Egran 2001), and DgF = (NDF – ADF) + WIP*. *: water insoluble pectins (Table 1). Mortality: from digestive disorders measured from weaning (28-35d) to slaughter (63-77d of age), on at least 40 rabbits/diet. According to studies, some WIP values were calculated by reformulation from feed ingredients.

Figure 8. Post weaning mortality rate of the rabbit is globally reduced when digestible fibre (DgF) is added in iso-ADF diets

The favourable effect of the DgF, compared to starch intake, was first demonstrated by (Perez et al. 2000) with four iso-ADF diets: mortality was significantly reduced when DgF replaces starch. As shown in the Figure 8, this role of DgF on digestive health was confirmed (four studies on six), although a large variability remained among the studies. A similar relationship is obtained when we related the criteria "TDF-ADF" to the mortality. The favourable effect of DgF (compare to starch) on health would originate from a stimulated caecal fermentative activity (Garcia et al. 2002), and possibly from their moderate effect on the rate of passage (Gidenne et al. 2004a).

From a set of 15 diets (five studies) when ADF and the ratio DgF/ADF are varying (within a study) (Figure 9), we observed a close relationship (R² = 0.69) between the ratio DgF/ADF and the HRi. This suggests that a too high incorporation of DgF, with respect to lignins and cellulose, should be avoided to minimise the Health Risk index during fattening. It is thus recommended that the ratio DgF/ADF remain under 1.3 for diets having an ADF level over 15% (see Table 4).

Therefore a balanced supply of low and high digested fibre fraction is required to reduce the risk of digestive trouble for the rabbit after weaning.

When a sufficient supply of lignocellulose (at least 18%) is provided, it is advisable to replace some starch by digestible fibre fractions. The HRi is improved while the feed efficiency is weakly modified (Perez et al. 2000; Gidenne et al. 2004b; Tazzoli et al. 2009; Trocino et al. 2011). Furthermore, a substitution of protein by DgF also led to a significant improvement of the digestive health status of the growing rabbit, without significant impairment in growth performances (Xiccato et al. 2011; Gidenne et al. 2013).

### Impact of quickly fermentable fibre on digestive physiology and health of the growing rabbit

Another way to analyse the role of cell-wall polysaccharides that are rapidly fermented (and highly digested) 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 (Gomez-Conde et al. 2009). Reversely, a higher level of NDSF improved the mucosal morphology and functionality and its immune response (Gomez-Conde et al. 2007). However, the NDSF criteria remain difficult to analyse, and precision is relatively low for complete feeds with low content of pectins or soluble fibre.

Accordingly, another approach is actually assessed to estimate the content of the quickly fermentable fibre, or soluble fibre "SF" by difference between the TDF (total dietary fibre) and the a+NDFom, with the latter that must be corrected for its crude protein content. SF would be thus easier to handle in a routine laboratory for feed analysis. It would recover the part of TDF that comprises the non-starch, non-NDF polysaccharides, including pectic substances, β-glucans, resistant starch, oligosaccharides, fructans and gums.
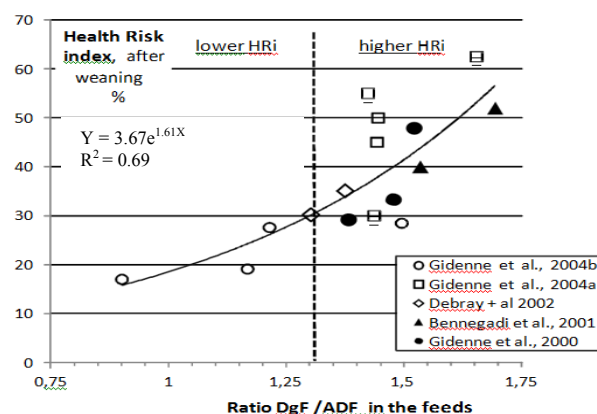
Data from five studies and 16 diets, without antibiotics, varying in their ratio DgF/ADF (within a study dietary ADF is varying). ADF: acid detergent fibre according to the Van-Soest sequential procedure (Egran 2001), and DgF = (NDF – ADF) + WIP*.*: water insoluble pectins (Table 1). Mortality: from digestive disorders measured from weaning (28-35d) to slaughter (63-70d of age), on at least 40 rabbits/diet. According to studies, some WIP values were calculated by reformulation from feed ingredients.

