Fibre digestion

Thierry Gidenne, R Carabaño, Abad-Guamán R., Garcia J, C. De Blas

5 Fibre Digestion

T. Gidenne,[1] R. Carabaño,[2] R. Abad-Guamán,[3] J. García[2] and C. de Blas[2]
[1]INRA, Occitanie-Toulouse, France; [2]Departamento de Producción Agraria, Universidad Politécnica de Madrid, Ciudad Universitaria, Madrid, Spain; [3]Universidad Nacional de Loja, Ecuador

5.1 Introduction

Dietary fibre (DF) is the major fraction of the rabbit diet, where it accounts for 0.35–0.55 of the total diet (Table 5.1). The importance of fibre is due to its influence on the rate of passage of digesta and mucosa functionality, and its role as a substrate for microbiota. All of these factors are related to rabbit digestive health (see Chapter 10) and performance. The concept of DF, both its quantification and its characterization, has been discussed extensively. The difficulty in reaching agreement on the concept of DF is based on its complex physical structure and the chemical composition of cell walls, the considerable diversity of cells types (and, accordingly, of cell walls) that constitute different plant tissues and the wide and different physiological effects of the different constituents. This all implies that the quantitative analysis of the whole components of this fraction cannot be obtained by any analytical method or combination of methods.

This chapter initially considers the definition and structure of the different classes of fibre and of cell wall constituents, followed by a description of some analytical methods employed for animal or human feeds. Second, the effects of fibre on rabbit digestion are described.

5.2 Dietary Fibre in Animal Feeds

5.2.1 Plant cell wall and dietary fibre: definition

The terms 'cell wall' and 'dietary fibre' are often imprecisely used because they refer to a common plant structure. However, they do not describe the same chemical components and therefore do not have the same meaning. Accordingly, it is useful to define separately these two terms. The term 'plant cell walls' (PCWs) must be employed when describing the structure of the plant cell, which is extremely complex. PCWs are not uniform: the type, size and shape of the walls are closely linked to the function of the cell within the plant (e.g. skeletal tissue, seeds). In general, PCWs consist of a series of polysaccharides often associated and/or substituted with glycoproteins (extensin), phenolic compounds and acetic acid, together with, in some cells, the phenolic polymer lignin. 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 a few cellulosic microfibrils and some non-cellulosic components such as pectic substances. During plant ageing, some cells develop a thick secondary cell wall consisting of cellulose embedded in a polysaccharide and lignin matrix (McDougall *et al.*, 1996).

Thus, in brief, the PCW is formed of cellulose microfibrils (the backbone) embedded in a matrix of lignins, hemicelluloses, pectins and proteins (Fig. 5.1).

The concept of DF developed for human nutrition in the 1960s (Hipsley, 1953) was extended to all mammals and is regularly revisited (Elleuch *et al*., 2011) and often restricted to the polysaccharides of the plant cell wall of fruit and legumes. It is defined as the feed components resistant to mammalian endogenous enzyme digestion and absorption, and that can be partially or totally fermented in the gut. This empirical 'catch-all' definition describes mainly PCW constituents, but also other substances including resistant starch, oligosaccharides, fructans, and protein linked to the cell wall (De Vries and Rader, 2005). Based on this concept both the EU and the USA have developed their own DF definitions (EU, 2011; FDA, 2016). Another approach is the DF for herbivores defined by Mertens (2003) as the indigestible or slowly digested organic matter of feeds that occupies space in the gastrointestinal tract, mainly insoluble fibre. It excludes rapidly fermenting and soluble carbohydrates (e.g. oligosaccharides, fructans) or resistant starch.

Table 5.1. Levels of fibre (g kg^{-1} dry matter) in complete experimental feeds used for the growing rabbit (n = 111) (Villamide *et al*., 2009).

Residue analysed	Mean	Range
Neutral detergent fibre (aNDFom)	368	248–443
Acid detergent fibre (ADFom)	196	135–284
Acid detergent lignin	56	27–195
Hemicelluloses	172	59–251
Cellulose	140	42–220
Crude fibre	166	122–244
Soluble fibre[a]	109	61–188
Total dietary fibre[b]	478	352–560
Other feed constituents		
Starch	176	82–324
Sugars[c]	53	31–163
Crude protein	176	134–232
Ether extract	32	10–71

ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, NDF assayed with a heat-stable amylase and expressed exclusive of residual ash; CP: crude protein; EE, ether extract; OM, organic matter.
[a]Calculated as: OM – CP – EE – aNDFom – starch – sugars.
[b]Calculated as: aNDFom + soluble fibre.
[c]Estimated according to ingredient composition (FEDNA, 2003).

5.2.2 Biochemical characteristics of dietary fibre

Biochemical features of dietary fibres are one of the main factors responsible for variations in their physiological effects (digestion, etc.). The chemical features of DF are highly variable, depending on many factors such as molecular weight, the nature of the monomers and types of linkages. With the exception of lignin, cell wall constituents are predominantly polysaccharides composed of neutral and/or acidic sugars. They can be determined using sophisticated extraction techniques, and examples of their concentration in some feedstuffs are given in Table 5.2.

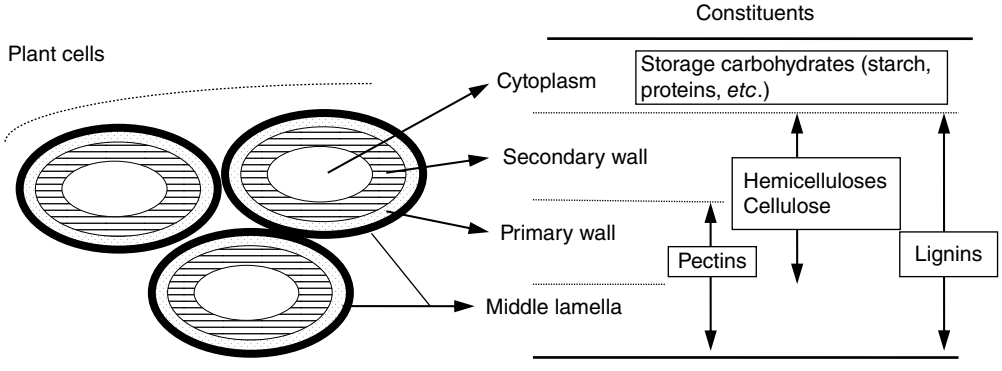

Fig. 5.1. Schematic representation of plant cell walls and their main constituents.

Table 5.2. Proximate composition of cell wall constituents (g kg^{-1} DM) in some raw materials used in rabbit feeds, according to several methods of analysis.

Ingredients	Wheat straw	Wheat bran	Dehydrated lucerne	Beet pulp	Sunflower meal	Soybean hulls	Grape pomace
aNDFom[a]	800	450	460	470	480	620	640
ADFom[b]	540	110	340	220	320	440	540
ADL[c]	160	30	80	20	110	20	340
NDSF[d]	–	30	180	300	–	220	–
Crude fibre[e]	400	100	270	190	260	360	260
WIP[f]	22	29	76	270	80	100	80
WICW[g]	840	450	470	580	390	720	690
TDF[h]	850	460	480	680	410	–	720
IDF[h]	820	450	420	550	370	–	680
INSP[i]	550	360	330	640	260	550	360
Rhamnose	<10	<10	<10	110	<10	110	<10
Arabinose	20	80	20	180	30	40	<10
Xylose	180	160	60	20	50	70	80
Mannose	<10	<10	<10	10	10	60	20
Galactose	<10	<10	<10	40	10	20	20
Glucose	330	90	190	190	110	290	190
Uronic acids	20	20	70	180	50	60	50
SNSP[j]	10	30	30	100	10	20	10
Crude protein N x 6.25	30	150	160	90	340	110	130

[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 *et al*., 2003).
[e]According to the Weende method (AOAC, 2000: official method 962.10).
[f] Water-insoluble pectins (uronic acids + neutral sugars of pectins insoluble in hot water).
[g]Water-insoluble cell wall, including lignins (Carré and Brillouet, 1989).
[h]McCleary *et al*., 2010.
[i]Insoluble non-starch polysaccharides, not including the lignins, determined by direct monomeric analysis of cell wall polysaccharides (Englyst, 1989; Barry *et al*., 1990).
[j]Water-soluble non-starch polysaccharides (Brillouet *et al*., 1988; Englyst, 1989).

There are two main groups of DF components according to their location, chemical structure and properties (Fig. 5.2):

- Cell wall components:
  - water-soluble non-starch polysaccharides (e.g. part of β-glucans, arabinoxylans and pectic substances).
  - water-insoluble polymers: lignin, cellulose, hemicelluloses and pectic substances.
- Cytoplasm components:
  - oligosaccharides, fructans, resistant starch and mannans.

Water-soluble polysaccharides and oligosaccharides include several classes of molecules with a degree of polymerization ranging from about 15 to >2000 (β-glucans). Most of them are insoluble in ethanol (80% v/v). Examples include soluble hemicelluloses such as arabinoxylans (in wheat, oats and barley, approximately 20–40 g kg^{-1} dry matter (DM)) and β-glucans (in barley or oat, approximately 10–30 g kg^{-1} DM), oligosaccharides such as α-galactosides (in lupin, pea or soya seeds, 50–80 g kg^{-1} DM) and soluble pectic substances (fruit or beet pulp, from 100 to 400 g kg^{-1} DM). Because of their highly variable structures, no satisfactory method is at present available to determine precisely the amount of these compounds in animal feeds.

