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2 Digestion of Sugars and Starch

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It is possible to classify the carbohydrate fractions of plants incorporated into animal feed into two groups: (i) those that are hydrolysable by the endogenous intestinal enzymes of the animal (these polysaccharides are located predominantly within the plant cell); and (ii) those that are hydrolysable only by enzymes produced by the digestive microbiota (these are mainly cell-wall polysaccharides and are considered in Chapter 5). The former can be further separated into two main groups: first, the simple sugars and oligosaccharides, which are present at low levels (<50 g kg^{-1}) in rabbit feeds; and second, the polysaccharides represented mainly by the starches (contributing 100–250 g kg^{-1}). The structure and digestion of starch are described later in this chapter. First, the digestion of simple sugars and oligosaccharides are considered.

2.1 Simple Sugars and Oligosaccharides

These two types of carbohydrate are often classed simply under one general term, 'the sugars'. Nevertheless, from a biochemical point of view, it is convenient to distinguish clearly between simple sugars and oligosaccharides because they are not digested by the same processes. For instance, the α-galactosides are only hydrolysed by bacterial enzymes, whereas simple sugars are very rapidly hydrolysed by enzymes of the host and absorbed by intestinal mucosa.

2.1.1 Definition, structure and analysis

Sugars are generally found at low concentrations in animal feeds, although the level of sucrose can reach 500 g kg^{-1} in some raw materials such as molasses (Table 2.1). Among the sugars found in common raw materials, glucose and fructose are the two major types, found as monosaccharides or as the disaccharide sucrose (glucose + fructose, α[1→2]β). Furthermore, compared with other mammals, lactose (glucose + galactose, α[1→4]) is at a very low level (50 g kg^{-1} dry matter (DM)) in the milk of the rabbit female (Maertens *et al.*, 2006), and thus is not used in feeding the young rabbit. Other disaccharides can also occur in the feed: maltose (two glucose units, α[1→4]), which mainly originates from starch hydrolysis, and melibiose (galactose + glucose, α[1→6]), which is found in some roots.

Oligosaccharides are defined as molecules with a low degree of polymerization (dp). Maltotriose corresponds to three units of glucose linked by α(1→4) bonds and originates from starch hydrolysis. The α-galactosides (dp 3–5) are a group of oligosaccharides (raffinose, stachyose, verbascose and ajugose) that are not digestible by the endogenous enzymes of the animal, but are rapidly degraded and fermented by

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Table 2.1. Level of total sugars[a] in some raw materials (INRA-CIRAD-AFZ Feed Tables, 2018).

Raw material	Sugars (g kg^{-1} air dry)
Beet molasses	466
Sugarbeet	160
Sugarbeet pulp	66
Citrus pulps	200
Apple pomace	140
Sweet lupin	64
Soybean meal	83
Sunflower meal	57
Brewer's grain	9
Wheat bran	67
Barley	21
Winter pea	39

[a]Analysed as total sugars soluble in ethanol (80%, v/v).

the caecal microbiota. They are found mainly in legume seeds or extracted legume meals. Pea seeds contain 50 g kg^{-1}, lupin seeds 40 g kg^{-1} and soybean meal 50 g kg^{-1}, all on a DM basis (INRA-CIRAD-AFZ Feed Tables, 2018).

Simple sugars and oligosaccharides are solubilized in boiling ethanol (80%, v/v). Following acid hydrolysis (with hot concentrated sulphuric acid), the total sugars can be quantified (as monosaccharides) either through colorimetric or chromatographic methods. The choice of extraction process is important when analysing the sugars in a raw material or a feed. For instance, the cold extraction (ethanol 40%, v/v, at ambient temperature) recommended in the European Community (EC) method (AFNOR, 1985) would not be adequate for extracting all sugars. This would explain the unexpected low values sometimes found for total sugars in soybean meals and legume seeds. Attention must be paid to the colorimetric determination of sugar in digesta and faeces because of interference by bile pigment (particularly for the glucose determination in a starch analysis procedure). It is thus recommended to avoid the glucose-oxidase/peroxidase technique and to use the hexokinase/glucose-6-phosphate-dehydrogenase system (Kozlowski, 1994).

2.1.2 Digestion

Compared with starch, glucose and fructose are rapidly absorbed by the intestinal mucosa. However, fructose could be absorbed more slowly than glucose in pigs (Carré, 1992). In contrast with glucose, fructose is probably not absorbed through an energy-dependent mechanism. The level of sugars (soluble in 80% ethanol) in the ileal contents, including therefore ethanol-soluble α-glucosides and glucose resulting from digestion of starch, reached 25 g kg^{-1} DM for adult rabbits fed a standard commercial diet (Gidenne and Ruckebusch, 1989), indicating that the flow of sugars entering the caecum is not negligible. Further studies are necessary to evaluate the digestion of sugars, especially in the young animal.

Complete digestion of α-galactosides has been observed in rats and pigs (Goodlad and Mathers, 1991). It is assumed that they are also totally digested by the caecal microbiota in rabbits, although this has not yet been measured.

2.2 Starch

2.2.1 Definition, structure and analysis

Starch (α-glucan) is a major reserve polysaccharide of green plants and probably the second most abundant carbohydrate in nature next to cellulose. In some cases, the reserve polysaccharides of the plant are α-fructans, such as inulin (linear α-fructan, dp ≈30) in the Jerusalem artichoke (*Helianthus tuberosus*) or levan (branched α-fructan, dp ≈100) in some grasses. Starch is found in nature as granules either in seeds, roots or tubers. The shape of the starch granule depends on the botanical source and many different sizes and forms are found – from tiny granules in oats or rice (5–6 μm) to larger granules in banana (38–50 μm). The interior of a granule is composed of alternating crystalline and amorphous regions. The disruption of this organization is the basis of gelatinization. The starch granule is modified by either chemical or physical treatment (e.g. heat, pressure). A prerequisite for digestion is that the enzymes are adsorbed onto the starch granule. Hydrolysis may then proceed either through surface erosion or penetration via pinholes. Analysis and functional aspects of starch have been reviewed by Englyst *et al.* (2007).

From a biochemical point of view, starch is a polysaccharide composed simply of D-glucose units. Starch basically consists of a mixture of

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two types of chains: (i) amylose, a linear chain of glucose (α[1→4] links); and (ii) amylopectin, a branched chain (α[1→4] + α[1→6] links). However, the polymeric structure of starch is more complicated, and its primary structure is not yet fully understood. Starch is the subject of many investigations because of its multiple uses in chemistry, the food industry, fermentation processes and so on.

It is now recognized that some amylose molecules have several branches. In addition, the presence of materials intermediate between amylose and amylopectin has been suggested in amylomaize (maize rich in amylose) and wrinkled-pea starches (Hizukuri, 1996). The relative proportion of amylose and amylopectin may vary considerably according to the plant source, and this may significantly affect its digestion (Table 2.2). For instance, maize rich in amylose has a lower digestibility than standard maize. In addition, the starch granule is sometimes encapsulated in a protein matrix that reduces its accessibility to enzymes. Thus, starch degradation is dependent on the biochemical and physical structure of the granule.