Figure 9. The health risk index (HRi) of the growing rabbit depends from a balance between low-digested (ADF) and high digested (DgF) fibre fractions
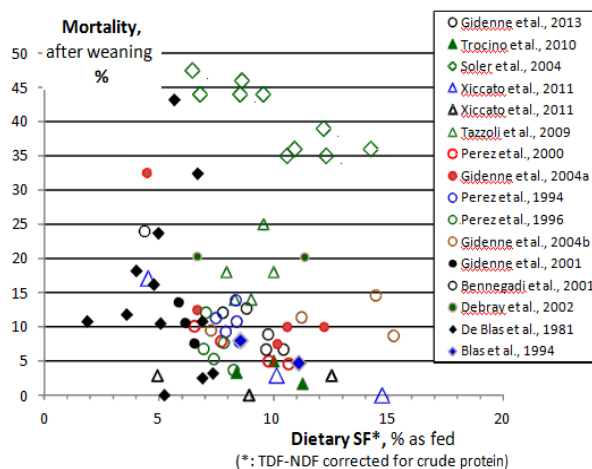
Data from 16 studies and 78 diets, without antibiotics and without selection for the fibre level. Mortality: from digestive disorders measured from weaning (28-35d) to slaughter (63-77d of age), on at least 30 rabbits/diet. According to studies, SF values are analysed (TDF-NDF), or calculated by reformulation from feed ingredients.

Figure 10. Overall relationship between the dietary soluble fibre level and the post-weaning mortality of the growing rabbits

The soluble fibre level is generally increased in a complete feed by supplying raw materials rich in pectins, such beet pulps, citrus or apple pulp, and thus most of the studies in fact relate "pulps levels"

to performances of physiological data. Accordingly, the SF dietary level is positively related with the faecal digestibility of insoluble fibre fractions (NDF and ADF, Trocino et al. 2013). The soluble fibre level favours the microbial activity with higher fermentation levels, lower pH, as reviewed by Trocino et al. (2013). As a consequence, the soluble fibre level is likely to affect ileal and, especially, caecal microbiota (Gomez-Conde et al. 2007; 2009) by modifying the amount and type of substrate reaching the caecum. These changes in microbiota may also modify the immune response observed in young rabbits fed soluble/insoluble fermentable fibre.

But, regardless of the advantages and disadvantages of the different methods and calculation procedures, the choice of the method to quantify SF will depend on the correlation with *in vivo* data collected in animals, and particularly the impact on the digestive health.

The meta-analysis presented in the Figure 10 evidenced that there is no clear global relationship between the soluble fibres, analysed as TDF-NDF, and the post-weaning mortality, although a small tendency to a reduction of the mortality might be observed. However, to look more precisely at this effect we should select studies comparing diets having a similar level of ADF (or NDF), as shown in Figure 11. But, even for the six studies selected (same dataset than Figure 8), with iso-NDF diets, we observed a very large variation of mortality for the same concentration of SF. Furthermore, for studies having a moderate mortality level (<15%), only two studies on four relate SF to mortality, and a low number of animals was often used.

As for the criteria DgF, we calculate the ratio SF on ADF for the same set of studies used for the Figure 9. But, the relationship between the HRi and the ratio SF/ADF is here not significant (R²<0.10), although a tendency for a lower HRi is globally observed (Figure 12). This lack of relationship seems logical since SF did not include the hemicelluloses fractions, that are however in large amounts in rabbit feeds.

It is also possible to estimate the quickly fermentable fibres (or SF) "by difference" with the following calculation: SF = (organic matter) − [NDF − (crude protein) − Starch-Sugars]. However, with the same set of 6 studies the relationship with post-weaning mortality is not improved at all.

Accordingly, criterion that quantify the quickly fermentable fibres or soluble fibre seemed not to improve the mortality prediction. Thus it remains very risky to recommend a SF concentration in rabbit feeds in order to reduce the risk of digestive troubles. Nevertheless, it seems that over a SF level of 7% the mortality rate seems lower, but in fact this level is generally reached in feeds that follow the current recommendations for ADF and DgF (Table 4).

Moreover, criterions for quickly fermentable fibres correspond to a lower amount of fibre residue than for DgF criteria, and due to a higher analytical error this could add further imprecision in recommendations.
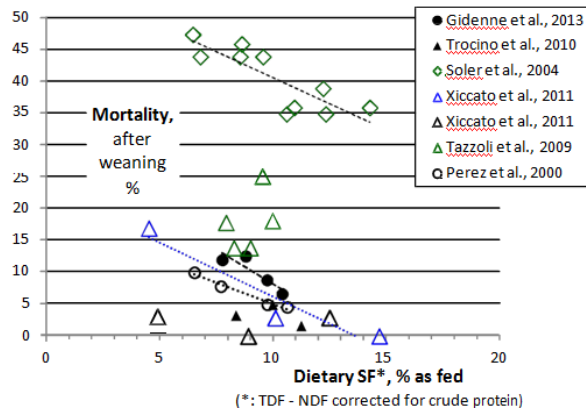
Data from six studies and 31 diets, without antibiotics: within a study the dietary SF level is varying, but the NDF levels are similar. Mortality: from digestive disorders measured from weaning (28-35d) to slaughter (63-77d of age), on at least 30 rabbits/diet. According to studies, SF values are analysed (TDF-NDF), or calculated by reformulation from feed ingredients.