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

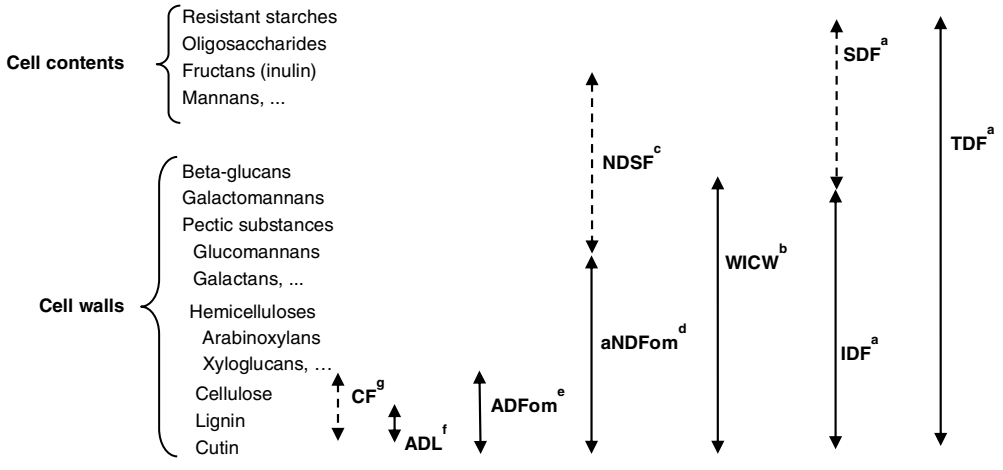

Fig. 5.2. Dietary fibre fractions and their quantification by some gravimetric methods pertinent in animal feed analysis.
[a]Soluble dietary fibre, insoluble dietary fibre and total dietary fibre (Mc Cleary *et al*., 2010; AOAC procedures 985.29 and 991.43, including resistant starch).
[b]Water insoluble cell wall (Carré and Brillouet, 1989).
[c]Neutral detergent soluble fibre (Hall *et al*., 1997).
[d]Neutral detergent fibre assayed with a heat-stable amylase and expressed free of ash.
[e]Acid detergent fibre expressed free of ash.
[f]Acid detergent lignin.
[g]Crude fibre.

dicotyledonous plants, such as in legume seeds (40–140 g kg^{-1} DM in soybean, pea, faba bean and white lupin), and also in fruit and pulp. Pectic substances correspond to several classes of polymers, including pectins (rhamnogalacturonan backbone and side chains of arabinose and galactose) 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 tetra-acetic acid (EDTA) (present in the solution for determining neutral detergent fibre (NDF) so pectins are not completely recovered in NDF analysis, as described below). Pectins of the middle lamellae serve as an adhesive in plant tissue, cementing plant cells together.

Cellulose is the major structural polysaccharide of the PCW and the most widespread polymer on earth. It is a homopolymer (in contrast to hemicelluloses and pectins), 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 is usually around 8000–10,000. Individual glucan chains aggregate (hydrogen bonding) to form microfibrils and may 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^{-1} DM in the hulls of legume and oilseeds, 100–300 g kg^{-1} DM in forages and beet pulp and 30–150 g kg^{-1} DM in oilseeds or legume seeds. Most cereal grains contain small quantities of cellulose (10–50 g kg^{-1} DM), except for oats (100 g kg^{-1} 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 the primary cell wall in dicotyledonous plants (legumes, seeds), whereas mixed linked glucans (β[1→3,4]) and arabinoxylans are the predominant hemicelluloses in cereal 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) and 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 constitute 100–250 g kg^{-1} DM in forages and agro-industrial by-products (brans, oilseeds and legume seeds, hulls and pulp) and about 20–120 g kg^{-1} DM in grains and roots.

Lignin is a non-carbohydrate constituent of the cell wall. It can be described as a highly branched and complex three-dimensional network (with a high molecular weight), built up from three phenylpropane units (cony-ferulic, coumarilic and sinapinic acid). Lignin networks 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^{-1}. The degree of lignification of the PCW may reach 120 g kg^{-1} with ageing in forages or up to 590 g kg^{-1} in grape-seed meal.

Other constituents are also present in PCWs, but in smaller quantities. Minerals, such as silica, are essentially found in graminaceous leaves. Phenolic acids are chemically linked to hemicelluloses and lignin 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 can encrust and embed the cell walls, making them impermeable to water.

Other phenolic compounds can also be mentioned – for example, condensed tannins, which may exist in higher plants. They form cross-linkages with protein and other molecules. They may be included in the sum of indigestible polysaccharides plus lignin. However, condensed tannins, lignins and indigestible proteins are closely related because indigestible complexes of these substances are common in plants (Van Soest, 1994). Moreover, according to heat treatment applied to the raw material (drying by heating), Maillard reaction products are formed thus adding to the lignin content, and also resistant starch fractions are formed adding to the fibre content. Similarly, extrusion may increase the content of soluble fibre according to the water content of the process used (FAO, 1998), and heat treatment during processing may enhance the viscosity properties of soluble fibre (Svihus and Zimonja, 2011).

5.2.3 Methods for estimating the dietary fibre content of animal feeds

According to the DF definition, DF can only be truly measured by the digestive process of the animal. Currently no method is totally adequate to quantify or fractionate DF due to its complexity. Although many methods have been developed to estimate the DF content in animal feeds, none of them corresponds to a precise DF fraction. Detailed reviews have been published on this subject (Hall, 2003; Mertens, 2003; De Vries and Rader, 2005; Gidenne, 2015). Enzymatic methods have been developed, for scientific purposes, to quantify total dietary fibre (TDF), including DF and associated compounds (lignins, cutin, etc.). They simulate the digestion by incubating samples with enzymes (amylases, proteases, and amyloglucosidase), then precipitating soluble fibre with ethanol and discounting the ash and protein content. However, the traditional gravimetrical methodology (AOAC 985.29/991.43) does not quantify soluble oligosaccharides having a low molecular weight as recommended by the new DF definitions. To solve this issue, the analytical method incorporates the use of liquid chromatography to measure the oligosaccharides in the residual liquid (AOAC 2009.01/2011.25), but this action makes difficult the applicability of this method in laboratories. The techniques currently used in rabbit feeding are described below (see also Fig. 5.2).

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 a fibrous residue after acidic followed by basic hydrolysis. The main drawback of this method 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% lignin. As a consequence, crude fibre digestibility could be higher than that of nitrogen-free extract in some feeds. For these reasons, this method is not very useful in explaining the effects exerted by fibre on the animal but is useful for predicting dietary energy within a raw material or to verify the fibre content compared to tables.

The main alternative to the crude fibre method is the sequential procedure of Van Soest, developed in 1967 and successively updated (Mertens, 2003). The NDF method was designed to isolate insoluble DF components in PCWs by using a hot neutral detergent solution – cellulose, hemicelluloses and lignins – as the majority of pectin substances are partially solubilized. This method has been criticized due to its variability among laboratories, especially when the results are compared with those obtained with other feed constituents (Xiccato *et al.*, 1996). This variability is partially due to the different procedures that can be used to perform the method (with heat-stable amylase and/or sodium sulfite or not, ash free or not), but usually described with the same reference (Uden *et al.*, 2005).

The acid detergent fibre (ADF) (AOAC, 2000: official method 973.18) method isolates cellulose and lignins using a hot acid detergent solution. It is designed to be performed after NDF analysis, as pectins are retained when it is performed directly. As with the crude fibre method, the ADF method is very useful in predicting dietary energy value (Wiseman *et al.*, 1992). Finally, the lignin fraction is isolated from the ADF residue after removal of the cellulose by using a strong acid solution at room temperature (Robertson and Van Soest, 1981).The main advantage of this sequential methodology is to calculate estimates of cellulose (ADF – acid detergent lignin (ADL)) and hemicellulose (NDF – ADF) fractions. In addition, it is relatively quick, simple and economical, and has an acceptable reproducibility when used in a standardized methodology (EGRAN, 2001).

These methods have been complemented by the estimation of the fibre dissolved by the neutral detergent solution (NDSF: neutral detergent soluble fibre) (Hall *et al.*, 1997), which mainly includes fructans, galactans, β-glucans and pectic substances. However, the determination of NDSF is too difficult to be used as routine analysis, as occurs with other methods used to estimate soluble fibre. Van Soest *et al.* (1991) also proposed estimating soluble fibre as the difference between TDF and NDF. This showed a good reproducibility for complete rabbit diets and raw materials with low or medium soluble fibre concentration, but results were impaired with materials rich in soluble fibre (Xiccato *et al.*, 2012). The calculation above rendered higher soluble fibre values than other alternatives, such as estimating soluble fibre by difference between TDF and insoluble fibre, quantified either using *in vitro* or enzymatic methodology (Abad *et al.*, 2013). However, these three procedures provided highly correlated values of soluble fibre, similar amounts of soluble fibre fermented in the ileum or in the whole digestive tract, in spite of the differences reported for their faecal digestibility (Abad-Guamán *et al.*, 2015a) and also were similarly correlated with some physiological traits such as caecal pH (Abad-Guamán, 2015). The difference TDF – NDF (both corrected for ash and protein) would be preferred to estimate dietary soluble fibre because NDF is a simple and extended measurement in most laboratories.

The quantification of soluble fibre in the digesta is more complex than in the diet/ingredients because the ethanol used to precipitate soluble fibre (in TDF analysis) also precipitates partially the intestinal mucins leading to an overestimation of ileal TDF content (Abad *et al.*, 2013; Montoya *et al.*, 2015). This seems to be the reason why the ileal digestibility of soluble fibre and TDF showed very low or even negative values both in rabbits (Gidenne, 1992; Martínez-Vallespín *et al.*, 2013) and pigs (Jørgensen *et al.*, 1996; Wilfart *et al.*, 2007). A simple correction of digesta TDF values by the mucin carbohydrate fraction retained in the TDF residue was proposed by Abad *et al.* (2013) and its application is essential when determining ileal digestibility of soluble fibre or TDF (especially in diets with low TDF or soluble fibre content) due to the relatively high ileal mucin content. This correction is less important at faecal level because mucins are extensively fermented in the caecum (>0.85) (Abad-Guamán *et al.*, 2015a).

Another approach is to estimate DF as the sum of non-starch polysaccharides (NSP) and lignin. Several methods are available to estimate total, soluble and insoluble NSP (Bach Knudsen, 2001; De Vries and Rader, 2005), where the non-fibrous components are extracted by solubilization, enzymatic hydrolysis or a combination of both procedures. Once isolated, the fibre residue can be quantified gravimetrically (by weighing

the residue) or chemically (by hydrolysing 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. In this way the total DF can be quantified (NSP and lignin) and separated into insoluble and soluble fibre (in aqueous solution), and the monosaccharide composition obtained. The combination of the monosaccharide composition of fibre with additional chemical information may allow a better description of fibre structure that influences its physicochemical properties and, accordingly, the effect exerted on an animal's digestive physiology and digestibility. However, these methodologies are complex, expensive and have relatively low reproducibility (especially for monomers determination). They are difficult to implement as routine analysis.