Numerous processes currently used in animal feed manufacturing are able to modify the starch granule, either slightly by steaming in the pelleting process or strongly by using temperature combined with hydration and pressure in the extrusion process. The interaction between starch and water is well known: the starch structure is strongly hydrophilic and the ratio of starch to water is inversely correlated to the gelatinization temperature (Champ and Colonna, 1993). In general, with an excess of water and temperatures over 55°C, the granules swell and solubilize (disorganization/dispersion of the structure); this is the gelatinization step (in the case of pure starch, a viscous solution results). Following cooling, the chains of glucose can reassociate. This is termed retrogradation and can lead to forms of starch that are resistant to amylases. Complete gelatinization of the starch granule is essential for the correct determination of the starch using enzymatic procedures.

Starch is usually determined in animal feeds or raw materials through the Ewers EC (optical rotation determination) or enzymatic methods (hydrolysis followed by glucose determination). The two techniques provide, in general, very well-correlated data, with slightly higher values for the Ewers EC method (+0.5–4%). The differences between the two methods are greater for legume than for cereal materials. The difference lies in the fact that the Ewers EC method may interfere with unextracted sugars or acid-labile polysaccharides. For example, the recommended starch method for beet pulp is the enzymatic one. Specific procedures are also recommended for starch determination in digesta and faeces (Kozlowski, 1994). If no previous ethanol extraction of samples has been carried out when using enzymatic procedures, starch, ethanol-soluble α-glucosides and glucose are considered as a whole.

2.2.2 Digestion of starch in the different parts of the gastrointestinal tract

Starch is almost completely digested in the digestive tract of rabbits, as in other livestock species. For this reason, the faecal excretion of starch is

Table 2.2. Starch and amylose concentrations in some raw materials, and respective faecal digestibility for the rat.

Source	Starch (g kg^{-1} DM)	Amylose (proportion of starch)	Faecal digestibility of starch in the rat[a]
Soft wheat	650–700	0.25–0.30	0.98–1.00
Maize	650–800	0.20–0.24	0.98–1.00
Maize rich in amylose	500–650	0.60–0.65	0.66–0.77
Smooth pea	430–480	0.31–0.35	0.99
Fava bean	300–430	0.31–0.34	0.99
Banana (green)	150–250	0.15–0.18	0.49
Cassava roots	800–850	0.17	0.95–0.97
Potato (uncooked)	600–650	0.20	0.27–0.28

[a]Champ and Colonna (1993).

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generally minimal (less than 0.02 of intake), although in some cases it can reach 0.10 of intake, depending mainly on the age of the rabbit and the source of the starch, both of which will be discussed later.

It is acknowledged that starch digestion takes place mainly in the small intestine. However, starch may also be degraded to some extent in other parts of the digestive tract, such as the stomach and large intestine. It would be of particular interest to evaluate the degradation of starch (or of intermediate α-glucosides and glucose not absorbed in the small intestine) by the microbiota in the large intestine and to review the factors affecting the ileal flow of starch and the possible consequences on caeco-colic fermentative activity, as well as on the stability of the microbial ecosystem and digestive health. Therefore, the following section discusses various aspects of the process of starch digestion in the different parts of the gastrointestinal tract of rabbits.

Gastric digestion

There are no reliable measurements of the extent of starch hydrolysis in the stomach. It has been observed that the starch concentration in the gastric digesta is clearly less than in the diet (Fraga *et al.*, 1984; Blas, 1986). Wolter *et al.* (1980) observed that, for restricted-fed rabbits slaughtered 4 h after feeding, 0.31 of the starch ingested had been hydrolysed in the stomach. However, this is probably an overestimate because of dilution of the diet with soft faeces intake (from caecotrophy), and of the intake of marker from soft faeces that was not taken into account.

Amylase in the stomach originates essentially from the soft faeces (bacterial origin) and saliva, and remains at a constant level from week 4 of life independently of starch intake (Blas, 1986). The low gastric pH is the main factor limiting its activity. Marounek *et al.* (1995) did not find amylase activity in the contents of the stomach of 4-week-old and 3-month-old rabbits, with enzyme-substrate incubations undertaken at pH 2.5. On the other hand, Sequeira *et al.* (2000) observed amylase activity in the gastric contents of 9-week-old rabbits with incubations carried out at pH 6.9.

In fact, the amylase activity of the stomach contents disappears completely if the pH is lower than 3.2 (Blas, 1986), and the gastric pH in the antrum is usually around 2 (see Chapter 1). However, the buffering capacity of the diet, soft faeces and saliva probably prevents immediate acidification. For instance, Blas (1986) found a pH of 4–4.5 in the stomach contents of growing rabbits 150 min after feeding following a 24-h fast; and Herrmann (1989) even reported a pH of >5 in certain areas of the stomach after high feed intake. However, rabbits that are fed *ad libitum* have 20–30 'voluntary meals' a day, and the gastric pH is thus normally <2.5. Nevertheless, as a consequence of physiological hypochlorhydria in young rabbits, the gastric pH is >5 in 3-week-old rabbits and still >4 in 4-week-old rabbits, as reviewed by Gidenne and Fortun-Lamothe (2002). On the other hand, while soft faeces are being stored the pH of the fundus can rise to 4.0–5.1, whereas the pH of the antrum always remains below 2 already at 3 weeks of age (Orengo and Gidenne, 2007).

Under these less acidic conditions, the amylase in the stomach contents, especially that of microbial origin from soft faeces, maintains appreciable activity (Alexander and Chowdhury, 1958; Hörnicke and Mackiewicz, 1976; Blas, 1986; Vernay, 1986). Table 2.3 illustrates this process of gastric fermentation (originating from starch, sugars and perhaps other carbohydrates), demonstrating that the concentration of lactate in both the gastric digesta (and not in

Table 2.3. Effect of caecotrophy prevention on lactate concentration in the gastric contents and blood of rabbits (adapted from Vernay, 1986).

	Control	Caecotrophy prevention for 4 days
Feed intake (g day^{-1})	124	127
Gastric contents (mM)		
Fundus	4.7	1.9
Corpus	3.4	1.9
Antrum	2.4	1.6
Blood (mM in plasma)		
Venous		
Gastric	3.4	1.9
Ileal	3.8	2.1
Caecal	2.8	1.9
Portal	3.6	2.5
Arterial (abdominal aorta)	3.0	1.8

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that of the other parts of the digestive tract) and the blood falls significantly when caecotrophy is prevented.

Intestinal digestion

As stated above, starch digestion takes place mainly in the small intestine, and the most important enzyme involved is pancreatic amylase. Other enzymes of the epithelial cells of the intestinal mucosa are also necessary (maltase, amyloglucosidase), resulting finally in the release of glucose, which in principle is absorbed *in situ*.

