Carbohydrates in fish nutrition: digestion and absorption in postlarval stages

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Abstract

This review summarizes information regarding digestion and absorption of carbohydrates in cultivated fish. Relevant results of studies of digestive enzymes, e.g. amylase, chitinase, cellulase and brush border disaccharidases are presented. Fish amylases appear to be molecularly closely related and to have characteristics comparable to mammalian amylases. Whether chitinases and cellulases are endogenous enzymes of some fish species is still a matter of speculation, although recent molecular evidence, at least for chitinase seems to settle the issue in favour of endogenous sources. Feed and intestinal microbes may be the source of polysaccharidases in fish feeding on nutrients-containing non-starch polysaccharides. Knowledge regarding monosaccharide transport in fish intestine as interpreted from studies of brush border membrane vesicles, everted sleeves of fish intestinal sections and molecular biology is discussed. Glucose transporters of the intestinal brush border show characteristics similar to those found in mammals. A tabulatory presentation of experimental details and results reported in the literature regarding starch digestibility is included as a basis for discussion. Although numerous investigations on digestion of starch and other carbohydrates in fish have been published, the existing information is highly fragmentary. As yet, it is impossible to derive a cohesive picture on the integrated process of carbohydrate hydrolysis and absorption and interaction with diet composition for any of the fish species under cultivation. The physiological mechanisms behind the species differences are not known.

KEY WORDS: digestion, enzymes, fish, glucose, non-starch polysaccharides, starch

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Introduction

Carbohydrate-containing feedstuffs are available in great quantities at low prices. Accordingly, omnivorous cyprinids and cichlids represent the majority of cultivated species on a worldwide basis. Cultivation of these species has long traditions in Asian and African countries and supplies large populations with highly needed, high-quality protein. However, in Western countries salmonids, basses and other carnivorous species are desired by a market able to pay high prices, supporting expanding aquaculture industries. Grains or grain products are the main carbohydrate sources in diets for cultivated fish (Tacon 1993).

Carbohydrates in fish feed range from highly digestible mono-, di- and oligosaccharides to insoluble and indigestible hemicelluloses and cellulose, with sources ranging from seaweed, algae and plankton to refined grain and soybean products. Carbohydrates from plants, algae, plankton and other feeds comprise oligo- and polysaccharides of monomers with various substitutions, whereas starches constitute the major carbohydrate component of grains. In soybeans, we find sucrose, oligosaccharides and complex non-starch polysaccharides, mainly hemicelluloses and pectins, but no starch. In the context of carbohydrate nutrition and as an important part of food for fish feeding on crustaceans, one has to consider chitin (1,4β-*N*-acetyl glucosamine), the second most abundant carbohydrate (after cellulose).

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Diets used in fish farming contain highly variable amounts of carbohydrates depending on the cultivated species. Even within fish species we find carbohydrate contents of diets to vary substantially. Developments in nutritional physiology, aquaculture technology and economical constraints have triggered use of cheaper feed ingredients with higher carbohydrate contents. Carbohydrates are used in fish diets primarily as energy sources and for their binding properties. Starches, pectins and hemicelluloses have pellet-binding characteristics of great importance to feed manufacturers. Therefore, carbohydrates may be added to the feed in excess of the amounts that can be efficiently utilized for energy by the fish. A discussion of metabolic and energetic aspects of carbohydrate utilization may be found in a recent review (Hemre *et al.* 2002).

Fish species differ greatly in their ability to digest carbohydrates. This variability reflects anatomical and functional differences of the gastrointestinal tract and associated organs. Digestive functions capable of hydrolyzing a greater variety of carbohydrate-containing feedstuffs have developed in herbivorous and omnivorous fish in contrast to carnivorous fish. Digestive organs of fish vary from short and simple to complex ruminant-type, reflecting the variation in nutrient sources. Not surprisingly, considering the long evolutionary history of fishes and their varied ecological niches, the diversity of fish intestine seems to exceed that of other vertebrates.

Digestive physiology

Enzymes involved in carbohydrate hydrolysis

All species of fish investigated to date, possess the enzymatic apparatus for hydrolysis and absorption of simple and more complex carbohydrates. Moreover, digestion and absorption of carbohydrates appear to take place along the same general routes in herbivorous, omnivorous and carnivorous species. Polysaccharides are broken down by α- and β-endoglucosidases whereas di- and oligo-saccharides are hydrolyzed by various brush border enzymes into their constituent monosaccharides. Digestion continues intracellularly in the enterocytes that possess various enzymes with disaccharidase activities. Fish species with high intestinal microbial activity may be supplied with additional carbohydrate energy through alternative routes.

Amylase

It is somewhat peculiar, with all the emphasis on replacing fish foodstuffs with carbohydrates, that some of the basics of the digestive machinery are still relatively poorly understood. Clearly, the importance of the availability of amylases and other carbohydrases in this context cannot be understated, but surprisingly little is known about the enzymes themselves and even less about their regulation. Further, only cursory attention has been devoted to endogenous dietary inhibitors of carbohydrases. Researchers have often been content to monitor adjustments of α-amylase to dietary changes without really addressing any of the underlying mechanisms. In addition, it appears that more emphasis has been put on developmental changes in enzyme activity – associated with first feeding or dietary preferences in hatchlings – than on activity patterns during juvenile or adult growth phases.

Intestinal α-amylase (α(1 → 4)-D-glucan glucanohydrolase, EC 3.2.1.1) is a good case in point. The enzyme catalyzes the endohydrolysis of α(1 → 4)-glucosidic linkages in starch and similar molecules to shorter oligosaccharides, including maltotriose and maltose. Subsequent hydrolysis by disaccharidases or glucosidases delivers carbohydrate units – usually monosaccharides – that can be transported across the villi. Fish also possess additional glucosidase activities, including an acid α-glucosidase (at times termed γ-amylase); however, these enzymes are usually present only in liver and involved in the breakdown of endogenous glycogen (Mehrani & Storey 1993) by a route that bypasses the better known glycogen phosphorylase (Moon *et al.* 1999) or at least complements its activity.