Figure 11. Relationship between the dietary soluble fibre level and the post weaning mortality of the growing rabbits, for feeds having a similar NDF level within a study
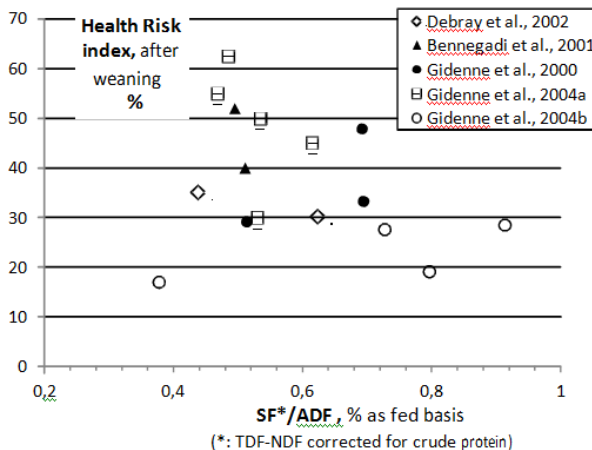
Data from five studies and 16 diets, without antibiotics, varying in their ratio SF/ADF, (within a study dietary ADF is varying). Health risk index = mortality + morbidity rate by diarrhoea, measured from weaning (28-35d) to slaughter (63-70d), on at least 40 rabbits/diet. According to studies, SF values were analysed (TDF-NDF) or calculated by reformulation from feed ingredients.

Figure 12. Relationship between ratio SF/ADF and post-weaning health risk index of the growing rabbits

More research is needed to elucidate the health response of rabbits to the soluble fibre intake, with large-scale studies comparing the health of large groups of rabbits (over 100). In perspectives, the effects of the fibre fractions that are rapidly fermented should be precised. The main problem is to obtain an analytical method sufficiently robust (Xiccato et al. 2012) and that could be used routinely in feed control laboratory.

Table 4. Fibre requirements to prevent the digestive troubles after weaning, for the rabbit bred in rational breeding systems.

Unit[1]	Post weaning (28-42 days old)	End of fattening (42-70 days old)
Lignocellulose "ADFom"	190	170
Lignins "ADL"	55	50
DgF/ADF	<1.3	<1.3
Cellulose "ADF-ADL"	130	110
Ratio lignins/cellulose	>0.40	>0.40
Hemicelluloses "NDF-ADF"	>120	>100

[1]g/kg as fed basis, corrected to a dry matter content of 900 g/kg

Dietary fibre recommendations to reduce the risk of digestive disorders in the weaned rabbit

Table 4 presents a summary of the fibre requirement for post-weaned and growing rabbits. To reduce the risk of digestive troubles after weaning, for the rabbit bred in rational breeding systems, one criteria is not sufficient for fibre recommendation. Three key points must be taken into account.
1) The first criteria to be controlled is the level of ADF that should be over 19% in a complete pelleted feed (Table 4).
2) Secondly, the quality of the lignocelluloses plays also a role in the digestive health, and the minimum level of lignins should be 5% in a feed.
3) Third, the balance between the low-digested "ADF" and high digested fibre fraction should be respected: the ratio DgF/ADF should under 1.3, to avoid a too high intake of highly fermentable polysaccharides (pectins, beta-glucans).

CONCLUSIONS AND PERSPECTIVES

The favourable impact of quantity and quality of low-digested fibre fractions on digestive health has been demonstrated and fibres requirements are now more precise. However, the analysis of the cell wall polysaccharides that are quickly fermented remains a challenge for the future. A criterion, such TDF-aNDFom, needs to be validated in term of reproducibility and repeatability for feed analyses. It should also be more precisely related to the digestive health of the young rabbit. In perspectives, the fibre requirements of the young rabbit before weaning should also be studied and specified. The nutritional preparation of the young before weaning is probably a key step determining the digestive health of the growing rabbit. However, the knowledge of the digestive tract maturation, including the microbes implantation, in the young rabbit needs to be improved, to provide new concept for the nutrition of the young in relation to dietary fibre.

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