Studies to assess the capacity of rabbits to digest starch in the small intestine vary widely in methodological aspects, such as: (i) target samples (pancreatic tissue or secretion, intestinal mucosa, intestinal contents); (ii) time of sampling (evening, morning, without or with a previous fasting period); (iii) conditions in assaying the enzyme activity (with or without considering optimal kinetic parameters, concerning substrate and enzyme concentrations, incubating period, temperature, pH); and (iv) units used to express enzyme activity (as relative to protein in pancreatic tissue or secretion, as relative to pancreatic tissue or secretion, as relative to intestinal mucosal protein or tissue, as relative to intestinal contents, as total in pancreatic tissue, intestinal mucosa or intestinal contents kg^{-1} live weight). All these methodological variations make it difficult to compare the results about the age-dependent evolution of the intestinal capacity to digest starch and the possible role of starch intake in modulating this capacity. Nevertheless, the ontogenic development of starch digestion is well established. Amylase activity increases rapidly between weeks 2 and 7 of life (Corring *et al.*, 1972; Blas, 1986; Scapinello *et al.*, 1999; Gutiérrez *et al.*, 2002a; Toral *et al.*, 2002; Gallois *et al.*, 2008a) and is still increasing in 3-month-old rabbits (Marounek *et al.*, 1995; Dojanã *et al.*, 1998). Similarly, the amyloglucosidase activity of the jejunal mucosa generally increases between 37 and 60 days of age (Otutumi *et al.*, 2005). However, the ontogenic development of intestinal maltase activity remains controversial. According to Toofanian (1984) and Gallois *et al.* (2008a), maltase activity increases very rapidly between weeks 2 and 4 of life, but not afterwards; others have reported increasing maltase between 32 and 42 days of life (Debray *et al.*, 2003; Gidenne *et al.*, 2007), and even between 1 and 3 months (Marounek *et al.*, 1995). Still further studies have reported no changes in maltase activity between 25- and 35-day-old rabbits (Gutiérrez *et al.*, 2002a) or between 32- and 42-day-old rabbits (Scapinello *et al.*, 1999). Finally, Otutumi *et al.* (2005) found similar maltase activity in the jejunal mucosa of 37- and 60-day-old rabbits, while it was higher in jejunal contents for 60- than for 37-day-old rabbits, maltase activity being expressed per mg of, respectively, mucosa or contents.

Modulating the intestinal capacity for starch digestion according to diet is usually approached by varying starch intake. In many species, it is acknowledged that the digestive potential of the small intestine adapts to higher starch intake by increasing pancreatic amylase, intestinal maltase and amyloglucosidase secretion. Blas (1986) found higher amylase activity in the pancreatic secretions of both growing (28- and 42-day-old) and adult rabbits with a higher starch intake in samples obtained 150 min after feeding following a 24-h fast (no differences were found in basal samples taken following a 24-h fast). Similar results have been observed in adult rabbits in both pancreatic and intestinal tissue, and also in the intestinal contents (Abbas *et al.*, 1991).

However, more recent studies that have investigated differences in the starch intake of young rabbits by modifying the dietary starch concentration (consequently changing other components, such as fibre) seem to disagree with the above-mentioned adaptability of the digestive potential. For instance, Debray *et al.* (2003) found no differences either in pancreatic amylase or in intestinal mucosal or intraluminal maltase in 42-day-old rabbits consuming twice the amount of starch than controls. Furthermore, Gidenne *et al.* (2007) reported higher amylase activity in the total intestinal contents in 42-day-old rabbits consuming one-third less starch than controls, while Gutiérrez *et al.* (2002a) reported no changes in pancreatic amylase in 35-day-old rabbits consuming twice the amount of starch (by replacing lactose) than controls and higher pancreatic amylase and jejunal mucosal maltase when consuming one-third less starch.

Finally, studies in young rabbits inducing differences in starch intake by stimulating feed

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intake through early weaning or milk restriction have reported contradictory results. Corring *et al.* (1972) found that pancreatic amylase activity in 30-day-old rabbits was higher in those weaned at 21 days than in those remaining suckling. Gutiérrez *et al.* (2002a) found even wider differences in 35-day-old rabbits depending on whether they were weaned at 25 days old or remained suckling, although jejunal mucosal maltase decreased in those that were early weaned as a consequence of impairing mucosal morphology. Conversely, higher feed and starch intake before weaning in milk-restricted rabbits had no effect on amylase and maltase activities in the intestinal contents at weaning or 10 days later (Scapinello *et al.*, 1999). Gallois *et al.* (2008a) reported lower amylase activity in the intestinal contents but higher maltase activity in the intestinal mucosa of 28-day-old rabbits that were still suckling than in those early weaned at 21 days of age (without negative effects of early weaning on mucosal morphology).

Caecal fermentation

Starch undigested in the small intestine is very quickly hydrolysed and fermented by the microbiota in the caeco-colic segment to lactate and volatile fatty acids (VFAs), absorbed *in situ*. Different studies have demonstrated the presence of amylase activity in the hindgut (Yoshida *et al.*, 1968; Blas, 1986; Makkar and Singh, 1987; Marounek *et al.*, 1995). Some data suggest that amylase could be of microbial origin and also from the ileal digesta flow. For instance, amylase activity in the caecum and the colon is even greater in germ-free rabbits than in normal rabbits (Yoshida *et al.*, 1968), and is more than twice as high in the rabbit caecum than in the rumen of steers (Makkar and Singh, 1987). Blas (1986) observed that amylase activity in the caecal contents hardly varied with age in 4- to 8-week-old rabbits, but was four times greater with a diet rich in starch than with a low-starch diet. On the other hand, Marounek *et al.* (1995) found amylase activity in caecal contents to be 28% higher in 3-month-old rabbits than in 4-week-old rabbits.

Stable high counts of amylolytic bacteria have been reported in the caecal contents of 2- to 7-week-old rabbits (Padilha *et al.*, 1995). Several strains of caecal bacteria (*Actinomyces israelii*, *Dichelobacter nodosus*, *Mitsuokella multiacidus*, *Bacteroides* spp., *Eubacterium* spp., *Clostridium* spp.) have been shown to produce extracellular or membrane-bound α-amylases (Sirotek *et al.*, 2006). In contrast, the *in vitro* fermentation of soluble potato starch, used as a substrate for determining α-amylase, by inocula prepared from caecal contents of 36- or 78-day-old rabbits, was slow and poor (long lag phase, long time to reach maximum fermentation rate, low maximum fermentation rate), especially in the younger rabbits. This suggested that the mean retention time of digesta in the caecum (6–12 h) would not allow a complete fermentation of the starch in the caeco-colic segment (Lavrenčič, 2007). It could be hypothesized that a low availability of glucose exo-splitting enzymes (β-amylase, amyloglucosidase) is a limiting factor. In a subsequent study with caecal inocula from 78-day-old rabbits, Kermauner and Lavrenčič (2012) observed that the kinetics of *in vitro* fermentation varied according to the source of starch. The time required to reach the maximum fermentation rate was shorter with cooked potato, intermediate with raw potato and wheat, and longer with maize and starch isolated from potato, wheat or maize. The gas production at 10 h of incubation varied on the contrary, which suggested that the process of isolation of starch from potato and wheat produced changes in the properties of starch that negatively affected its fermentation, and that only cooked potato starch could be extensively fermented *in vivo* while the fermentation of maize starch would be negligible. As shown later, the amount of starch passing the ileo-caecal junction being fermented *in vivo* can be calculated from the difference between its ileal and faecal digestibility.