First, the discussion had to be settled that amylase was indeed of endogenous origin. Potentially, enzymes can be contributed by the diet or produced by intestinal bacteria, and although such exogenous contributions can be substantial, this possibility can be excluded with a minimum of experimental effort by withdrawal of diet or application of selective antibiotics. In contrast to mammals, where amylase is produced by salivary and pancreatic cells, the only source of α-amylase in fish appears to be the exocrine pancreas. High amylase activity in liver and bile in some species like carp and goldfish (Table 1)

Table 1 Amylase activities in the liver, intestine and bile of selected teleosts at 37 °C (Hidalgo *et al.* 1999)

	Liver (U mg^{-1} protein)	Intestine (U mg^{-1} protein)	Bile (U mL^{-1})
Carp (*Cyprinus carpio*)	108.0 ± 7.3	72.5 ± 8.5	4.79
Goldfish (*Carassius auratus*)	23.8 ± 4.2	75.5 ±15.8	1.61
Tench (*Tinca tinca*)	13.1 ± 1.3	19.4 ± 2.7	n.a.
Seabream (*Sparus aurata*)	2.7 ± 0.4	1.75 ± 0.28	0.84
Trout (*Oncorhynchus mykiss*)	0.0	1.30 ± 0.07	0.0
Eel (*Anguilla anguilla*)	0.76 ± 0.08	0.46 ± 0.05	0.067

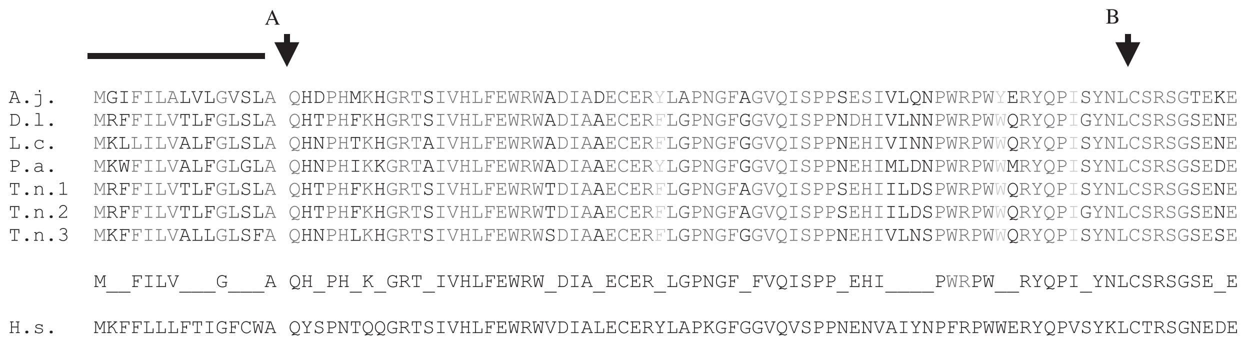

Figure 1 N-terminal amino acid sequences of teleostean and human α-amylase deduced from cDNAs. Only the N-terminal 93 residues of amylases are collated. The bar indicates the putative N-terminal signal peptide. Arrow 'A' indicates the N-terminal, usually blocked, glutamine residue of the enzyme. Arrow 'B' indicates the first of 10 conserved cysteine residues that form five characteristic disulphide bridges in the enzyme molecule. A.j., *Anguilla japonica* (Japanese eel; GenBank AB070721); D.l. *Dicentrarchus labrax* (seebass; AJ310653); L.c., *Lates calcarifer* (barramundi; AF416651); P.a., *Pleuronectes americanus* (winter flounder; AF252633); T.n., *Tetraodon nigroviridis* (puffer; AJ308233) amylases 1–3. H.s., *Homo sapiens* (NM000699). Human pancreatic α-amylase is included for comparison only. Identical residues in the fishes are listed between the teleostean and the human sequences.

may be because of the infiltration of endocrine and exocrine pancreatic tissue into the liver of these species.

Digestive α-amylase has been localised throughout the entire gastrointestinal tract of many fish species (Kawai & Ikeda 1972; Chiu & Benitez 1981; Fagbenro 1990; Ugwumba 1993; Sabapathy & Teo 1993; Chakrabarti *et al*. 1995; Kuz'mina 1996a; Peres *et al*. 1998; Hidalgo *et al*. 1999; de Seixas *et al*. 1999; Fagbenro *et al*. 2000; Tengjaroenkul *et al*. 2000; Alarcón *et al*. 2001; Fernandez *et al*. 2001). The enzyme is present in the distal parts of the intestine and in some species also in the oesophagus. Not surprisingly, the exocrine pancreas has the highest activities (Overnell 1973; Yardley & Wild 1991). Together with other pancreatic enzymes, amylase activity is detected within the lumen of the intestine, in the chyme as well as attached to the mucosal membrane (Ugolev & Kuz'mina 1994; Hoehne-Reitan *et al*. 2001). Whether specific receptors anchor the enzymes to the mucus is not known, and the modes of attachment are in need of clarification.

Characteristics of amylase differ between species regarding pH optima and temperature stability. Studies of six Mediterranean sparid fishes have shown pH optima between 4 and 9 (Fernandez *et al*. 2001; Alarcón *et al*. 2001); all, but one, showed more than one pH peak, and some possessed two isoforms of the enzyme. Amylase from two tilapias, *Oreochromis niloticus* and *Sarotherodon melanotheron*, shows molecular masses in the same range as the sparid amylases, around 56 kDa; their pH optima were in the neutral range. Other features were in common to the α-amylase family of enzymes (Yamada *et al*. 1991; Moreau *et al*. 2001), and both enzymes occurred in two isoforms. Similar results have been observed in other fish species (Munilla-Moran & Saborido-Rey 1996b). In addition to the common characteristics, fish amylases also reveal distinct differences, for example regarding dependence on ions and ion concentrations (Munilla-Moran & Saborido-Rey 1996a).