Dietary insoluble fibre can also be estimated by using near infra-red spectroscopy (NIRS). NIRS could be used in predicting dietary DM, protein, fat, starch and even 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).

In conclusion, the determination of the fibre content of a compound feed (Table 5.1) or raw material (Table 5.2) 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, predict the nutritive value).

5.2.4 Physicochemical properties of fibre related to digestion: particle size

As described below, a part of the fibre effects on rabbit digestive physiology is related to the large-size fibre particles that influence the passage rate of digesta. Particle size can be measured by dry or wet sieving on pelleted feeds (Lebas and Lamboley, 1999; Nicodemus *et al.*, 2010) or on ingredients, and varies largely depending on the fibre source (Table 5.3). Dietary particle size is modified considerably during feed manufacturing (see Chapter 11) and it is essential to determine the particle size profile on pellets (Lebas and Lamboley, 1999; Nicodemus *et al.*, 2010) instead of the meal. In this way Morisse (1982), by milling a feed one or three times with the same sieve size (4 mm), observed an increase in the proportion of fine particles (<0.25 mm) from 0.31 to 0.74. It is important to consider that the determination of particle size in complete diets includes fibrous but also starch-rich particles. To assess the amount of large fibre, the NDF content in particles larger than 0.3 mm should be determined, which would be at least around 180 g kg^{-1} DM (in low-fibre diets that meet fibre-requirements), showing higher values for fibre-rich diets of around 300 g kg^{-1} DM (Nicodemus *et al.*, 2010; Romero *et al.*, 2011). These authors did not report a relevant influence of particle size of balanced diets on NDF digestibility.

Other physicochemical properties such as digesta viscosity, cation-exchange or hydration capacity of fibre might influence fibre digestibility but they do not seem to affect performance or health (García *et al.*, 2000; Volek *et al.*, 2005).

5.3 Dietary Fibre Digestion by the Rabbit

5.3.1 Fibre digestion before the hindgut

Traditionally, microbial fermentation of DF has been considered to occur in the hindgut (after the small intestine), that is, the caecum and the proximal colon for the rabbit. However, there is evidence that some components of the PCWs are

Table 5.3. Particle size determined by wet sieving of some commercial sources of fibre after pelleting (García *et al.*, 1999, 2002b).

	Paprika meal	Olive leaves	Lucerne hay	Soybean hulls	NaOH-barley straw	Sunflower husk	Grape-seed meal
<0.160 mm	0.84	0.54	0.54	0.24	0.23	0.02	0.31
>0.315 mm	0.07	0.38	0.29	0.53	0.54	0.74	0.45
>1.250 mm	0.00	0.09	0.02	0.04	0.11	0.04	0.02

degraded prior to entering the caecum. This has also been observed in other non-ruminant species such as pigs and poultry.

The extent of precaecal fibre digestion in rabbits varies largely according to the methods used to analyse the fibre fraction: from 0.07 to 0.19 for crude fibre (Yu *et al*., 1987), from 0.05 to 0.43 for NDF (Gidenne and Ruckebusch, 1989; Merino and Carabaño, 1992; Blas *et al*., 2003; Abad-Guamán, 2015), from 0 to 0.37 for NSP (Gidenne, 1992; Carabaño *et al*., 2001) and from 0.05 to 0.28 for TDF (Fig. 5.3a). It must be pointed out that the values obtained using NDF and crude fibre with respect to those obtained with NSP might be overestimated due to solubilization and filtration of cell wall components that would be considered digested. In fact, up to 0.06 of dietary NDF was not recovered during the

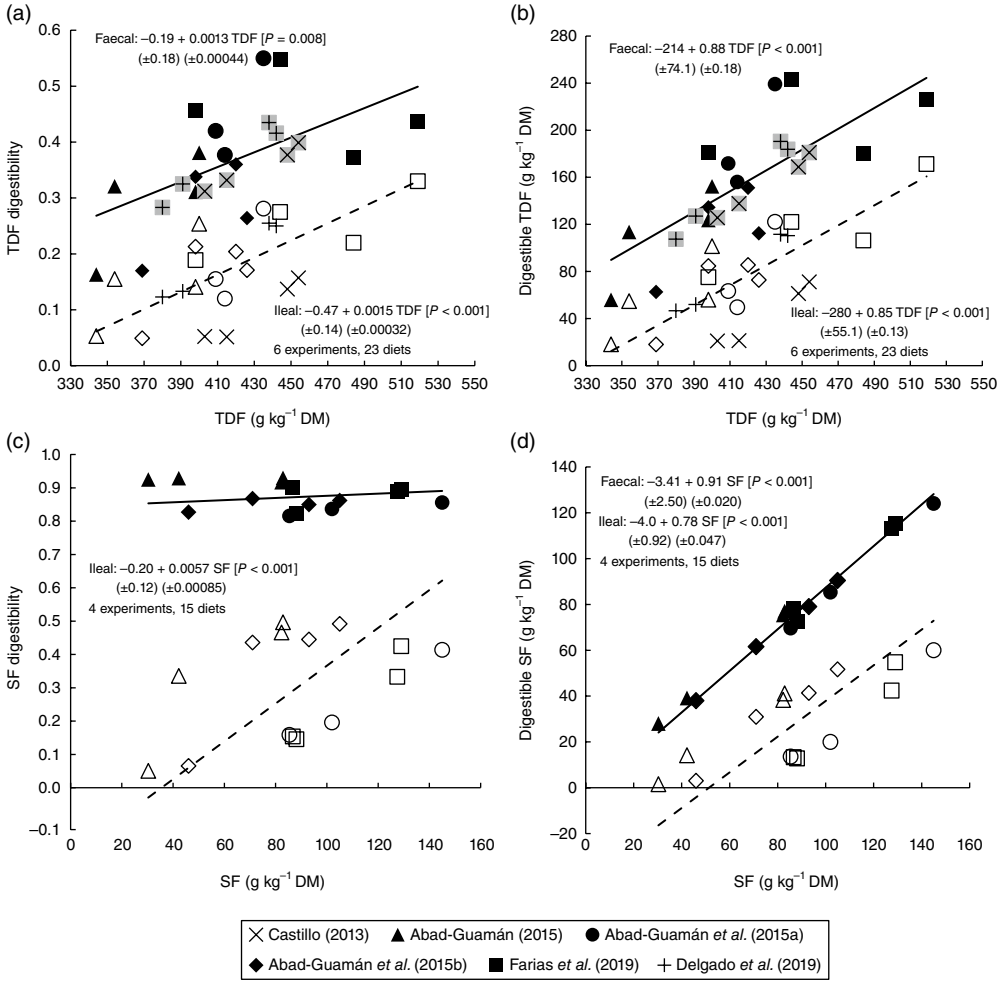

Fig. 5.3. Relationship between dietary soluble fibre (SF) and total dietary fibre (TDF) and its ileal and faecal digestibility (a and c) and digestible content (b and d) (all corrected by digesta mucin content). Original observations plotted with the mean regression line across studies (black symbols and continuous line: faecal values; white symbols and dashed line: ileal values). Increase of TDF was predominantly obtained by increasing beet pulp. Dietary neutral detergent fibre (NDF) (corrected for ash and protein) ranged from 290 to 396 g kg^{-1} dry matter (DM) (but 20 out of 23 diets were between 307 and 336 g NDF kg^{-1} DM), and acid detergent lignin from 31.0 to 66.4 g kg^{-1} DM.

two-step pepsin/pancreatin *in vitro* NDF digestibility (Abad-Guamán, 2015). A similar situation might also occur with TDF because the soluble fibre is not completely recovered (Mañas and Saura-Calixto, 1993).

A review of the variation of the ileal TDF and soluble fibre digestibility compared with the faecal one (both corrected by intestinal mucins) is reported in Fig. 5.3. Data were obtained from six experiments in which the increase of dietary TDF was mostly obtained from increasing the soluble fibre level, and analysed according to St. Pierre (2001). There is a positive effect of the level of dietary TDF and soluble fibre on the amount of digested TDF and soluble fibre at ileal and faecal level, and on their ileal and faecal digestibility. These results imply that around 0.45 (from 0.15 to 0.80) of total digestible fibre (including soluble fibre) is degraded before the caecum, which is similar to that observed in pigs (Bach Knudsen, 2001). Surprisingly, soluble fibre accounts for around a third of this value, whereas the remaining two-thirds correspond to insoluble fibre, in spite of the relatively short oro-ileal mean retention time of the digesta (around 5 h; Gidenne, 1994; García *et al*., 1999). A higher ileal digestibility of the soluble fibre fraction compared to the insoluble one would be expected, because of a higher accessibility for bacterial enzymes (Marounek *et al*., 1995). When NSPs have been analysed, arabinose and uronic acids, typical monomers of pectic substances, have been found to be largely digested before the ileum (from 0.2 to 0.5). On the other hand, glucose and xylose, the major monomers in most fibre sources, showed a much lower ileal digestibility (0–0.2). These results suggest that in the ileum the cell wall polysaccharides can be partially hydrolysed to carbohydrates with lower molecular weight (to shorter polysaccharides or to oligosaccharides) rather than being extensively fermented, as hypothesized by Abad-Guamán *et al*. (2015a). Consequently, this fraction would not be retained with the insoluble fibre, nor would it be precipitated with ethanol. This hypothesis is coherent with the higher volatile fatty acid (VFA) concentration in the caecum than in the ileum (88.2 versus 24.3 mmol g^{-1} fresh digesta, Ocasio-Vega *et al*., 2018a), the higher *in vitro* gas production in the caecum than in the ileum (Abad-Guamán *et al*., 2018) and the negative relationship between the amount of TDF degraded before the caecum and the caecal pH (Fig 5.4a).