2.2.3 Factors affecting starch digestibility

As stated above, starch digestion is primarily affected by the age of the rabbit and by the dietary level and origin of the starch. Other factors may also have some influence, such as the feed manufacturing process or the use of exogenous enzymes as dietary supplements.

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Age and starch in the diet

ADULT RABBITS. Table 2.4 shows starch digestibility data obtained from different studies in adult rabbits. Faecal losses of starch are always very low (<0.01 of intake). These losses are greater for maize than for other sources (Gidenne and Perez, 1993b; Amber, 1997), although the differences are quantitatively of little relevance. The faecal digestibility of starch is largely independent of starch intake. Accordingly, de Blas *et al.* (1995) found no variation between lactating and non-lactating rabbit does, with the former consuming more than twice the amount of starch. As expected, almost all of the starch is hydrolysed before reaching the caecum: its ileal digestibility is about 0.97 and is largely independent of both the starch intake and the source of starch. Only Pinheiro (2002) detected a reduction in the ileal digestibility of starch when replacing half of the starch from wheat with purified potato starch (0.983 versus 0.967), the latter being very resistant to *in vitro* digestion with thermostable amylase during 270 min at pH 4.5 and 37°C (Pinheiro and Gidenne, 2000). Consequently, the ileal flow of starch is low: 1.0–3.2 g day^{-1} (Gidenne, 1992), 0.3–1.3 g day^{-1} (Amber, 1997), 0.2–2.3 g day^{-1} (Gidenne *et al.*, 2000) and 0.5–1.1 g day^{-1} (Pinheiro, 2002). The amount of starch fermented in the caecocolic segment of adult rabbits is between 0.01 and 0.07 of the starch intake.

GROWING RABBITS. A review of 43 studies involving 125 different diets, from 1982 to 2019, reveals that faecal digestibility in growing rabbits (4–11 weeks old) is higher than 0.96 (averaging 0.984) in most cases, independent of the age of the rabbits, starch intake and starch source (barley, wheat, wheat bran, maize, oats, triticale, cassava, purified maize starch or purified potato starch). There are some exceptions that will be discussed later, essentially suggesting an interaction between the age of rabbit and some starch sources.

The effect of rabbit age is very limited for the majority of starch sources. In fact, faecal digestibility of starch during week 4 of life, in still-suckling young rabbits beginning to consume solid feed, is almost total (0.98–0.99) with starch from barley, wheat or pea, with minor but significant reduction when including pea (Blas, 1986; Gidenne *et al.*, 2007). However, as shown in Table 2.5 and Fig. 2.1, faecal losses of starch can increase in some cases, usually for maize and particularly for the youngest rabbits. The resistance of maize starch to digestion is also seen in pigs and ruminants, but not in poultry. The endosperm structure of maize seeds and their

Table 2.4. Ileal and faecal digestibility of starch in adult rabbits fitted with an ileal cannula.

Source of starch	Dietary level of starch (g kg^{-1} DM)	Digestibility of starch		Reference
		Ileal	Faecal	
Purified maize starch	158	0.945	0.993	Gidenne (1992)
	353	0.947	0.996	
Barley	306	0.982	0.995	Merino and Carabaño (1992)
Purified maize starch	256	–	0.997	Gidenne and Perez (1993b)
Maize	292	–	0.990	Gidenne and Perez (1993b)
Barley	283	–	0.998	Gidenne and Perez (1993b)
Pea	280	–	0.996	Gidenne and Perez (1993b)
Purified maize starch	280	0.992	0.998	Amber (1997)
	353	0.991	0.999	
Maize	103	0.972	0.989	Amber (1997)
	255	0.971	0.990	
Barley	102	0.970	0.990	Amber (1997)
	251	0.984	0.994	
Wheat, wheat bran	116	0.987	0.992	Gidenne *et al.* (2000)
Wheat	329	0.930	0.997	Gidenne *et al.* (2000)
Wheat, wheat bran	220	0.983	0.997	Pinheiro (2002)
Purified potato starch, wheat	223	0.967	0.996	Pinheiro (2002)

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Table 2.5. Faecal digestibility of starch from several studies on growing rabbits showing values of <0.96.

Source of starch	Age (weeks)	Faecal digestibility of starch	Reference
Wheat bran, barley	8	0.945	Parigi-Bini *et al*. (1990)
Maize	7–9	0.945	de Arruda *et al*. (2002)
Wheat, wheat bran	8–11	0.950	Cossu *et al*. (2004)
Maize, wheat bran	9	0.957	Michelan *et al*. (2006)
Barley (coarse), wheat bran; with coarse lucerne	6	0.932	Romero *et al*. (2011)
Barley (coarse), wheat bran; with fine lucerne	6	0.954	Romero *et al*. (2011)
Barley (fine), wheat bran; with coarse lucerne	6	0.950	Romero *et al*. (2011)
Barley (fine), wheat bran; with fine lucerne	6	0.954	Romero *et al*. (2011)
Wheat bran	8	0.959	Trocino *et al*. (2011)
Barley, wheat bran	7	0.933	Volek and Marounek (2011)
Barley, wheat bran, oats	7	0.947	Volek and Marounek (2011)
Barley, oats, wheat bran	7	0.953	Volek and Marounek (2011)