The first teleostean amylase cDNA to be sequenced was from winter flounder (*Pleuronectes americanus*) (Douglas *et al*. 2000); a few other sequences have been added since (see Fig. 1). If we can extrapolate from the flounder and a puff-erfish (*Tetraodon nigroviridis*) to all teleosts, then fish α-amylases follow the common vertebrate arrangement of an open reading frame consisting of approximately 1500 nucleotides, with an assumed signal peptide of 15 amino acid residues. The enzyme monomer contains some 500 amino acids, yielding the well-characterized active monomer of the enzyme with a molecular mass between 55 and 56 kDa. The high degree of conservation is apparent in the deduced amino acid sequences for the N-terminal regions of the fish amylases compiled in Fig. 1. The sequences are also highly conserved at the nucleotide level, with sequence identities above 70%. Just like mammalian pancreatic amylase, the fish amylase gene contains nine exons and eight introns (Fig. 2).

In addition to the amylase sequences listed in Fig. 1, a few more partial cDNA sequences are available in the literature. The medaka (*Oryzias latipes*) sequence covers the C-terminal region of the enzyme and is too incomplete to be included into our analysis. Interestingly, one of the incomplete sequences of the zebrafish (*Danio rerio*) appears to be quite different from the other teleostean amylases sequenced to date (Fig. 3). Rather than depicting the phylogenetic relationship of the zebrafish to the other teleosts, we would argue

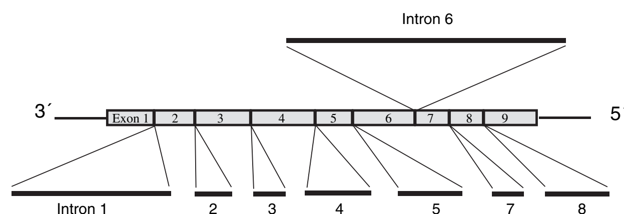

Figure 2 Exon–intron arrangement in the pufferfish (*Tetraodon nigroviridis*) α-amylase gene. This picture is for amylase 3, probably more appropriately termed amylase 2C, as this is related to pancreatic amylases (amy2) of other vertebrates. The total number of residues in the entire gene without untranslated regions is 3859 bp. Lengths of introns and exons are drawn to scale – the UTRs are not. Drawn from GenBank accession no. AJ308233 [Bernot, A. (2001), Analysis of 148 kb of genomic DNA of *Tetraodon nigroviridis* covering an amylase gene family, unpubl. data].

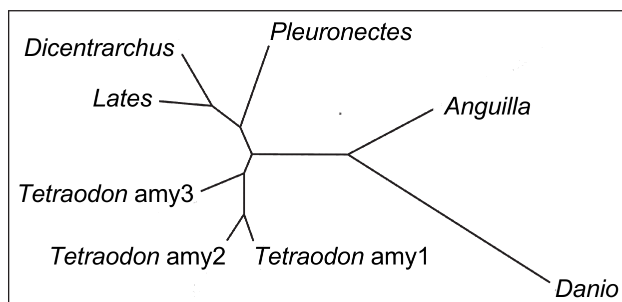

Figure 3 Unrooted tree of teleostean α-amylases. Drawn using ClustalW, using the open reading frames of teleostean amylase sequences available on the GenBank database. For accession numbers, see legend of Fig. 1, except zebrafish (*Danio rerio*) CB 362341 (incomplete cDNA).

that the cDNA characterized in *D. rerio* might represent a different lineage or family of amylases than the closely related enzymes found in the other species of teleosts. In a BLASTN analysis (Altschul *et al.* 1997), the short zebrafish sequence also maps to different regions of the *Takifugu rubripes* database (available at http://fugu.hgmp.mrc.ac.uk) than the amylase sequences of the other teleosts.

Insufficient attention has been devoted to the presence of multiple amylase genes – a very likely scenario not only considering the alleged genome duplication that the teleostean lineage underwent some 300 million years ago (Taylor *et al.* 2001). In addition to the protein data reviewed above, multiple amylases have been distinguished in Southern blots for a few species of flatfish (Douglas *et al.* 2000). In the pufferfish, a single large genomic sequence contains three α-amylases in series, interrupted by two, relatively short noncoding sequences of 3600 nucleotides (amy1–amy2) and 791 nucleotides (amy2–amy3), respectively, with some intriguing differences. The current nomenclature for the pufferfish amylases (amylases 1–3) is somewhat unfortunate, as technically, these three amylases represent variations on the pancreatic amylase theme, generally termed amylase 2. All three open reading frames show little sequence variability, and as a consequence, in a simple phylogenetic analysis based on the open reading frames, the three enzymes cluster closely together (Fig. 3). Intriguing differences are discovered when these three genes are analysed with inclusion of their respective introns. Although this analysis could be strengthened even further by consideration of all exons and introns, our brief analysis focuses on exons 2 and 3 and the intervening intron 2. In the two exons, amylases 1 and 2 only differ by approximately 4%, while sequence differences for the 250 bp intron amount to more than 20% (Fig. 4). As introns tend to evolve more rapidly than exons, we conclude that indeed these two sequences represent two different amylase genes derived by gene duplication. Amylase 3 is similarly conserved in the exons, again arguing for gene duplication of one of the other amylase genes, but the intron 2 for this gene is less than 1000 bps, with very little resemblance to the intron 2 of the other two genes. We think that together these data support the idea that fish posses multiple amylase genes, possibly arranged into different amylase families. However, it should be kept in mind that the electrophoretic variants mentioned above, could also be generated by other routes. For instance, at least for mammalian amylases, multiple posttranslational modifications have been described, including methylation, *N*-α-acetylation, deamidation and glycosylation.