The pattern of fermentation in the ileum differs from that observed in the caecum, by a

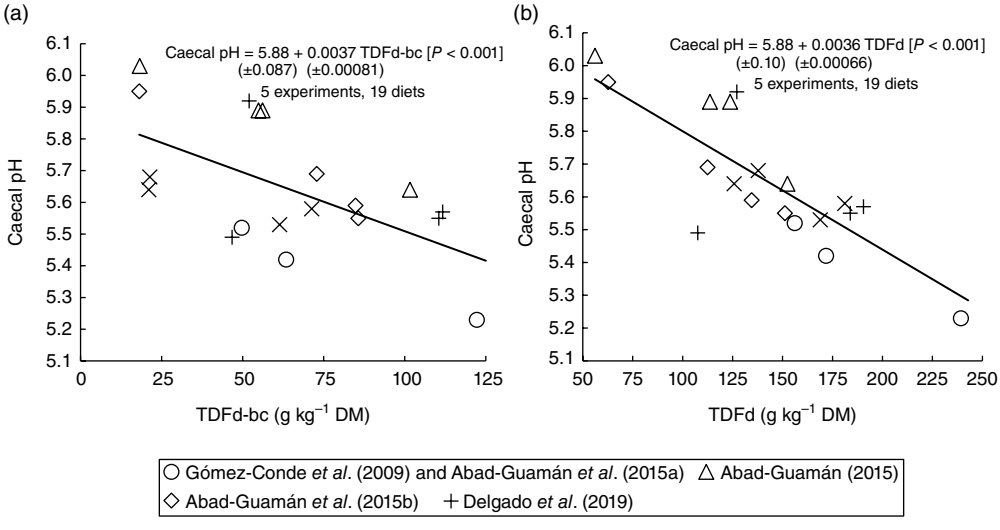

Fig. 5.4. Relationship between caecal pH and (a) total dietary fibre digested before the caecum (TDFd-bc); (b) total dietary fibre digested in the whole digestive tract (TDFd); both corrected by digesta mucin content. Original observations plotted with the mean regression line across studies. Total dietary fibre ranged from 344 to 454 g kg^{-1} dry matter (DM), neutral detergent fibre (corrected for ash and protein) ranged from 290 to 336 g kg^{-1} DM, and acid detergent lignin from 31.0 to 58.3 g kg^{-1} DM.

higher molar proportion of acetic acid mainly at the expense of butyric acid (Ocasio-Vega *et al*., 2018a). It might be related to the probable minor fermentation of endogenous substances in the ileum compared with the caecum (Marounek *et al*., 2000; Miner-Williams *et al*., 2009; Abad-Guamán *et al*., 2015a, 2015b), and it would be in agreement with the lower acetate to butyrate ratio when *in vitro* caecal fermentation is done with predigestion (where the added enzymes are fermented) than without it (Ocasio-Vega *et al*., 2018b).

5.3.2 Caecal digestion of fibre

Fibre degradation is ultimately determined by microbial activity, digesta retention time in the caecum and fibre chemical composition and structure.

Microbial activity

Most of the effects exerted by fibre on the rabbit digestive physiology depend on its hydrolysis and fermentation by the digestive microbiota. However, it is difficult to study the influence of any dietary component on the microbiota, as traditional cultivation techniques only recover around one-quarter of the intestinal microbiota. Now, molecular microbiology provides evidence of the age-related changes for the intestinal microbiota, while the diet seems to have a minor impact (Rodríguez-Romero *et al*., 2012; Combes *et al*., 2013, 2017), although some relevant effects related to DF were also reported (Gómez-Conde *et al*., 2007, 2009). Other indirect techniques are used, such as the VFA concentration, microbial nitrogen synthesized or fibrolytic activity. The caecal bacteria secretes enzymes able to hydrolyse the main components of DF. 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; Falcão e Cunha *et al*., 2004). These results support the faecal digestibility of the corresponding TDF constituents in rabbits (Table 5.4), 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 dietary factors affecting the variability of fibrolytic activity have not been extensively studied, but it seems that quantity of fibre consumed little affects the caecal fibrolytic activity (Gidenne *et al*., 2000, 2002). In return, the quality of the fibre strongly influences the fibrolytic activity, with sugarbeet pulp increasing caecal pectinolytic and cellulolytic activity, and wheat-bran-based diets increasing xylanolytic activity (Falcão e Cunha *et al*., 2004).

Table 5.4. Faecal digestibility of DF fractions in experimental diets.[a]

Class of dietary fibre	n[b]	Mean	Range
Total dietary fibre (TDF)	49	0.43	0.22–0.66
	23[c]	0.36	0.16–0.55
Neutral detergent fibre (aNDFom)	182	0.33	0.03–0.71
	23[d]	0.24	0.07–0.40
Soluble fibre (TDF – NDF)	55	0.90	0.73–1.00
	15[c]	0.87	0.82–0.93
Uronic acids	7	0.58	0.30–0.85
Hemicelluloses (aNDFom – ADF)	127	0.46	0.00–0.82
Cellulose (ADFom – ADL)	52	0.27	0.01–0.59
Lignin (ADL)	34	0.11	–0.08 to 0.25

ADFom, acid detergent fibre expressed exclusive of residual ash; ADL, acid detergent lignin; aNDFom, NDF assayed with a heat-stable amylase and expressed free of ash.

[a]Falcão e Cunha and Lebas (1986), Gidenne (1987, 1992), Fraga *et al*. (1991), Gidenne et al. (1991, 1998, 2000, 2001, 2002, 2004a, b), García *et al*. (1992, 1993, 1999, 2002b), Gidenne and Perez (1993, 1994, 2000), Perez (1994), Bellier and Gidenne (1996), Gidenne and Jehl (1996), Motta-Ferreira *et al*. (1996), Carabaño *et al*. (1997), Falcão e Cunha *et al*. (1998, 2000, 2004), Gidenne and Bellier (2000), Nicodemus *et al*. (2002), Xiccato *et al*. (2006, 2008, 2011), Volek *et al*. (2005, 2007), Carraro *et al*. (2007), Tazzoli *et al*. (2009, 2013, 2015), Trocino *et al*. (2010, 2011, 2013b), Castillo (2013), Abad-Guamán (2015), Abad-Guamán et al. (2015a,b), Farias *et al*. (2019), Delgado *et al*. (2019).

[b]n = number of diets. [c]values corrected for intestinal mucins (Fig. 5.4c). [d]values corrected also for protein.

Fermentation time

The digesta retention time in the caeco-colic segments can be estimated from the difference in ileo-rectal mean retention time (i-r MRT, h) and minimal transit time (TTm, h) obtained using ileally cannulated animals. The latter value is relatively constant with a range from 3.5 to 4.5 h, averaging 3.7 h (Gidenne, 1994; García *et al.*, 1999). Several studies (Gidenne *et al.*, 1991; Gidenne and Perez, 1993; Gidenne, 1994; García *et al.*, 1999) have measured the i-r MRT for diets based on lucerne hay, wheat bran and fibrous by-products. This trait was linear and negatively correlated with dietary NDF content, which varied from 220 to 470 g kg^{-1} (DM basis). The regression equation obtained was

$$\text{i-r MRT} = 26.5(\pm 4.9) - 0.0368(\pm 0.015)\ \text{NDF (DM)}, R^2 = 0.35, n = 13, P = 0.03.$$

According to this equation, the i-r MRT of an average rabbit diet containing 360 g NDF kg^{-1} diet DM would be 13.2 h. The time of fermentation (i.e. retention in the caecum and proximal colon) could be estimated as 9.5 h (13.2–3.7 h).

Ileo-rectal MRT is also related to the weight of the caecal contents (CCW as a proportion of body weight; P = 0.04; Gidenne, 1992; García *et al.*, 1999, 2000). Some results are shown in Fig. 5.5, where CCW has been related to dietary NDF content, but the type of fibre (particle size and lignin content) also influences CCW (Nicodemus *et al.*, 1999, 2006). Sampling time significantly affects this trait (see Chapter 1), so that only results obtained using the same methodology have been chosen (García *et al.*, 2002a), and analysed according to St. Pierre (2001). Based on a wide range of level and sources of fibre, a quadratic relationship was found between the dietary NDF content and the CCW, with a minimal CCW for a dietary NDF content of 405 g kg^{-1} DM. The additional effect of the degree of lignification of NDF on CCW indicates an additional influence of source of fibre. Diets containing low-lignified beet pulp or high-lignified sunflower husk tended to give, respectively, higher and lower CCW values at the same NDF level.

Another factor related to fermentation time is particle size. As was observed by Björnhag (1972), the particle size of fibre influences the entry of digesta in the caecum. Also, Gidenne (1993) observed that particles <0.3 mm were retained for longer (≥10 h, on average) than particles >0.3 mm, because the proximal colon returns small particles and selects large particles to be excreted as hard faeces, which was confirmed using different sources of fibre (García *et al.*, 1999).

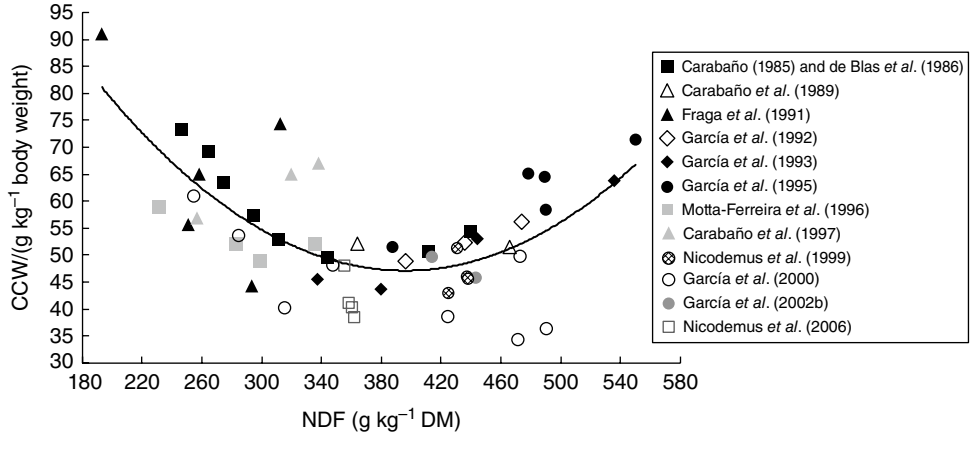

Fig. 5.5. Effect of dietary neutral detergent fibre (NDF) content on the weight of caecal contents (CCW) (data reviewed by García *et al.*, 2002a). Original observations plotted with the mean regression line (considering an average ADL/NDF value of 0.187) across studies. (CCW = 173[±18.2] – 0.60 NDF [±0.097] + 0.00074[±0.0013] NDF^2 – 20.7[±8.67] ADL/NDF, n = 52, P < 0.001 for NDF and P = 0.021 for ADL/NDF, that ranged from 0.05 to 0.73). ADL, acid detergent lignin.