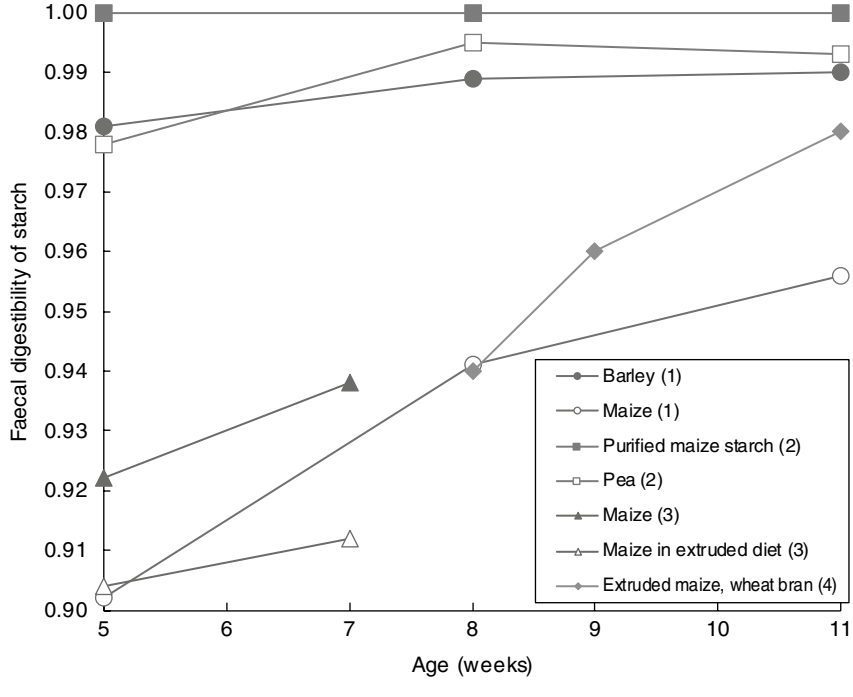

Fig. 2.1. Effect of age and starch source on the faecal digestibility of starch in growing rabbits. 1, Blas *et al*. (1990); 2, Gidenne and Perez (1993a); 3, Maertens and Luzi (1995); 4, Cossu *et al*. (2004).

resistance to grinding are considered the main factors behind this lower degradation (Rooney and Pflugfelder, 1986). These disappear in the process of manufacturing purified maize starch. Nevertheless, some studies using maize as the only or main starch source have reported faecal digestibility of starch similar to that from other sources, from 0.98 to 1.00 (Toral *et al*., 2002;

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Xiccato *et al.*, 2002; Furlan *et al.*, 2003). It must be stated that the differences between varieties of a particular grain, with special reference to the amylose:amylopectin ratio, may affect faecal losses of starch. This may also help to explain some values of <0.96 of faecal digestibility of starch from wheat, barley, oats and wheat bran (Parigi-Bini *et al.*, 1990; Cossu *et al.*, 2004; Romero *et al.*, 2011; Trocino *et al.*, 2011; Volek and Marounek, 2011) (see Table 2.5).

In different studies with both growing and adult rabbits, comparing various dietary starch levels (Blas, 1986; Blas *et al.*, 1990; Parigi-Bini *et al.*, 1990; Gidenne, 1992; de Blas *et al.*, 1995; Amber, 1997; Gidenne and Perez, 2000; de Arruda *et al.*, 2002; Gutiérrez *et al.*, 2002a; Pinheiro *et al.*, 2009; Trocino *et al.*, 2011, 2013; Volek and Marounek, 2011; Xiccato *et al.*, 2011; Castillo, 2013; Tazzoli *et al.*, 2013, 2015; Delgado *et al.*, 2017), the faecal digestibility of starch decreased systematically in diets with lower starch content in comparison with those of higher starch content (even with the same source of starch). Although statistically significant, this decrease remains small and may often be considered irrelevant. There is no clear explanation for these results. In fact, a lower dietary starch level usually corresponds to a higher fibre level, and thus it can be hypothesized that a faster rate of passage leads to a lower efficiency in starch degradation. A presence of endogenous α-linked glucose polymers (e.g. dextrans in the microbial reserves) being proportionally more important in diets lower in starch can also be hypothesized, particularly when increasing dietary soluble fibre, caecal fermentative activity and microbial presence in faeces (Delgado *et al.*, 2017).

Results on the ileal digestibility of starch in growing rabbits are summarized in Table 2.6, with values ranging between 0.87 and 0.99 and averaging 0.938. No clear evidence of effect of the dietary starch level or source on its ileal digestibility has been detected, although studies with sources of more resistant starch (e.g. maize) are not available. However, some other factors can be considered as affecting the efficiency of starch digestion in the small intestine. In this context, the ileal digestibility of starch in 36-day-old rabbits was lower than in 43-day-old rabbits when the dietary starch level was increased enough (Soler, 2014). On the other hand, Gómez-Conde *et al.* (2007) found that the ileal digestibility of starch increased when the dietary neutral detergent-soluble fibre (NDSF) rose in iso-starch diets, concurrently with improved intestinal mucosal morphology (higher villous height to crypt depth ratio) and functionality (higher sucrose activity). Similarly, other studies observed higher ileal digestibility of starch when soluble fibre (SF, calculated as total dietary fibre – neutral detergent fibre) increased at the expense of starch, also in association with higher villous height to crypt depth ratio and/or higher ileal flow of mucin (Castillo, 2013; Delgado *et al.*, 2017). Conversely, Martínez-Vallespín *et al.* (2013) did not find differences in ileal digestibility of starch when varying the level of NDSF in iso-starch diets or when NDSF increased at the expense of starch, and no effect of including SF or NDSF to the detriment of starch on intestinal mucosa morphology was observed in some studies (Xiccato *et al.*, 2008, 2011; Trocino *et al.*, 2013; Soler, 2014). This discrepancy in the effect on intestinal mucosa could be due to differences in age or the time elapsed since weaning and to the influence of simultaneous collateral variations mainly in the level or nature of some fibrous constituents. Interestingly, the effect of SF on villous height to crypt depth ratio in piglets seems to be beneficial or detrimental depending on whether the viscosity increases below or above a certain threshold (Bach-Knudsen *et al.*, 2008) and to be detrimental or not depending on the nutrient responsible for the increase in viscosity (McDonald *et al.*, 2001; Hedemann *et al.*, 2006).

Consequently, the ileal flow of starch has been found to range between 0.1 and 2.3 g day^{-1} in 35- or 36-day-old rabbits (Gutiérrez *et al.*, 2002a; Nicodemus *et al.*, 2003; Gómez-Conde *et al.*, 2007; Castillo, 2013; Soler, 2014), between 0.1 and 0.4 g day^{-1} in 39-day-old rabbits (Delgado *et al.*, 2017) and between 1.1 and 1.7 g day^{-1} in 46-day-old rabbits (Romero *et al.*, 2011). The amount of starch fermented in the caeco-colic segment of 4- to 6-week-old rabbits is between 0 (Delgado *et al.*, 2017) and 0.11 (Gutiérrez *et al.*, 2002a) of the starch intake, without excluding possible higher proportions if sources of more resistant starch are used. As a reference, in the study of Delgado *et al.* (2017), even with high-starch diets (averaging 204 g kg^{-1} DM), the amount of starch fermented in the caeco-colic segment of 6-week-old rabbits was very low

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Table 2.6. Ileal digestibility of starch in growing rabbits slaughtered between 19.00 and 22.00 h.