Even though in the interest of fish aquaculturists, unfortunately, even less is known about the regulation of amylase activity, secretion or biosynthesis in fishes. If mammalian models apply, we can expect that transcription and secretion are under positive hormonal control by vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP), cholecystokinin (CCK) and possibly other hormones activating pancreatic protein kinase A and protein

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                                  Exon 2
T.n.amy1 ATTTCTCCTCCAAGTGAGCACATCATTCTAGACAGCCCCTGGAGGCCCTGGTGGCAGAGATATCAGCCAATCAGCTACAACCTGTGCTCCAGATCAGGTAGTGAGAATGAGCTGAGAGAC
T.n.amy2 -----------------------------------------------------------------------TG----------------------C-A----------------------

                                   Intron 2
T.n.amy1 ATGATCACCAGGTGCAACAATGTTGGGgtaagacattgagcagatataatcccacattgaaaaacttcttggtaatttatgttacatcacctattgtcaacaatcaaaaaagtattgatg
T.n.amy2 ---------------------------------------------------------------------------c-------xx---gc-t-c-------------gt--catgc-g--

T.n.amy1 ctttttttgtttttgttttttttagttttacaataatatgacttaattatatgttgatacatatttxtgaaatcagcacaaactgaagtcccaacgatgtgtgataagagccagtcaaa
T.n.amy2 a cxxxxxxxa--aa---------gta------g----------g-g---c-------g-----g g---g--a-t--t---t---tt--------a----c---gg-c---a--g--g

                                     Exon 3
T.n.amy1 aggtgttgactatttctctttggttttaaattatagGTCAACATCTATGTGGATGTTGTCATTAACCACATGTGCCGTGCCAGCGGTGGTGAGGGGACACACTCCTCCTGTGGAAGCTGG
T.n.amy2 ----a-ca-g--------------a------------------------------C-------------------G----TG--------------------------------------

T.n.amy1 TTCAATGCAGGCGCGAAAGACTTTCCCTCTGTCCCCTACTCCTACTTGGACTTTAATGATTACAAGTGCAAAACTAGCAGTGGAAACATTGAAAGTTATGATGCTGATGTGAATCAG
T.n.amy2 -----------------------------------------------------C-----------------------------CG-A-----G------C---xxx-C---C-----
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Figure 4 Alignment comparison of cDNAs of exons 2 and 3 and the intervening intron 2 of pufferfish (*Tetraodon nigroviridis*) α-amylases 1 and 2. Sequences are from GenBank [accession no. AJ308233; Bernot, A. (2001) Analysis of 148 kb of genomic DNA of *Tetraodon nigroviridis* covering an amylase gene family, unpubl. data]. Sequence identity is 96% in the exons and less than 80% in the introns, ignoring the gaps. '-' represents identical nucleotide and 'x' represents gap. Additional information on the *Tetraodon nigroviridis*) genome project is available at http://www.genoscope.cns.fr.

kinase C pathways. Teleosts contain extensive networks of VIP and PACAP-immunoreactive cells in their gastrointestinal tracts (Olsson & Karila 1995); alas, the well established link between VIP release and amylase expression and secretion in mammals (Thwaites *et al.* 1989) remains to be confirmed for the fishes. Yet, cod VIP was virtually equipotent with porcine VIP in stimulating amylase release from guinea-pig pancreatic acini (Thwaites *et al.* 1989).

Amylase activities of tissues and intestinal contents vary between species and appear higher in herbivorous and omnivorous than in carnivorous fish (Hoffer & Sturmbauer 1985; Sabapathy & Teo 1993; Ugolev & Kuz'mina 1994; Hidalgo *et al.* 1999). In some carnivores α-amylase levels are very low (*cf.* trout in Table 1, Hidalgo *et al.* 1999). In a study of intestinal amylase activities in intestinal content from Atlantic salmon values were mostly below detection limits (Brudeseth 1996).

Pancreatic tissue is considered to be the source of amylase activity detected in the contents of mid and distal parts of the intestine. However, the origin of amylase observed in the very proximal parts of the gastrointestinal tract has not been documented. The amylase present in the proximal regions may originate from pancreatic tissues, as the species in question mostly lacked distinct stomach pouches. At present, it is not possible to identify the true source of the amylase; i.e. to distinguish between dietary contributions or reflux from the lower intestine. Moreover, amylase may also be produced by the microflora of the digestive tract (Sugita *et al.* 1997).

In a number of fish species activities of intestinal amylase correlates positively with dietary carbohydrate level and feeding intensity (Kawai & Ikeda 1972; Cowey & Walton 1989; reviewed by Kuz'mina 1996b). The ability to adapt amylase secretion to match carbohydrate level in the diet and to feed intake may be restricted to herbivorous and omnivorous fish. In rainbow trout, in contrast, increasing the level of dietary starch has actually been found to reduce amylase activities in the chyme (Spannhof & Plantikow 1983). In the latter study, the amylase reduction was considered to be because of inhibition of the enzyme after adsorption to the starch molecules. Additional potato starch inhibited the activities of the remaining amylase in extracts from chyme of starch-fed trout. Endogenous amylase inhibitors present in wheat and other grains might also bring about enzyme inhibition (Sturmbauer & Hoffer 1985), possibly in a species-specific manner. For instance, inhibition of intestinal amylase by compounds from wheat was more pronounced in carp than in rainbow trout (Spannhof & Plantikow 1983). Most amylase inhibitors are proteins or peptides (Franco *et al.* 2002). While the above species difference in amylase inhibition may be due partly to the specific sets of enzymes with different substrate preferences or because of the species-specific enzyme turnover, it should not come as a surprise that the suite of proteolytic enzymes in the carp were less effective in deactivating the amylase inhibitors than the trout proteases (Spannhof & Plantikow 1983).