Digestion rate

The rate of fermentation of PCW constituents is a primary factor influencing fibre digestion in rabbits, because of the relatively short mean retention time (about 10 h) in the hindgut. As stated above, the PCW biochemical structure is the main factor affecting the fibre degradation rate. Dietary soluble fibre is the substrate more easily fermented, as well as uronic acids (important constituent of pectins). In fact, the increase of dietary soluble fibre improved the faecal TDF digestibility as reviewed by Trocino *et al.* (2013a). Accordingly, the faecal digestibility of soluble fibre (0.87–0.90) is much higher than for NDF (0.24–0.34), and closer to that of starch (0.99) in this group of diets (Fig. 5.3c; Table 5.4). The differences in the degradation rate of fibrous ingredients are described by the caecal (and ileal) *in vitro* gas production (Fig. 5.6) and confirm the higher fermentation rate of soluble fibre-rich ingredients. The caecal microbial NDF degradation rate may be derived from *in situ* rumen measurements (García *et al.*, 1995; Escalona *et al.*, 1999). From these data, it can be concluded that the relative value of fibre is highly dependent on the time of fermentation. For instance, paprika meal has a relatively high degradation rate at 10 h and a low degradation rate at 72 h, whereas the opposite occurs for NaOH-treated wheat straw.

Lignin and cutin are considered almost totally undegradable, although positive values for lignin digestibility have been obtained that might indicate a solubilization rather than digestion. Lignin is covalently linked to hemicelluloses (Van Soest, 1994) and, consequently, the degree of lignification of NDF negatively affects the level of digestible hemicelluloses ($n = 127$; $r = -0.57$; $P < 0.001$), but not that of cellulose. The two raw materials that increase the digestible hemicellulose level in the diet are beet pulp (low lignified and with a high hemicellulose to cellulose ratio of 1.1 compared to lucerne at 0.4) and wheat bran (with the highest hemicellulose to cellulose ratio at 3.2).

The faecal digestibility of different fibre fractions of several fibrous ingredients is presented in Table 5.5. The highest values for TDF and NDF (0.70, 0.84–0.62) were obtained for beet pulp, poorly lignified and having a lengthy fermentation time in the caecum. The lowest NDF digestibility (0.03–0.10) was found for olive leaves, grape-seed meal and sunflower hulls.

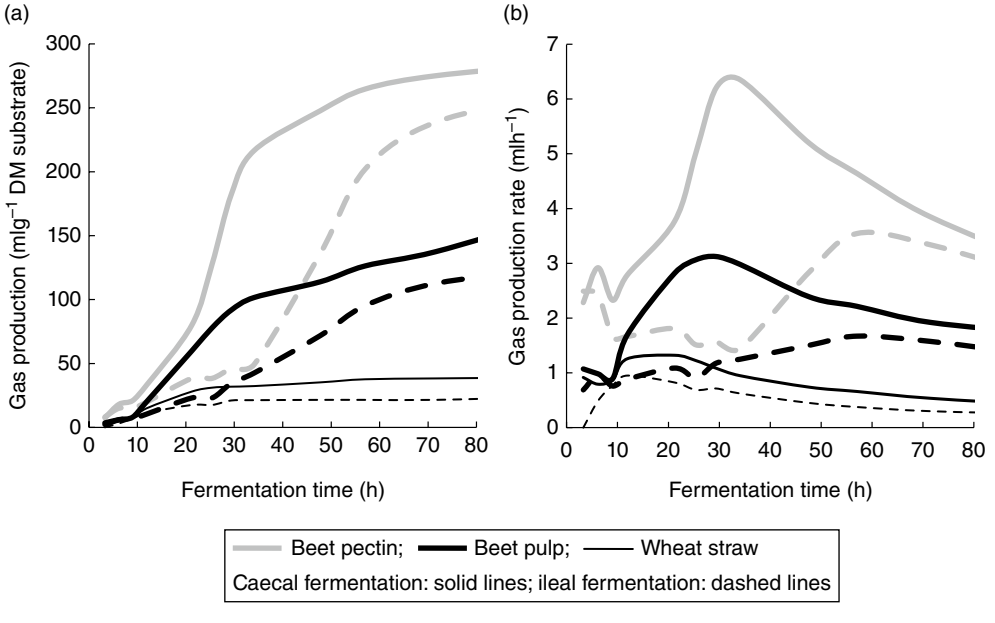

Fig. 5.6. *In vitro* (a) gas production and (b) gas production rate of three ingredients differing widely in the type of fibre (Ocasio-Vega *et al*., unpublished data).

Table 5.5. Faecal digestibility of fibre fractions of several feedstuffs in rabbits[a].

Feedstuff	NDFd	NSPd	TDFd	SDFd	Reference
Dehydrated lucerne	0.21–0.28	0.38–0.49	–	–	Gidenne *et al*. (1992)
Dehydrated lucerne (*n* = 12)	0.25–0.41	–	–	–	Perez (1994)
Lucerne hay (*n* = 6)	0.17–0.28	0.40	–	–	García *et al*. (1995, 1999)
Wheat bran	0.53	–	–	–	Gidenne (1987)
Wheat bran (*n* = 8)	0.25–0.41	–	–	–	Blas *et al*. (2000)
Beet pulp	0.84	–	–	–	Gidenne (1987)
Beet pulp	0.62	–	0.70	0.80	Abad-Guamán (2015)
Apple pulp	0.48	–	0.62	0.95	Abad-Guamán *et al*. (2015b)
Paprika meal	0.35	0.69	–	–	García *et al*. (1999)
Soybean hulls	0.28	0.35	–	–	García *et al*. (1999)
Sunflower hulls	0.10	0.22	–	–	García *et al*. (1999)
Barley straw, NaOH-treated	0.17	0.25	–	–	García *et al*. (1999)
Olive leaves	0.03	0.16	–	–	García *et al*. (1999)
Grape-seed meal	0.09	–	–	–	García *et al*. (2002b)
Palm kernel meal	0.43	–	–	–	Carrión *et al*. (2011)
Brewers grains	0.28	–	–	–	Guermah *et al*. (2016)
Maize silage	0.13	–	–	–	Guermah *et al*. (2016)

[a]Faecal non-starch polysaccharides digestibility (NSPd), and total dietary fibre and soluble DF digestibility (TDFd and SDFd; both corrected for faecal mucins).

They are highly lignified (≥290 g lignin kg^{-1} NDF) and the latter has a low proportion (0.26) of fine particles (<0.3 mm).

Accordingly, fibre digestion by the rabbit is moderate on average, but high for soluble fractions or low-lignified insoluble fibre that requires a shorter fermentation time. The degradation of these types of DF seems to begin along the stomach and small intestine and would be completed in the caecum.

Fermentation pattern

VOLATILE FATTY ACIDS. VFAs are the main products of carbohydrate microbial fermentation. Their concentration in the caecum has been used as an indirect estimation of microbial activity and is mainly affected by the quality of the DF, although great dietary changes are required to significantly modify VFAs due to their high variability. VFAs are rapidly absorbed in the hindgut and provide a regular source of energy for the rabbit. Butyrate seems to be a preferential source of energy for the hindgut, whereas acetate is mainly metabolized in the liver for lipogenesis and cholesterogenesis (Vernay, 1987). Furthermore, VFAs have been suggested to enhance colon mucosal growth (Chiou *et al*., 1994). Although VFAs have been proposed as a protective factor against pathogen microbiota (*Escherichia coli*) infections *in vitro* (Prohaszka, 1980; Wallace *et al*., 1989), no clear effects have been observed in rabbits *in vivo* (Gidenne and Licois, 2005).

Carbohydrate uptake by the caecal microbiota includes most of the cell wall constituents, in addition to undigested starch in the small intestine and endogenous mucopolysaccharides. Additionally, protein residues from the ileal digesta (undigested dietary protein, mucosal-cell protein, enzymes) can be utilized (after deamination) as an energy source for the microbial population. In this way, Vernay and Raynaud (1975) observed a caecal VFA concentration of 17.8 mmol l^{-1} in fasted rabbits, suggesting that a significant amount of endogenous materials can be fermented in the caecum, in agreement with the high caecal mucin degradability observed (Abad-Guamán *et al*., 2015a,b). In fact, a decrease of ileal protein digestibility increased caecal acidity, suggesting

an important role of protein in caecal fermentation (Gidenne and García, 2006). However, the relative contribution of these nitrogenous sources to total caecal VFA production remains unknown.

The caecal VFA concentration determined in 78 experimental diets averaged 57.4 mmol l^{-1} and ranged from 18.1 to 99.8 mmol l^{-1}. It increased with dietary uronic acid and NDF levels and decreased with the degree of lignification of NDF (García *et al*., 2002a). This result is in agreement with Trocino *et al*. (2013a), which reviewed the results from 63 diets and found a positive effect of dietary soluble fibre on caecal VFA concentration. Chiou *et al*. (1994) also observed a negative effect of lignin on caecal VFA concentration using isolated components of DF (cellulose, pectins and lignin) and lucerne hay.

The caecal VFA profile is specific to the rabbit, with a predominance of acetate (77 mmol 100 ml^{-1} on average, range 65–87 mmol 100 ml^{-1}), followed by butyrate (17 mmol 100 ml^{-1} on average, range 6–28 mmol 100 ml^{-1}) and then propionate (6 mmol 100 ml^{-1} on average, range 3–11 mmol 100 ml^{-1}). These molar proportions are affected by fibre level. For instance, the proportion of acetate increases and that of butyrate generally decreases when the fibre level increases, whereas the propionic acid proportion is only positively correlated to dietary uronic acids concentration (García *et al*., 2002a).

CAECAL pH. Caecal pH is frequently determined in digestion studies because it gives quickly an estimation of the extent of the fermentation. Caecal pH is negatively related to both dietary uronic acid and digestible NDF concentrations (García *et al*., 2002a), to the dietary soluble fibre (Trocino *et al*., 2013a), and accordingly to the total digested TDF (Fig. 5.4b). Consequently, caecal pH decreases with the inclusion of ingredients such as beet and fruit pulps, soy hulls and lucerne in the diet; the opposite occurs with cereal straw, sunflower hulls and grape-seed meal.