Source of starch	Dietary level of starch (g kg^{-1} DM)	Age (days)	Ileal digestibility of starch	Reference
Wheat, wheat bran	75	35	0.918	Gutiérrez *et al*. (2002a)
Wheat, wheat bran	103	35	0.916	Gutiérrez *et al*. (2002a)
Wheat	168	35	0.914	Gutiérrez *et al*. (2002a)
Wheat	226	35	0.884	Gutiérrez *et al*. (2002a)
Wheat	215	35	0.955	Nicodemus *et al*. (2003)
Wheat, wheat flour, wheat bran	279	39	0.968	Nicodemus *et al*. (2004)
Heat-treated wheat; 79 g NDSF/kg DM	211	35	0.932	Gómez-Conde *et al*. (2007)
Heat-treated wheat; 103 g NDSF/kg DM	208	35	0.950	Gómez-Conde *et al*. (2007)
Heat-treated wheat; 131 g NDSF/kg DM	205	35	0.968	Gómez-Conde *et al*. (2007)
Wheat, wheat bran	113	28	0.941	Gallois *et al*. (2008b)
		42	0.954	
Barley (coarse), wheat bran; with coarse lucerne	152	46	0.899	Romero *et al*. (2011)
Barley (coarse), wheat bran; with fine lucerne	154	46	0.923	Romero *et al*. (2011)
Barley (fine), wheat bran; with coarse lucerne	152	46	0.947	Romero *et al*. (2011)
Barley (fine), wheat bran; with fine lucerne	150	46	0.937	Romero *et al*. (2011)
Wheat, barley, wheat bran; 119 g SF/kg DM	202	35	0.924	Castillo (2013)
Wheat, barley, wheat bran; 89 g SF/kg DM	239	35	0.902	Castillo (2013)
No starchy ingredients; 183 g NDSF/kg DM	30	35	0.961	Martínez-Vallespín *et al*. (2013)
Wheat, wheat bran; 187 g NDSF/kg DM	82	35	0.940	Martínez-Vallespín *et al*. (2013)
Wheat; 147 g NDSF/kg DM	83	35	0.937	Martínez-Vallespín *et al*. (2013)
Wheat, wheat bran; 144 g NDSF/kg DM	130	35	0.947	Martínez-Vallespín *et al*. (2013)
Wheat bran; 255 g NDSF/kg DM	67	36	0.974	Soler (2014)
		43	0.976	
Wheat; 158 g NDSF/kg DM	186	36	0.870	Soler (2014)
		43	0.958	
Wheat, wheat bran; 130 g SF/kg DM	183	39	0.985	Delgado *et al*. (2017)
Wheat, wheat bran; 78 g SF/kg DM	224	39	0.977	Delgado *et al*. (2017)
Wheat	184	35	0.901	Delgado *et al*. (2019)

NDSF, neutral detergent-soluble fibre; SF, soluble fibre (as total dietary fibre – neutral detergent fibre, both corrected by organic matter and crude protein).

(between 0 and 0.4 g day^{-1}), whereas the amount of total dietary fibre fermented in this segment was much higher (between 12.1 and 3.4 g day^{-1}). Similarly, with a moderate amount of dietary starch (113 g kg^{-1} DM, Gallois *et al*., 2008b), the amount of starch fermented in the caeco-colic segment of 28-day-old rabbits was only 0.1 g day^{-1}, while the amount of cellulose

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digested in the whole gastrointestinal tract was estimated to be 0.9 g day^{-1}. In contrast, with a high-starch diet (226 g kg^{-1} DM, Gutiérrez *et al.*, 2002a), the amount of starch fermented in the caeco-colic segment of 5-week-old rabbits was much higher (2.1 g day^{-1}), similar to the amount of cellulose digested in the whole gastrointestinal tract (estimated to be 2.1 g day^{-1}).

Other studies have indirectly approached the efficiency of the small intestine for starch digestion by measuring the starch concentration in the terminal ileum of growing rabbits, as summarized in Fig. 2.2. Within these studies, large variations in sampling times make it difficult to establish clear conclusions, because the composition of the ileal contents varies according to the feed-intake pattern of the animal, including caecotrophy. Nevertheless, these results suggest that the amount of starch reaching the caecum increases at earlier ages when the rabbit is fed with high-starch diets, and that interactions with the starch source cannot be excluded. Thus, purified potato starch (uncooked), known to be resistant to intestinal digestion in the piglet, seems to be highly digested in the intestine of the young rabbit, with a low ileal starch concentration (Pinheiro, 2002), while it reached 109–129 g kg^{-1} DM with maize-rich diets (Blas *et al.*, 1994; Gidenne *et al.*, 2005a). Similarly, Gutiérrez *et al.* (2002b) found a higher starch concentration in the ileum of 35-day-old rabbits when using pea instead of wheat (60 and 36 g kg^{-1} DM, respectively). It has also been found that, compared with ileal digesta, caecal digesta with a maize-rich diet contains 50% less starch in 38-day-old rabbits and 32% less in 49-day-old rabbits, while these figures are 24% and 3%, respectively, with a diet containing less maize (Blas *et al.*, 1994). Finally, as mentioned above, amylase activity in the caecal contents does not differ throughout the growing period, whereas pancreatic amylase secretion increases during this period.

Feed manufacturing process

Grinding determines the size of the dietary particles and therefore could affect the digestibility of the starch by its influence on the surface of exposure to digestive enzymes and on the digestive transit. Thus, using a 4.5-mm or 1.5-mm screen to grind barley and lucerne, Romero *et al.* (2011) observed lower ileal digestibility of starch with coarse barley as a result of the higher proportion of starch provided by particles >1.250 mm. This effect was stronger when combined with coarse lucerne than with fine lucerne, probably because