Chitinase

Chitin, a linear homopolymer of β(1 → 4)-linked *N*-acetylglucosamine (NAG) units, is a common component of some bacterial cell walls and lower plants and a major constituent of the exoskeleton of arthropods and cuticles of annelid and molluscs. Therefore, it is not unlikely that some fish species will encounter this carbohydrate derivative in

their diet. This is especially relevant for species consuming arthropods, as it has been estimated that over 20% of the gross energy is contained in the chitin-rich exoskeleton of arthropods and terrestrial vertebrates may recover as much as 90% of their dietary chitin (Karasov 1989). Degradation of chitin is brought about by sequential action of chitinase (EC 3.2.1.14) which attacks internal β(1 → 4)-linkages between NAG units and β-*N*-acetylglucosaminidase-type enzymes (EC 3.2.1.52) that degrade NAG-dimers and trimers into the monomers.

Chitinase is present in the digestive system of many fish species regardless of dietary habits (Smith *et al.* 1989), but its presence is not restricted to the digestive tract (Lindsay *et al.* 1984). The function of chitinase in blood and various tissues is unknown (Lindsay *et al.* 1984), although a link between human chitinase and defence against chitinous pathogens has been proposed. This enzyme activity tends to be associated with those species consuming chitinous prey and lacking mechanical structures to disrupt their exoskeleton. Chitinase activity in cod (*Gadus morhua*) reaches a peak when the animals feed on crustaceans, most likely because of high concentrations of chitinase in the prey. However, increasing evidence points towards some fish having the ability to produce endogenous intestinal chitinase (Danulat 1986). Chitinases with different pH-optima and tissue distribution have been identified (Jeuniaux *et al.* 1982).

Chitinolytic activity is found in chyme throughout the gastrointestinal tract and, analogous to amylase, also in gut wall homogenates. Highest concentrations are localized to stomach and pyloric tissue, indicating that these organs or the diet are the main sources of the enzymes.

The overall importance of intestinal chitinase to carbohydrate digestion and nutrition is still under debate. Only limited information exists on chitin digestibility. Most studies are *in vitro* studies involving test tube assays for chitinase employing chitin that has been solubilized in ice-cold concentrated HCl, clearly not a pretreatment present in fish intestines. Therefore, it is not overly surprising that rainbow trout show high chitinase activity, contrasted by low chitin digestibilities (Lindsay *et al.* 1984). An investigation of the *N*-acetyl β-glucosaminidase- and chitinase-producing ability of 283 strains of *Aeromonas* species from the intestinal tract of common carp (*Cyprinus carpio)* showed that more than 90% of the strains were able to produce one or more of enzymes with such specificity (Sugita *et al.* 1999). The results indicate that the microflora also may play a role in the hydrolysis and apparent absorption of carbohydrates from chitin. Interestingly, dietary chitin increased the activity of the seabream (*Sparus aurata*) innate immune system, indicating that some unforeseen metabolic costs may be involved in chitin digestion (Esteban *et al.* 2001).

Nevertheless, new molecular evidence supports the notion of endogenous chitinase produced by fishes. For instance, using BLASTN (Altschul *et al.* 1997) with the nucleotide sequence for toad pancreatic chitinase (Oshima *et al.* 2002) produces several promising hits in the *Takifugu rubripes* transcriptome database that are already annotated as 'chitinase-like'. Curiously, these sequences were isolated from the pufferfish gut and not the pancreas. We can take this as initial molecular evidence for presence of endogenous chitinase in teleosts, corroborating previous enzyme activity measurements.

Lysozyme

Another enzyme potentially degrading chitin is lysozyme (EC 3.2.1.17) that weakly attacks internal links between NAGs, in addition to its main function as an endoglucosidase hydrolyzing β(1 → 4)-linkages between NAG and *N*-acetylmuramate. Usually, (C-type) lysozyme activity in fish plasma, skin and head–kidney is mentioned in the context of immune function in the degradation of outer layers of some bacteria (Paulsen *et al.* 2001, Savan & Sakai 2002). Recently a g-type lysozyme has been characterized in a flounder; the enzyme is expressed in fish intestine, especially after exposure to bacteria (Golovanova *et al.* 1999). Hesitatingly extrapolating from expression work to functional protein, it is therefore conceivable that teleostean lysozyme contributes to 'chitinase' activity in intestine. However, some contradictory molecular evidence predicts that encoded g-type lysozyme proteins are not secreted, as they lack a signal peptide, and therefore an intracellular function has been postulated (Irwin & Gong 2003).

Cellulase and other polysaccharidases

Cellulase activity has been observed in several fish species indicating that fish may be able to utilize cellulose and similar fibrous carbohydrates (Chakrabarti *et al.* 1995). Whether the observed cellulase activities are of endogenous or microbial origin is under debate (Lindsay & Harris 1980; Chiu & Benitez 1981). In a study of digestive enzymes in grass carp (*Ctenopharyngodon idella*), cellulase was observed both in hepatopancreas and intestine (Das & Tripathi 1991), and dietary cellulose level affected the activities significantly. The fact that cellulase activity was reduced to approximately one-third when the antibiotic tetracycline had been added to the diet, indicates that microorganisms may supply an important

part of the cellulolytic activity in the intestinal tract of fish. The residual activity measured in the presence of the antibiotic, indicate that a fraction of the activity may be of endogenous origin (Das & Tripathi 1991). However, because of the abundance of antibiotic resistance, there is the distinct possibility that tetracycline-resistant organisms may account for the remaining activity. In the catfish (*Clarias isheriensis*), fed an omnivorous diet, but feeding mainly on the pond plankton *Cyanophycea*, high cellulase activities were found in the stomach, and in proximal and distal parts of the mid intestine (Fagbenro 1990).

Intestinal microbes from carp (*C. carpio*) are able to metabolize oligosaccharides commonly found in soy and other beans with the liberation of short chain fatty acids, carbon dioxide and methane gas (Kihara & Sakata 2002). Moreover, the hindgut chamber of the king angelfish (*Holacanthus passer*) contains a large number of microorganisms with the ability to hydrolyze complex carbohydrates (Diaz & Espana 2002). The hindgut of this species is highly vascularized indicating importance for absorption.