From a chemical point of view, caecal pH is expected to be related to VFAs and ammoniacal nitrogen, the main sources of hydrogen and hydroxide ions. However, a poor or absent relationship has been found between these variables, which only account for 12% of the variability observed in caecal pH (García *et al*., 2002a). This may be due to the presence of buffer substances in the caecum from endogenous or feed origin, which may also explain the stability of caecal pH among animals fed different diets. In fact, 68% of the variability of caecal pH is explained by the pH of the dry caecal contents (free from VFA and ammoniacal nitrogen), which is negatively related to the base-buffering capacity of the dry caecal contents (García *et al*., 2000).

References

Abad, R., Ibáñez, M.A., Carabaño, R. and García, J. (2013) Quantification of soluble fibre in feedstuffs for rabbits and evaluation of the interference between the determinations of soluble fibre and intestinal mucin. *Animal Feed Science and Technology* 182, 61–70.

Abad-Guamán, R. (2015) Identification of the method to quantify soluble fibre and the effect of the source of fibre on the ileal, and faecal digestibility of soluble and insoluble fibre in rabbits. Doctoral thesis, Universidad Politécnica de Madrid, Spain.

Abad-Guamán, R., Carabaño, R., Gómez-Conde, M.S. and García, J. (2015a) Effect of type of fibre, site of fermentation, and method of analysis on digestibility of soluble and insoluble fibre in rabbits. *Journal of Animal Science* 93, 2860–2871.

Abad-Guamán, R., Delgado, R., Carabaño, R. and García, J. (2015b) Efecto de las fracciones soluble e insoluble de la fibra de la pulpa de manzana sobre la digestibilidad ileal y fecal en conejos. *Proceedings XL Symposium de Cunicultura*, Santiago de Compostela, Spain, pp.56–60.

Abad-Guamán, R., Larrea-Dávalos, J.A., Carabaño, R., García, J. and Carro, M.D. (2018) Influence of inoculum type (ileal, caecal and faecal) on the in vitro fermentation of different sources of carbohydrates in rabbits. *World Rabbit Science* 26, 227–240.

AOAC (Association of Official Analytical Chemists) (2000) *Official Methods of Analysis*, 17th edn. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Bach Knudsen, K.E. (2001) The nutritional significance of 'dietary fibre' analysis. *Animal Feed Science and Technology* 90, 3–20.

Barry, J.L., Hoebler, C., David, A., Kozlowski, F. and Gueneau, S. (1990) Cell wall polysaccharides determination: comparison of detergent method and direct monomeric analysis. *Sciences des Aliments* 10, 275–282.

Bellier, R. and Gidenne, T. (1996) Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit. *British Journal of Nutrition* 75, 353–363.

Björnhag, G. (1972) Separation and delay of contents in the rabbit colon. *Swedish Journal of Agricultural Research* 7, 105–114.

Blas, E., Fernández-Carmona, J., Cervera, C. and Pascual, J.J. (2000) Nutritive value of coarse and fine wheat brans for rabbits. *Animal Feed Science and Technology* 88, 239–251.

Blas, E., Falcao, L., Gidenne, T., Scapinello, C., Pinheiro, V., García, A.I. and Carabaño, R. (2003) Interlaboratory study on ileal digestibility in rabbits: the effect of digesta collection time and a simplification of the procedure. *World Rabbit Science* 11, 101–111.

Boulahrouf, A., Fonty, G. and Gouet, P. (1991) Establishment, counts and identification of the fibrolytic bacteria in the digestive tract of rabbit. Influence of feed cellulose content. *Current Microbiology* 22, 1–25.

Brillouet, J.M., Rouau, X., Hoebler, C., Barry, J.L., Carré, B. and Lorta, E. (1988) A new method for determination of insoluble cell-walls and soluble non-starchy polysaccharides from plant materials. *Journal of Agricultural and Food Chemistry* 36, 969–979.

Carabaño, R. (1985) Influencia de la composición química de la dieta sobre distintos paráemtros cecales de conejos en crecimiento y su relación con el aporte nutritivo de las heces coprófagas. Doctoral thesis, Universidad Politécnica de Madrid, Spain.

Carabaño, R., Fraga, M.J. and de Blas, J.C. (1989) Effect of protein source in fibrous diets on performance and digestive parameters of fattening rabbits. *Journal of Applied Rabbit Research* 12, 201–204.

Carabaño, R., Motta-Ferreira, W., de Blas, J.C. and Fraga, M.J. (1997) Substitution of sugarbeet pulp for alfalfa hay in diets for growing rabbits. *Animal Feed Science and Technology* 65, 249–256.

Carabaño, R., García, J. and de Blas, C. (2001) Effect of fibre source on ileal apparent digestibility of non-starch polysaccharides in rabbits. *Animal Science* 72, 343–350.

Carraro, L., Trocino, A., Fragkiadakis, M., Xiccato, G. and Radaelli, G. (2007) Digestible fibre to ADF ratio and starch level in diets for growing rabbits. *Italian Journal of Animal Science* 6, 752–754.

Carré, B. and Brillouet, J.M. (1989) Determination of water-insoluble cell-walls in feeds: interlaboratory study. *Journal of the Association of Official Analytical Chemists* 72, 463–467.

Carrión, S., De Blas, J.C., Méndez, J., Caídas, A. and García-Rebollar, P. (2011) Nutritive value of palm kernel meal in diets for growing rabbits. *Animal Feed Science and Technology* 165, 79–84.

Castillo, C. (2013) Effect of dietary soluble fibre and threonine on digestion and growth performance in post-weaning rabbits. Masters thesis, Agronomic Institute of Zaragoza, University of Zaragoza and Spanish Foundation for the Development of Animal Nutrition, Spain.

Chiou, P.W.S., Yu, B. and Lin, C. (1994) Effect of different components of dietary fiber on the intestinal morphology of domestic rabbits. *Comparative Biochemistry and Physiology* 108A, 629–638.

Combes, S., Fortun-Lamothe, L., Cauquil, L. and Gidenne, T. (2013) Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. *Animal* 7, 1429–1439.

Combes, S., Massip, K., Martin, O., Furbeyre, H., Cauquil, L., Pascal, G., Bouchez, O., Le Floc'h, N., Zemb, O., Oswald, I.P. and Gidenne, T. (2017) Impact of feed restriction and housing hygiene conditions on specific and inflammatory immune response, the cecal bacterial community and the survival of young rabbits. *Animal* 11, 854–863.

de Blas, J.C., Santomá, G., Carabaño, R. and Fraga, M.J. (1986) Fiber and starch levels in fattening rabbit diets. *Journal of Animal Science* 63, 1897–1904.

De Vries, J.W. and Rader, J.I. (2005) Historical perspective as a guide for identifying and developing applicable methods for dietary fibre. *Journal of the AOAC International* 88, 1349–1366.

Delgado, R., Abad-Guamán, R., Nicodemus, N., Carabaño, R., Menoyo, D. and García, J. (2019) Effect of dietary soluble fibre level and n-6/n-3 fatty acid ratio on digestion and health in growing rabbits. *Animal Feed Science and Technology* 255, 114222.

EGRAN (European Group on Rabbit Nutrition); Gidenne, T., Perez, J.M., Xiccato, G., Trocino, A., Carabaño, R., Villamide, M.J., Blas, E., Cervera, C., Falcão e Cunha, L. and Maertens, L. (2001) Technical note: attempts to harmonize chemical analyses of feeds and faeces for rabbit feed evaluation. *World Rabbit Science* 9, 57–64.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. and Attia, H. (2011) Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: a review. *Food Chemistry* 124, 411–421.

Englyst, H. (1989) Classification and measurement of plant polysaccharides. *Animal Feed Science and Technology* 23, 27–42.

Escalona, B., Rocha, R., García, J., Carabaño, R. and de Blas, C. (1999) Characterization of *in situ* fibre digestion of several fibrous foods. *Animal Science* 68, 217–221.

EU (2011) Regulation (EU) No 1169/2011 of the European parliament and of the Council on the provision of food information to consumers. *Official Journal of the European Union* L 304 pp. 18–63.

Falcão e Cunha, L. and Lebas, F. (1986) Influence chez le lapin adulte de l'origine et du taux de lignine alimentaire sur la digestibilite de la ration et l'importance de la caecotrophie. *4 emes Journees de la Reserche Cunicole*, Communication no. 8.

Falcão e Cunha, L., Ferreira, P. and Bengala Freire, J.P. (1998) Étude de l'effet de l'interaction fibre x lipides dans l'alimentation du lapin: croissance, digestibilité et paramètres fermentaires. *7emes Journees de la Recherche Cunicole*, 155–158.

Falcão e Cunha, L., Jorge, J., Freire, J.P. and Pérez, H. (2000) Fat addition to feeds for growing rabbits, differing in fiber level and nature: effects on growth rate, digestibility and caecal fermentation patterns. *World Rabbit Science* 8 (Suppl. 1, Vol. C), 191–198.

Falcão e Cunha, L., Peres, H., Freire, J.P.B. and Castro-Solla, L. (2004) Effects of alfalfa, wheat bran or beet pulp, with or without sunflower oil, on caecal fermentation and on digestibility in the rabbit. *Animal Feed Science and Technology* 117, 131–149.

FAO (1998) Carbohydrates in human nutrition. (FAO Food and Nutrition Paper 66). Available at: http://www.fao.org/3/W8079E/W8079E00.htm (accessed 1 August 2019).

Farias, C., Feijoo, D., Gratta, F., Brambillasca, S., Ocasio-Vega, C., Nicodemus, N., Carabaño, R. and García, J. (2019) *Efecto del nivel de fibra insoluble y soluble sobre la cantidad de fibra fermentada a nivel ileal, cecal y fecal en gazapos en crecimiento*. XVIII Jornadas sobre Producción Animal de AIDA, Zaragoza, Spain.

FDA (2016) Food and Drug Administration, Revision of the Nutrition and Supplement Facts Label, US Federal Register, Vol.81, No.103.