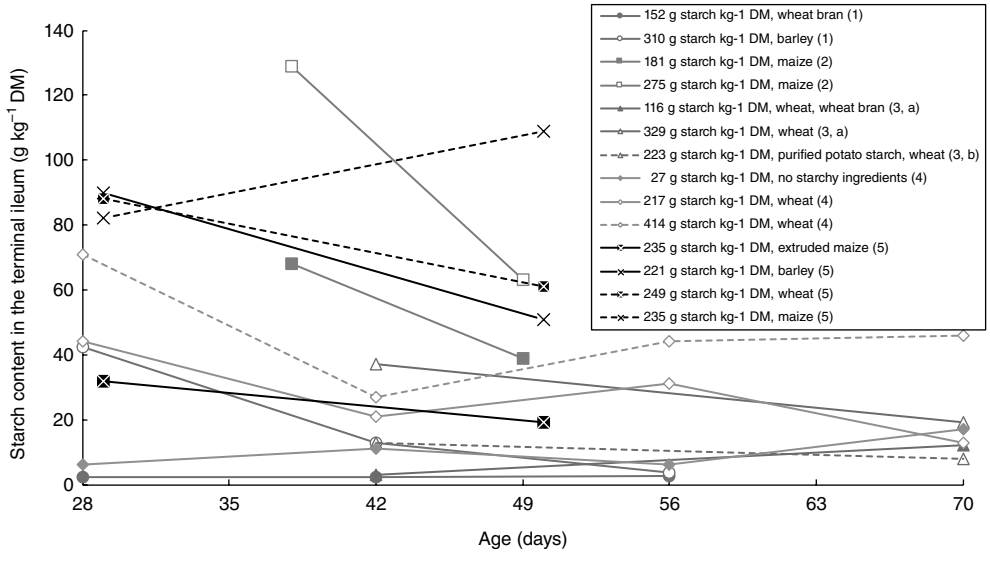

Fig. 2.2. Effect of age and dietary starch level or source on starch concentration in the terminal ileum of growing rabbits. 1, Sampling at 150 min after feeding following a 24-h fast (Blas, 1986); 2, sampling at 20.00 h, fed *ad libitum* (Blas *et al.*, 1994); 3, sampling at 10.00 and 18.00 h (a) or at 13.00 h (b), fed *ad libitum* (Pinheiro, 2002); 4, sampling at 13.00 h, fed *ad libitum* (Gidenne *et al.*, 2004); 5, sampling at 13.00 h, fed *ad libitum* (Gidenne *et al.*, 2005a). DM, dry matter.

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of the additional effect of the higher proportion of fibre provided by large particles that could stimulate the rate of passage in the small intestine (see Table 2.6). Faecal digestibility of starch was lower only with coarse barley combined with coarse lucerne (see Table 2.5), probably because caecal fermentation of starch with this combination was limited by the higher proportion of fibre provided by large particles reducing the caecal retention time.

The oral administration of cooked purified maize starch in adult rabbits causes a clear post-prandial response of glycaemia in peripheral blood. This is similar to that produced by glucose, but somewhat later and more prolonged (Fig. 2.3). However, uncooked purified maize starch hardly affects basal glycaemia. This is due to slower digestion leading to a prolonged but much less pronounced increase in glycaemia in the portal blood, which has little impact on glycaemia. It is unlikely that slower digestion results in greater faecal losses of starch, but it could affect the amount of starch fermented in the large intestine. In practice, it would be interesting to clarify whether, under normal feeding conditions, the feed manufacturing process (involving heating, moisture and pressure) affects starch digestion, especially in young rabbits. Unfortunately, there is little information available on this matter.

All of the results presented so far have been obtained with pelleted diets, as pelleting is the usual process in rabbit-feed manufacturing. Maertens and Luzi (1995) observed that extrusion of feed (which involves more intensive processing of the diet at a higher temperature, moisture and pressure) improved the *in vitro* solubility of dietary starch, but failed to reduce faecal losses of starch in 5- or 7-week-old rabbits fed on a maize-rich diet; on the contrary, these losses increased with the extruded diet, and the authors suggested that starch may be retrograded after cooling (see Fig. 2.1). The alternative is the inclusion of previously extruded or cooked starch sources in pelleted diets. Otutumi *et al.* (2005) reported improved faecal digestibility of starch from both extruded maize and sorghum in 5- or 9-week-old rabbits. Obviously, no effect of extrusion or cooking on faecal digestibility of starch was found when it was already 0.99 when using raw maize, wheat, triticale, pea or cassava (Gutiérrez *et al.*, 2002b; Furlan *et al.*, 2003, 2004, 2005; Otutumi *et al.*, 2005). On the other hand, these processes could reduce the

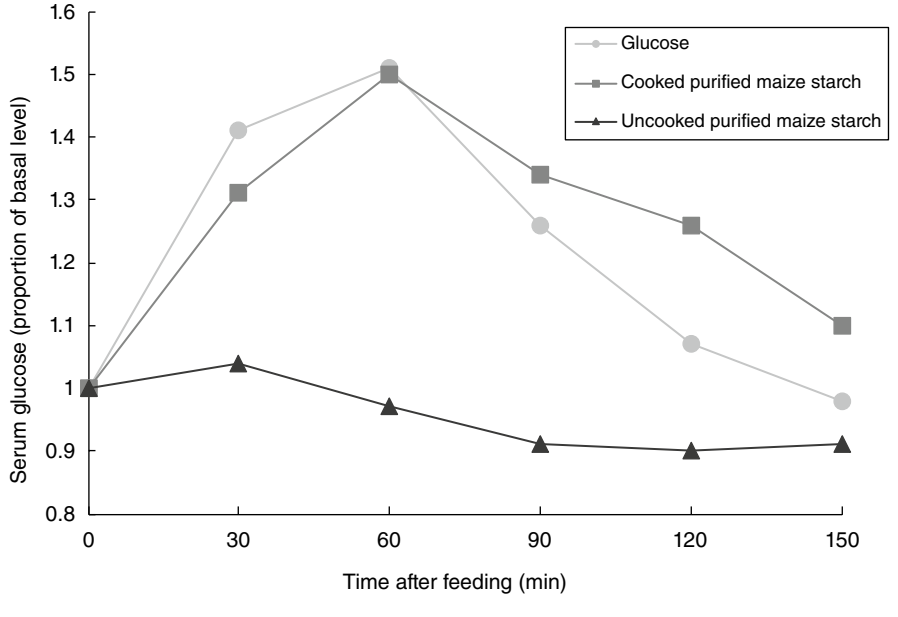

Fig. 2.3. Serum glucose in peripheral blood (marginal ear vein) of adult rabbits after oral administration of glucose or purified maize starch (0.5 g kg^{-1}) following a 12-h fast. (Adapted from Lee *et al.*, 1985.)

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amount of starch escaping from the small intestine. Significant decreases in the ileal starch concentration in 35-day-old rabbits have been reported by cooking pea or wheat (Gutiérrez *et al.*, 2002b), and especially by extruding maize in 29- or 50-day-old rabbits (Gidenne *et al.*, 2005a).

Enzyme supplementation

It is well established that the effectiveness of exogenous enzymes depends on their capacity to resist gastric pH and proteolytic attack by host digestive enzymes, as well as to survive the feed manufacturing process (Bedford, 1995). Yu and Tsen (1993) observed that the incubation of thermostable amylase with rabbit intestinal contents at pH 7.5 did not greatly reduce its activity, while the activity fell to 0.2 in 10 min and reached negligible values in 30 min when the incubation was performed with the contents of the stomach at pH ranging from 2.0 to 3.2.

Logically, enzyme supplements including α-amylase, even if thermostable, are ineffective in increasing the faecal digestibility of starch when it is already >0.99 in control diets (Fernández *et al.*, 1996; Sequeira and Villamide, 1999; Gutiérrez *et al.*, 2002b). However, a significant reduction of the ileal starch concentration in 35-day-old rabbits fed pea- or wheat-including diets has been reported (Gutiérrez *et al.*, 2002b).