The volatile fatty acids liberated by microbial fermentation, mainly acetate together with some propionate and butyrate, are taken up across the gut wall and can be detected in plasma in appreciable concentrations (Seeto *et al.* 1996). In three species of marine herbivores, the rates of turnover of volatiles are in the same order of magnitude as those found in the intestinal tracts of herbivorous reptiles and mammals (Mountfort *et al.* 2002), even though the ectothermic fishes were 'digesting' at much lower temperatures (17–23 °C). This result does not support the idea that high temperatures are a prerequisite for efficient fermentation systems to operate in marine herbivores (Kandel *et al.* 1995). The importance of volatile fatty acids to overall energy supply and metabolism has not yet been quantified for any of these herbivores but it may be substantial (Mountfort *et al.* 2002). Yet, it is unlikely to be a significant factor in carnivorous cold-water species, such as salmonids. Algae consumed by marine fishes contain much more complex and different carbohydrates than vascular plants with their largely cellulose and hemicellulose-based structural components. In addition to different sets of secondary metabolites, digestion is achieved in a differing ionic environment. Neither has attracted much attention by researchers as yet.

Disaccharidases

Homogenates of gastrointestinal mucosa of fish show the ability to hydrolyze a number of low molecular weight saccharides, including the disaccharides maltose ($\alpha(1 \rightarrow 4)$ glucopyranosyl glucose), sucrose ($\alpha 1 \rightarrow 4$glucopyranosyl fructose), and trehalose ($\alpha 1 \rightarrow \alpha 1$glucopyranosyl glucose) (Buddington & Hilton 1987; Krogdahl *et al.* 1999; Harpaz & Uni 1999). These brush border enzymes can also be detected in the lumen content (Fagbenro *et al.* 2000; Nordrum *et al.* 2003), most likely because of shedding of mucosal tissue. However, as these enzymes are shown to be active in the chyme, they may also hydrolyze substrates present in the chyme. Disaccharidases occur in the mucosa of pyloric, mid and distal segments of herbivorous, omnivorous and carnivorous fish (Ugolev & Kuz'mina 1994; Krogdahl *et al.* 1999). In general, the distal parts show lower activities than the mid and pyloric regions. The intestinal segments tend to possess a greater capacity for hydrolysis of maltose than other disaccharides (Buddington & Hilton 1987; Ugolev & Kuz'mina 1994; Krogdahl *et al.* 1999). Intestinal mucosa from Atlantic salmon shows maltase activities 10 times higher than the activities of trehalase and sucrase. Disaccharidase activities vary greatly between species and are highest in herbivorous, and lowest in carnivorous fish (Ugolev & Kuz'mina 1994). However, in comparison with a mammalian carnivore, the mink (*Mustela vision*), enzyme activities of Atlantic salmon are only one-tenth of those measured in the mink at the same temperature (Brudeseth 1996).

A comparison of maltase in an Antarctic (*Pagothenia bernacchii*) and a temperate fish species (*Anguilla anguilla*) showed the highest specific activities in the Antarctic fish (Maffia *et al.* 1993). The authors concluded from the studies that the adaptation of the enzyme appeared to involve alterations in protein–lipid interactions rather than alterations in protein structure, suggesting that increasing specific activity compensates the effect of temperature on enzyme activity.

In several studies, the activity of brush border disaccharidases did not respond to changes in the amount of dietary carbohydrate. The omnivorous red tilapia, a cross of two common tilapia species, did not increase intestinal disaccharidase activities in response to carbohydrate intake (Shiau & Liang 1995). Non-responsiveness to dietary carbohydrate has also been noted for strictly carnivorous species. In the rainbow trout, for instance, no differences were seen in specific disaccharidase activities throughout the intestinal tract between fish fed a diet without carbohydrates and fish fed a diet with 250 g kg^{-1} glucose (Buddington & Hilton 1987). Similar observations have been made in Atlantic salmon fed diets with 60 and 170 g kg^{-1} starch (Krogdahl *et al.* 1999). In fact, in the two salmonids, the results indicated inhibitory effects of high carbohydrate levels on specific disaccharidase activities. However, in a recent comparative experiment with rainbow trout and Atlantic salmon stimulating effects of

starch were observed (Krogdahl *et al.* 2004). In both species activities of the brush border enzymes maltase and sucrase as well as the cytosolic lactase were 25–100% higher in fish fed diets with 230 g kg^{-1} compared with 70 g kg^{-1} maize starch. The basis for the apparent conflicting results regarding responsiveness of disaccharidases to variation in dietary starch level is not clear. However, it should be kept in mind that in most reported studies, disaccharidase activity has been measured as specific activity or activity per weight of tissue, not as total enzyme capacity of the intestine. It appears that fish may adjust enzyme capacity more than one way, by adjusting total amount of tissue, concentration or efficiency. Further studies are needed to understand the mechanisms of and variation in adaptation of fish digestive functions to variations in dietary carbohydrate level.

Transport

For two reasons, intestinal transport has attracted a fair amount of attention in fishes. First, intestinal transport plays important roles in intermediary metabolism and as potential regulator of intestinal hormone release and second, because of the use of 'everted sleeves' and isolated brush border membrane vesicles (BBMV) these key phenomena have been well accessible experimentally. The field has been reviewed for mammals (Stevens *et al.* 1984; Karasov & Diamond 1983) and fishes (Collie & Ferraris 1995), therefore, in the present paper, only a short summary of the present state of knowledge will be given, focusing on monosaccharides. In mammals, the products of luminal and brush border carbohydrate hydrolysis, mainly D-glucose, D-galactose and D-fructose reach the blood by passing through the plasma membranes. However, paracellular pathways may also be of importance (Ballard *et al.* 1995), and activation of the cellular route is suggested to stimulate the paracellular transport. In fishes, in contrast, paracellular passage of monosaccharides appear to be negligible (Ferraris *et al.* 1990).