FEDNA (2003) Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos, 2ª ed. In: de Blas, J.C., Mateos, G.G. and Rebollar, P.G. (eds) *Fundación Española para el Desarrollo de la Nutrición Animal*. FEDNA, Madrid, Spain.

Fraga, M.J., Pérez, P., Carabaño, R. and de Blas, J.C. (1991) Effect of type of fiber on the rate of passage and on the contribution of soft feces to nutrient intake of finishing rabbits. *Journal of Animal Science* 69, 1566–1574.

García, G., Gálvez, J.F. and de Blas, J.C. (1992) Substitution of barley grain by sugar-beet pulp in diets for finishing rabbits. 2. Effect on growth performance. *Journal of Applied Rabbit Research* 15, 1017–1024.

García, G., Gálvez, J.F. and de Blas, J.C. (1993) Effect of substitution of sugarbeet pulp for barley in diets for finishing rabbits on growth performance and on energy and nitrogen efficiency. *Journal of Animal Science* 71, 1823–1830.

García, J., Pérez-Alba, L., Alvarez, C., Rocha, R., Ramos, M. and de Blas, C. (1995) Prediction of the nutritive value of lucerne hay in diets for growing rabbits. *Animal Feed Science and Technology* 54, 33–44.

García, J., Carabaño, R. and de Blas, J.C. (1999) Effect of fiber source on cell wall digestibility and rate of passage in rabbits. *Journal of Animal Science* 77, 898–905.

García, J., Carabaño, R., Pérez-Alba, L. and de Blas, C. (2000) Effect of fiber source on cecal fermentation and nitrogen recycled through cecotrophy in rabbits. *Journal of Animal Science* 78, 638–646.

García, J., Gidenne, T., Falcão e Cunha, L. and de Blas, C. (2002a) Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Animal Research* 51, 165–173.

García, J., Nicodemus, N., Carabaño, R. and de Blas, J.C. (2002b) Effect of inclusion of defatted seed meal in the diet on digestion and performance of growing rabbits. *Journal of Animal Science* 80, 162–170.

Gidenne, T. (1987) Effet de l'addition d'un concentré en fibres dans une ration à base de foin, distribuée à deux niveaux alimentaires chez la lapine adulte. 2. Mesures de digestiblité. *Reproduction Nutrition Development* 27, 801–810.

Gidenne, T. (1992) Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *British Journal of Nutrition* 67, 133–146.

Gidenne, T. (1993) Measurement of the rate of passage in restricted-fed rabbits: effect of dietary cell wall level on the transit of fibre particles of different sizes. *Animal Feed Science and Technology* 42, 151–163.

Gidenne, T. (1994) Effets d'une reduction de la tener en fibres alimentaires sur le transit digestif du lapin. Comparaison et validation de modéles d'ajustement des cinétiques d'excrétion fécale des marqueurs. *Reproduction Nutrition Development* 34, 295–307.

Gidenne, T. (2015) Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review. *Animal* 9, 227–242.
Gidenne, T. and Bellier, R. (2000) Use of digestible fibre in replacement to available carbohydrates – effect on digestion, rate of passage and caecal fermentation pattern during the growth of the rabbit. *Livestock Production Science* 63, 141–152.
Gidenne, T. and García, J. (2006) Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens, L. and Coudert, P. (eds) *Recent Advances in Rabbit Sciences*. ILVO, Belgium, pp. 229–238.
Gidenne, T. and Jehl, N. (1996) Replacement of starch by digestible fibre in the feed for the growing rabbit. 1. Consequences for digestibility and rate of passage. *Animal Feed Science and Technology* 61, 183–192.
Gidenne, T. and Licois, D. (2005) Effect of a high fibre intake on the resistance of the growing rabbit to an experimental inoculation with an enteropathogenic strain of *Escherichia coli*. *Animal Science* 80, 281–288.
Gidenne, T. and Perez, J.M. (1993) Effect of dietary starch origin on digestion in the rabbit. 2. Starch hydrolysis in the small intestine, cell wall degradation and rate of passage measurements. *Animal Feed Science and Technology* 42, 249–257.
Gidenne, T. and Perez, J.M. (1994) Apports de lignines et alimentation du lapin en croissance. I. Conséquences sur la digestion et le transit. *Annales de Zootechnie* 43, 313–322.
Gidenne, T. and Perez, J.M. (2000) Replacement of digestible fibre by starch in the diet of the growing rabbit. I. Effects on digestion, rate of passage and retention of nutrients. *Annales de Zootechnie* 49, 357–368.
Gidenne, T. and Ruckebusch, Y. (1989) Flow and passage rate studies at the ileal level in the rabbit. *Reproduction Nutrition Development* 29, 403–412.
Gidenne, T., Carre, B., Segura, M., Lapanouse, A. and Gomez, J. (1991) Fibre digestion and rate of passage in the rabbit: effect of particle size and level of lucerne meal. *Animal Feed Science and Technology* 32, 215–221.
Gidenne, T., Bellier, R. and van Eys, J. (1998) Effect of the dietary fibre origin on the digestion and on the caecal fermentation pattern of the growing rabbit. *Animal Science* 66, 509–517.
Gidenne, T., Pinheiro, V. and Falcão e Cunha, L. (2000) A comprehensive approach of the rabbit digestión: consequences of a reduction in dietary fibre supply. *Livestock Production Science* 64, 225–237.
Gidenne, T., Arveux, P. and Madec, O. (2001) The effect of the quality of dietary lignocelluloses on digestion, zootechnical performance and health of the growing rabbit. *Animal Science* 73, 97–104.
Gidenne, T., Jehl, N., Segura, M. and Michalet-Doreau, B. (2002) Microbial activity in the caecum of the rabbit around weaning: impact of a dietary fibre deficiency and of intake level. *Animal Feed Science and Technology* 99, 107–118.
Gidenne, T., Jehl, N., Lapanouse, A. and Segura, M. (2004a) Inter-relationship of microbial activity, digestion and gut health in the rabbit: effect of substituting fibre by starch in diets having a high proportion of rapidly fermentable polysaccharides. *British Journal of Nutrition* 92, 95–104.
Gidenne, T., Mirabito, L., Jehl, N., Perez, J.M., Arveux, P., Bourdillon, A., Briens, C., Duperray, J. and Corrent, E. (2004b) Impact of replacing starch by digestible fibre, at two levels of lignocellulose, on digestion, growth and digestive health of the rabbit. *Animal Science* 78, 389–398.
Gómez-Conde, M.S., García, J., Chamorro, S., Eiras, P., Rebollar, P.G., Pérez de Rozas, A., Badiola, I., de Blas, C. and Carabaño, R. (2007) Neutral detergent-soluble fibre improves gut barrier function in 25 d old weaned rabbits. *Journal of Animal Science* 85, 3313–3321.
Gómez-Conde, M.S., Pérez de Rozas, A., Badiola, I., Pérez-Alba, L., de Blas, J.C., Carabaño, R. and García, J. (2009) Effect of neutral detergent soluble fibre on digestion, intestinal microbiota and performance in twenty five day old weaned rabbits. *Livestock Science* 125, 192–198.
Hall, M.B. (2003) Challenges with nonfiber carbohydrate methods. *Journal of Animal Science* 81, 3226–3232.
Hall, M.B., Lewis, B.A., Van Soest, P.J. and Chase, L.E. (1997) A simple method for estimation of neutral detergent-soluble fibre. *Journal of the Science of Food and Agriculture* 74, 441–449.
Hall, M.B., Hoover, W.H., Jennings, J.P. and Webster, T.K.M. (1999) A method for partitioning neutral detergent-soluble carbohydrates. *Journal of the Science of Food and Agriculture* 79, 2079–2086.
Hipsley, E.H. (1953) Dietary "fibre" and pregnancy toxaemia. *British Medical Journal* 2, 420–422.
Jehl, N. and Gidenne, T. (1996) Replacement of starch by digestible fibre in the feed for the growing rabbit. 2. Consequences for microbial activity in the caecum and on incidence of digestive disorders. *Animal Feed Science and Technology* 61, 193–204.
Jørgensen, H., Zhao, X.Q. and Eggum, B. (1996) The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hindgut and energy metabolism in pigs. *British Journal of Nutrition* 75, 365–378.

Lebas, F. and Lamboley, B. (1999) Liquid phase sifting determination of the size of particles contained in pelleted rabbits feeds. *World Rabbit Science* 7, 229–235.

Mañas, E. and Saura-Calixto, F. (1993) Ethanolic precipitation: a source of error in dietary fiber determination. *Food Chemistry* 47, 351–355.

Marounek, M., Vovk, S.J. and Skrinova, V. (1995) Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. *British Journal of Nutrition* 73, 463–469.

Marounek, M., Skrivanova, V. and Duskova, D. (2000) In vitro caecal fermentation of nitrogenous substrates in rabbits. *Journal of Agricultural Science* 135, 437–442.

Martínez-Vallespín, B., Martínez-Paredes, E., Ródenas, L., Moya, V.J., Cervera, C., Pascual, J.J. and Blas, E. (2013) Partial replacement of starch with acid detergent fibre and/or neutral detergent soluble fibre at two protein levels: effects on ileal apparent digestibility and caecal environment of growing rabbits. *Livestock Science* 154, 123–130.

McCleary, B.V., DeVries, J.W., Rader, J.I., Cohen, G., Prosky, L., Mugford, D.C., Champ, M. and Okuma, K. (2010) Determination of total dietary fiber (CODEX definition) by enzymatic-gravimetric method and liquid chromatography: collaborative study. *Journal of the AOAC International* 93, 221–233.

McDougall, G.J., Morrison, I.M., Stewart, D. and Hillman, J.R. (1996) Plant cell walls as dietary fibre: range, structure, processing and function. *Journal of the Science of Food and Agriculture* 70, 133–150.

Merino, J. and Carabaño, R. (1992) Effect of type of fibre on ileal and faecal digestibilities. *Journal of Applied Rabbit Research* 15, 931–937.

Mertens D.R. (2003) Challenges in measuring insoluble dietary fibre. *Journal of Animal Science* 81, 3233–3249.

Miner-Williams, W., Moughan, P.J. and Fuller, M.F. (2009) Methods for mucin analysis: a comparative study. *Journal of Food Chemistry* 57, 6029–6035.