2.2.4 Consequences of starch digestion on fermentative activity in the caeco-colic segment

As discussed earlier, the ileal flow of starch is low, but varies according to age of the rabbit and to starch intake and quality; this consequently affects the activity of the caeco-colic microbiota. As variations in the level of starch intake are classically linked to inverse variations in fibre intake, the possible effect of undigested starch on microbial activity can only be elucidated by comparing diets with negligible differences in the diverse fibrous constituents, as with some diets formulated to compare different starch sources.

The effect of the starch source on caecal fermentation in 7- to 12-week-old rabbits fed *ad libitum* and slaughtered in the morning seems negligible. Thus, Belenguer *et al.* (2002) observed no differences in the total VFA concentration and in the fermentation pattern in caecal contents or in the concentration of purine bases (a marker of microbial concentration) in soft faeces depending on the starch source (maize versus barley), whether the source of fibre used was lucerne or sugarbeet pulp. Similarly, Gidenne *et al.* (2005a) found that the starch content of digesta in the terminal ileum varied widely depending on the starch source (from 19 kg^{-1} DM for extruded maize to 109 g kg^{-1} DM for maize, with intermediate values for wheat and barley) but no significant changes were detected in the total VFA concentration or in the fermentation pattern in caecal contents. Xiccato *et al.* (2002) found that the effect of dietary starch source on caecal fermentation was limited to a higher proportion of valerate, a minor VFA linked to amylolytic microbiota (Padilha *et al.*, 1995), with maize than with barley.

However, results from rabbits of similar age (10- to 12-week-old) but under feed restriction and slaughtered a few hours after feed distribution at 08.00 h could be different. Thus, in the caecal contents from rabbits fed a 'maize' diet compared with rabbits fed a 'wheat' diet, Belenguer *et al.* (2011) reported lower total VFA concentration when the fibre source was lucerne and not sugarbeet pulp, higher butyrate proportion at the expense of acetate whatever the fibre source and a higher concentration of purine bases when the fibre source was sugarbeet pulp and not lucerne. Additionally, *in vitro* incubations confirmed the effect on butyrate and acetate proportions and also revealed higher gas production with substrate and inoculum from rabbits fed a 'maize' diet whatever the fibre source. Likewise, Belenguer *et al.* (2012) obtained higher amino acid ^{15}N-enrichments in soft faeces from rabbits fed a 'maize' diet compared with rabbits fed a 'wheat' diet when the fibre source was sugarbeet pulp and not lucerne, suggesting that microbial protein synthesis and activity is stimulated when higher ileal flow of starch is enhanced by presence of fermentable fibre. Nevertheless, no effects on the total VFA concentration or the fermentation pattern in caecal contents were observed, perhaps because in this study caecotrophy was prevented 24 h before sampling.

In adult rabbits, the starch concentration in the ileum ranged from 3 to 27 g kg^{-1} DM, depending on the source of starch (purified maize

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starch, barley, pea, maize), while the faecal digestibility of hemicelluloses ranged from 0.37 to 0.54, although changes in the nature of dietary fibre were appreciable because of the high level of inclusion of starch sources (Gidenne and Perez, 1993b). However, Amber (1997) found that both ileal flow and the amount of starch fermented in the caeco-colic segment were slightly greater with a maize-rich diet than with a barley-rich diet, and no relevant differences in the faecal digestibility of the fibres were observed.

In any case, fibre remains the main factor determining fermentative activity. The influence of starch is much less important, although it could be relevant in young animals fed diets containing high levels of resistant starch and fermentable fibre.

2.2.5 Role of starch on digestive health

Suckling rabbits

Reviewing a total of 21 studies involving 54 different diets, from 1995 to 2019, it appears that dietary starch levels (also linked to fibre or fat changes) do not greatly affect the mortality rate of the young rabbits, from the time they begin to consume feed until weaning. In fact, the consumption of milk represents an important part of nutrient intake and contributes to health protection (Fortun-Lamothe and Boullier, 2007; Gallois *et al.*, 2007), thus explaining that the health status of the suckling rabbit is largely independent of the feed. In the context of epizootic rabbit enteropathy (ERE), Martínez-Paredes *et al.* (2009) reported very high mortality during week 6 of age with a weaning at 28 days old, but very low mortality in those that remained suckling until 42 days old, where the mortality rate clearly increased during week 8 of age.

Growing rabbits

It is well established that the susceptibility of rabbits to digestive disorders is greater after weaning, on account of the many physiological changes occurring around this time. An old hypothesis suggested that an overload of rapidly fermentable carbohydrates in the large intestine increases the risk of digestive disorders in weaned rabbits (Cheeke and Patton, 1980). The previous sections demonstrated that ileal starch flow is very limited. Some studies reported lower mortality as a consequence of the inclusion of enzymatic supplements containing α-amylase and reducing the starch concentration at the ileum (Gutiérrez *et al.*, 2002b; Cachaldora *et al.*, 2004). On the contrary, Remois *et al.* (1996) found the inclusion of thermostable amylase and/or amyloglucosidase in a rabbit diet to have no effect on mortality rate. Thus, the beneficial effect of enzyme complexes could be more related to other enzymes included (pectinases, β-glucanases, β-xylanases), which would cause partial hydrolysis of certain fibrous constituents and the formation of complex oligosaccharides with favourable effects on the microbiota and digestive health. Furthermore, in a large-scale study with different starch sources (maize, wheat, barley and extruded maize) and negligible variations in the fibre content, Gidenne *et al.* (2005a,b) did not observe differences in mortality in spite of the starch concentration at the ileum varying widely depending on the starch source. Accordingly, Gidenne and García (2006) proposed that the restriction of the dietary starch level could be higher than the usual one of 150–155 g kg^{-1} DM, or even removed. Thus, the effects of dietary carbohydrates on digestive health are mainly linked to changes in the fibre constituents (see Chapters 5 and 10). Recently, several studies in growing rabbits in poor sanitary conditions, mainly subject to ERE outbreaks, have shown that increasing the content of fibrous constituents (both insoluble and soluble) above the usual recommendations at the expense of starch content generally has a favourable impact on digestive health and has reduced the mortality rate (Fabre *et al.*, 2006; Carraro *et al.*, 2007; Xiccato *et al.*, 2008, 2011; Martínez-Vallespín *et al.*, 2011; Castillo, 2013; Grueso *et al.*, 2013; Soler, 2014; Delgado, 2017).

Adult rabbits

The relationship of starch intake to the incidence of digestive disorders in adult rabbits seems to be very limited within the usual dietary starch levels. De Blas *et al.* (1995) have suggested a trend towards an increase in the replacement rate of rabbit does (associated with more diarrhoea and sudden death at parturition) as

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the starch content of the diet increases while the fibre content decreases. However, other studies with changes in the starch content, at the expense of those of fibre or fat, have not reported relevant differences in the replacement rate of does (Lebas and Fortun-Lamothe, 1996; Pascual *et al.*, 1998, 1999; Quevedo *et al.*, 2006; Nicodemus *et al.*, 2010).

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