Monosaccharides may cross the brush border membrane by simple diffusion or by the aid of specific transporters. Transporters with different characteristics are localized in the brush border and the basolateral membrane. The piscine D-glucose transporters of the brush border show characteristics similar to those found in mammals, i.e. they are electrogenic and dependent on energy and Na^+ (Collie & Ferraris 1995). The glucose transporters show varying characteristics along the intestinal tract (Ahearn *et al.* 1992). Glucose affinity appears to increase from the proximal to the distal parts of the intestine, while the pyloric caeca display the lowest affinity. Variations in Na^+-dependence may indicate that the transporters of the distal parts of the intestine are more efficient in capturing glucose molecules from the chyme than the pyloric caeca. It is possible that differences in transporter characteristics are because of the differences in the molecular environment of the transporter. The mammalian transporter itself contains 11 transmembrane sections, a total of approximately 662 amino acid residues and a molecular mass of approximately 75 kDa (Turk *et al.* 1994). In addition, the transporter seems to be fairly conserved in the vertebrates, as Northern blots using a probe developed from the rabbit revealed the presence of related mRNAs in intestines of other mammals and the rainbow trout (Pajor *et al.* 1992).

Just like the mammalian glucose transporter, the piscine version also serves as a carrier for D-galactose, while inositol appears to be transported by a specific transporter, quite distinct from the glucose transporter, although inositol may inhibit glucose transport implying the existence of an allosteric inositol binding site (Vilella *et al.* 1989). Further, the kinetic constants for inositol transport differ substantially between carp and eel, which may be linked to the differing dietary preferences between these two species (Vilella *et al.* 1989). Competition between transport of D-glucose and D-galactose across the brush border membrane has been shown in tilapia, eel and other species (Storelli *et al.* 1986; Reshkin & Ahearn 1987a,b; Ahearn *et al.* 1992). Collie & Ferraris (1995) estimated site densities of Na^+-dependent glucose transporters in the intestine of channel catfish (*Ictalurus punctatus*) and came to the conclusion that the low rate of glucose absorption in fish intestine compared with mammals may be explained partly by lower densities of transporters and partly by smaller amounts of absorptive tissue.

Transport of glucose across the basolateral membrane of the absorptive cell in the fish intestine is also similar to that found in mammals (Collie & Ferraris 1995; Ferraris 2001). As in the case of the brush border membrane transporter, the rate of basolateral transport is smaller than in mammals. As expected, the transport is facilitated, Na^+-independent and competitively inhibited by D-galactose. In fact, D-galactose has a higher affinity for the transporter than D-glucose.

Herbivorous and omnivorous species like the carp and tilapia can adapt brush border glucose transport according to changes in dietary carbohydrate level by altering V_{max} (Collie & Ferraris 1995) without affecting the binding kinetics. Therefore, alterations in density of membrane transporters appear to be the predominant mechanism to adapt glucose flux capacity. However, in some species, increased flux rates may also be achieved through alterations in tissue mass.

Some recent molecular evidence supports the idea of presence of both GLUT4 and GLUT2-types in fish intestine. Expression of GLUT4 was confirmed in brown trout intestine by Northern blotting, but curiously, results from RT-PCR experiments were inconclusive (Planas *et al.* 2000). A GLUT2 that shares 58% sequence identity with avian GLUT2 has recently been characterized in rainbow trout liver, and there is evidence that this glucose transporter is also expressed in the rainbow trout intestine (Krasnov *et al.* 2001). Adding a further twist to the discussion on glucose transporters and hormonal control is the surprising postulate of GLUT2-presence (using mammalian antibodies) in adipocytes of a carp (*Catla catla*). The same tissue was also alleged to produce large amounts of insulin, adding adipose tissue as a potential control point for control of glucose metabolism (Roy *et al.* 2003). Presence of GLUT2-type transporters in tissues such as intestine and adipocytes may indicate that glucose uptake sensing is wider spread in fish tissues than initially thought. Independent of the actual route of uptake, intestinal glucose is highly responsive to various hormones, and can be activated by glucagon, the synthetic glucocorticoid dexamethasone (Fig. 5), glucagon-like peptide 1 (Soengas & Moon 1998) and 17α-methyltestosterone (Hazzard & Ahearn 1992).

As sodium is co-transported in active glucose uptake, uptake rates might be expected to vary with water salinity.

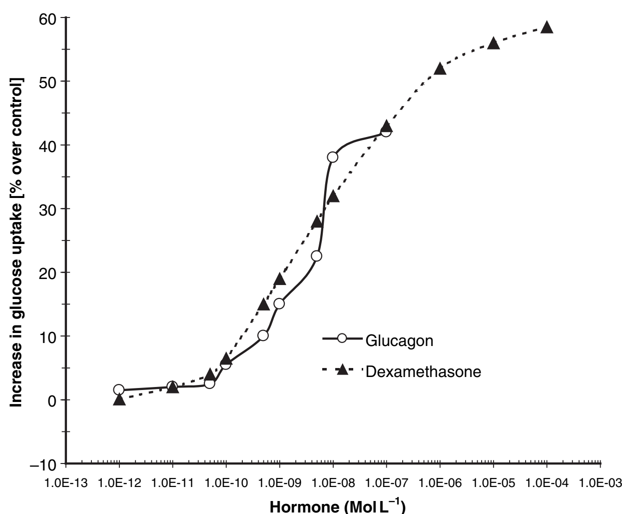

Figure 5 Effects of glucagon and dexamethasone on glucose transport in catfish enterocytes. Isolated catfish (*Ameiurus nebulosus*) enterocytes were exposed to hormones and glucose uptake was determined radiometrically. Data redrawn from Soengas & Moon (1998). Increase in the rate of glucose transport is expressed as a percentage over the rate in the control enterocytes. Maximum variation from the means was less than 7%.