Montoya, C.A., Rutherfurd, S.M. and Moughan, P.J. (2015) Nondietary gut materials interfere with the determination of dietary fiber digestibility in growing pigs when using the prosky method. *Journal of Nutrition* 145, 1966–1972.

Morisse, J.P. (1982) Taille des particules de l'aliment utilisé chez le lapin. *Revue Médecine Vétérinaire* 133, 635–642.

Motta-Ferreira, W., Fraga, M.J. and Carabaño, R. (1996) Inclusion of grape pomace, in substitution for lucerne hay, in diets for growing rabbits. *Animal Science* 63, 167–174.

Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (1999) Performance response of lactating and growing rabbits to dietary lignin content. *Animal Feed Science and Technology* 80, 43–54.

Nicodemus, N., García, J., Carabaño, R. and de Blas, J.C. (2002) Effect of inclusion of sunflower hulls in the diet on performance, disaccharidasse activity in the small intestine and caecal traits of growing rabbits. *Animal Science* 75, 237–243.

Nicodemus, N., García, J., Carabaño, R. and de Blas, C. (2006) Effect of a reduction of dietary particle size by substituting a mixture of fibrous by-products for lucerne hay on performance and digestión of growing rabbits and lactating does. *Livestock Science* 100, 242–250.

Nicodemus, N., Redondo, R., Pérez-Alba, L., Carabaño, R., De Blas, J.C. and García, J. (2010) Effect of level of fibre and type of grinding on the performance of rabbit does and their litters during the first three lactations. *Livestock Science* 129, 186–193.

Ocasio-Vega, C., Delgado, R., Abad-Guamán, R., Carabaño, R., Carro, M.D., Menoyo, D. and García, J. (2018a) The effect of cellobiose on the health status of growing rabbits depends on the dietary level of soluble fiber. *Journal of Animal Science* 96, 1806–1817.

Ocasio-Vega, C., Abad-Guamán, R., Delgado, R., Carabaño, R., Carro, M.D. and García, J. (2018b) In vitro caecal fermentation of carbohydrate-rich feedstuffs in rabbits as affected by substrate predigestion and donors' diet. *World Rabbit Science* 26, 15–25.

Perez, J.M. (1994) Digestiblité et valeur energetique des luzernes deshydratées pour le lapin: influence de leur composition chimique et de leur technologie de preparation. In: *Vièmes Journèes de la Recherche Cunicole, La Rochelle*, Vol. 2, pp. 355–364.

Prohaszka, L. (1980) Antibacterial effect of volatile fatty acids in enteric *E. coli* infections of rabbits. *Zentrabl Veterinaermed Reihe* B 27, 631–639.

Robertson, J.B. and Van Soest, P.J. (1981) The detergent system of analysis. In: James, W.P.T. and Theander, O. (eds) *The Analysis of Dietary Fibre in Food*. Marcel Dekker, New York, USA, pp. 123–158.

Rodríguez-Romero, N., Abecia, L. and Fondevila, M. (2012) Bacterial profile from caecal contents and soft faeces in growing rabbits given diets differing in soluble and insoluble fibre levels. *Anaerobe* 18, 602–607.

Romero, C., Nicodemus, N., Rodríguez, J.D., García, A.I. and De Blas, C. (2011) Effect of type of grinding of barley and dehydrated alfalfa on performance, digestion, and crude mucin ileal concentration in growing rabbits. *Journal of Animal Science* 89, 2472–2484.

St-Pierre, N.R. (2001) Invited review: integrating quantitative findings from multiple studies using mixed model methodology. *Journal of Dairy Science* 84, 741–755.

Svihus, B. and Zimonja, O. (2011) Chemical alterations with nutritional consequences due to pelleting animal feeds: a review. *Animal Production Science* 51, 590–596.

Tazzoli, M., Carraro, L., Trocino, A., Majolini, D. and Xiccato, G. (2009) Replacing starch with digestible fibre in growing rabbit feeding. *Italian Journal of Animal Science* 8, 148–150.

Tazzoli, M., Birolo, M., Filiou, E., Trocino, A., Zuffellato, A. and Xiccato, G. (2013) Increasing dietary energy with starch and soluble fibre and reducing adf at different protein levels for growing rabbits. *Agriculturae Conspectus Scientificus* 78, 235–239.

Tazzoli, M., Trocino, A., Birolo, M., Radaelli, G. and Xiccato, G. (2015) Optimizing feed efficiency and nitrogen excretion in growing rabbits by increasing dietary energy with high-starch, high-soluble fibre, low-insoluble fibre supply at low protein levels. *Livestock Science* 172, 59–68.

Trocino, A., Fragkiadakis, M., Radaelli, G. and Xiccato, G. (2010) Effect of dietary soluble fibre level and protein source on growth, digestion, caecal activity and health of fattening rabbits. *World Rabbit Science* 18, 199–210.

Trocino, A., Fragkiadakis, M., Majolini, D., Carabaño, R. and Xiccato, G. (2011) Effect of the increase of dietary starch and soluble fibre on digestive efficiency and growth performance of meat rabbits. *Animal Feed Science and Technology* 165, 265–277.

Trocino, A., García, J., Carabaño, R. and Xiccato, G. (2013a) A meta-analysis on the role of soluble fibre in diets for growing rabbits. *World Rabbit Science* 21, 1–15.

Trocino, A., Fragkiadakis, M., Majolini, D., Tazzoli, M., Radaelli, G. and Xiccato, G. (2013b) Soluble fibre, starch and protein level in diets for growing rabbits: effects on digestive efficiency and productive traits. *Animal Feed Science and Technology* 180, 73–82.

Uden, P., Robinson, P.H. and Wiseman, J. (2005) Use of detergent system terminology and criteria for submission of manuscripts on new, or revised, analytical methods as well as descriptive information on feed analysis and/or variability. *Animal Feed Science and Technology* 118, 181–186.

Van Soest, P.J. (1994) *Nutritional Ecology of the Ruminant*, 2nd edn. Cornell University Press, Ithaca, New York, USA.

Van Soest, P.J., Robertson, J.B. and Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber and on-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

Vernay, M. (1987) Origin and utilization of volatile fatty acids and lactate in the rabbit: influence of the faecal excretion pattern. *British Journal of Nutrition* 57, 371–381.

Vernay, M. and Raynaud, P. (1975) Répartition des acides gras volatils dans le tube digestif du lain domestique. II. Lapin soumis au jeûne. *Annales Recherches Vétérinaries* 6, 369–377.

Villamide, M.J., Carabaño, R., Maertens, L., Pascual, J., Gidenne, Falcão e Cunha, L. and Xiccato, G. (2009) Prediction of the nutritional value of European compound feeds for rabbits by chemical components and *in vitro* analysis. *Animal Feed Science and Technology* 150, 283–294.

Volek, Z., Marounek, M. and Skrivanová, V. (2005) Replacing starch by pectin and inulin in diet of early-weaned rabbits: effect on performance, health and nutrient digestibility. *Journal of Animal and Feed Sciences* 14, 327–337.

Volek, Z., Marounek, M. and Skrivanová,V. (2007) Effect of a starter diet supplementation with mannan-oligosaccharide or inulin on health status, caecal metabolism, digestibility of nutrients and growth of early weaned rabbits. *Animal* 1, 523–530.

Wallace, R.J., Falconer, M.L. and Bhargava, P.K. (1989) Toxicity of volatile fatty acids at rumen pH prevents enrichment of *Escherichia coli* by sorbitol in rumen contents. *Current Microbiology* 19, 277–281.

Wilfart, A., Montagne, L., Simmins, P., Van Milgen, J. and Noblet, J. (2007) Sites of nutrient digestion in growing pigs: effect of dietary fiber. *Journal of Animal Science* 85, 976–983.

Wiseman, J., Villamide, M.J., de Blas, C., Carabaño, M.J. and Carabaño, R.M. (1992) Prediction of the dietary energy value of feeds for rabbits. *Animal Feed Science and Technololgy* 39, 27–38.

Xiccato, G., Carazzolo, A., Cervera, C., Falcão e Cunha, L., Gidenne, T., Maertens, L., Perez, J.M. and Villamide, M.J. (1996) European ring-test on the chemical analyses of feed and faeces: influence on the calculation of nutrient digestibility in rabbits. In: Lebas, F. (ed.) *Proceedings of the 6th World Rabbit Congress, Toulouse*, Vol., 1. Association Française de Cuniculture, Lempdes, France, pp. 293–297.

Xiccato, G., Trocino, A., De Boever, J.L., Maertens, L., Carabaño, R., Pascual, J.J., Perez, J.M., Gidenne, T. and Falcão e Cunha, L. (2003) Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). *Animal Feed Science and Technology* 104, 153–168.

Xiccato, G., Trocino, A., Carraro, L. and Fragkiadakis, M. (2006) Digestible fibre to ADF ratio and protein concentration in diets for early-weaned rabbits. *Proceedings 3rd Rabbit Congress of the Americas*. Maringá, Brasil 35, 1–6.

Xiccato, G., Trocino, A., Carraro, L., Fragkiadakis, M. and Majolini, D. (2008) Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. *Proceedings 9th World Rabbit Congress*. Verona, Italy, pp 847–851.

Xiccato, G., Trocino, A., Majolini, D., Fragkiadakis, M. and Tazzoli, M. (2011) Effect of decreasing dietary protein level and replacing starch with soluble fibre on digestive physiology and performance of growing rabbits. *Animal* 5, 1179–1187.

Xiccato, G., Trocino, A., Tazzoli, M., Majolini, D., Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Abad, R., Blas, E., Cervera, C., Ródenas, L., Martínez, E., Falcao-E-Cunha, L., Bengala Freire, J.P., Maertens, L., Bannelier, C., Segura, M. and Gidenne, T. (2012) European ring-test on the chemical analyses of total dietary fibre and soluble fibre of compound diets and raw materials for rabbits. *Proceedings 10th World Rabbit Congress*. Sharm El-Sheikh, Egypt, pp. 453–471.

Yu, B., Chiou, P.W.S., Young, Ch.L. and Huang, H.H. (1987) A study of ratty T-type cannule and its ileal digestibilities. *Journal of the Chinese Society of Animal Science* 16, 73–81.