Indeed, such an effect has been described for the Mozambique tilapia (*Oreochromis mossambicus*) (Reshkin & Ahearn 1987b). Glucose transport in brush border vesicles increases in fish kept in full strength seawater compared with fish in fresh water. Seawater adaptation affects apparent glucose influx, apparent diffusional permeability, and apparent Na-affinity of the transporter.

Employing the everted sleeve method, however, both Atlantic salmon and rainbow trout kept in salt water showed lower rates of carrier-mediated glucose transport and lower V_{max} than fish kept in freshwater (Nordrum *et al.* 2000a) in contrast to the observations made with isolated brush border membranes. Whether the apparent difference in results from isolated membrane vesicles and everted sleeves are because of the species differences or methodological differences can only be elucidated through further studies.

The everted sleeve technique has also revealed zonation for capacity for glucose uptake and flux rates generally decreasing from the proximal to the distal part of the intestine of fish regardless of carbohydrate composition of the natural diet (Buddington *et al.* 1987; Bakke-McKellep *et al.* 2000; Nordrum *et al.* 2000a). In eight different fish species on the same diet, the rates of glucose uptake scaled with carbohydrate level in the natural diet, in increasing order from carnivorous, through omnivorous to herbivorous. Moreover, the herbivorous carp showed adaptation of intestinal uptake of glucose in correspondence with dietary glucose level, whereas no such relationship was observed in the rainbow trout (Buddington & Hilton 1987; Buddington 1987). The increase in absorptive capacity in the carp seemed to be brought about by increased amounts of absorptive tissue and stepped-up transport capacities per unit of tissue, while binding kinetics are unaltered. Under conditions of coupled hydrolysis and transport, experimental evidence was obtained showing differences in the gradients of absorption of various classes of carbohydrates in bream (*Abramis brama*) and carp (*Cyprinus carpio*) (Golovanova 1993). The maximal absorption of mono- and disaccharides per unit mass of intestine occurred in the second half of the intestine; absorption of hexoses formed during starch hydrolysis did not vary significantly along the intestine. The actual sugar activity per mass of every intestinal segment examined was maximal for both fish species in the anterior part of intestine for glucose, galactose, fructose and monosaccharides formed during starch hydrolysis. Two maxima of accumulation, in the anterior and central parts of intestine, were found for products of maltose and sucrose hydrolysis. In addition, a correlation was found between transport activity and functional state of the fish, namely, reduced monosaccharide

absorption was observed in intestine of starved carp, especially in the anterior part of the intestine (Golovanova 1993). In carnivorous fish most of the absorption of carbohydrate takes place in the anterior portion of the intestine (Buddington *et al.* 1987, Krogdahl *et al.* 1999).

Effects of environmental temperature on glucose transport have been studied in channel catfish (Houpe *et al.* 1996). According to the increased needs for energy by increasing environmental temperature, transport capacities were found to increase with increasing temperature. Uptakes in brush border membrane vesicles and everted sleeves showed corresponding results.

One final example should exemplify how rudimentary, at least in part, our understanding of carbohydrate digestion and utilization in fish really is. As shown above, chitin is degraded stepwise into NAG; yet we neither know the exact source of the β-*N*-acetylglucosaminidase responsible for production of the monomers, nor the fate of the NAG. We assume that uptake into the enterocytes is via facilitated diffusion. But what is next? Two options are likely, but the sites are unknown. First, NAG could be funnelled into one of the many synthetic pathways, requiring NAG or glucosamine. Second, the glucose-core of NAG could be channelled into glycolytic intermediates, in a pathway involving phosphorylation to NAG-6-phosphate, followed by deacetylation into glucosamine 6-phosphate and finally deamination/isomerization into fructose 6-phosphate and ammonia. Some of the latter enzymes are present in human erythrocytes, but nothing is known for the fishes.

A recurring theme in the above discussion is that the teleostean intestine shows zonation with respect to hydrolytic activities against carbohydrate substances, nutrient resorption and rates of glucose transport. These observations go together with zonation for enzymes of carbohydrate metabolism. For instance, in tilapia the greatest maltase activity (Tengjaroenkul *et al.* 2000), which is localized to the brush border of the columnar epithelial cells, coincides with the areas of peak amylase activity in the middle intestinal region (Nagase 1964). This area, incidentally, is also the area with the highest activity of a glucose-processing enzyme, namely glucose 6-phosphate dehydrogenase in the trout (Fig. 6), and in tilapia after exposure to cortisol (Fig. 7). At this point, it is not clear whether the main function of glucose 6-phosphate dehydrogenase is oxidation of glucose *per se*, pentose delivery for nucleotide biosynthesis or utilization of glucose to furnish reducing power for reductive syntheses. The different zonation of malic enzyme (malate dehydrogenase: decarboxylating, EC 1.1.1.40) seems to run counter to the latter

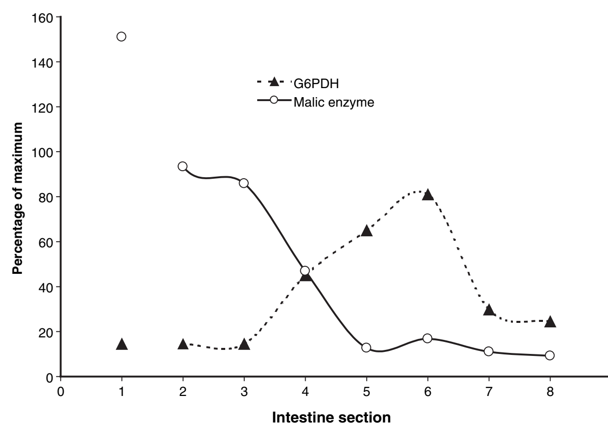

Figure 6 Zonation of glucose 6-phosphate dehydrogenase and malic enzyme in trout intestine. Rainbow trout (*Oncorhynchus mykiss*) intestine was cut into seven parts of equal length and then assayed for the activity of the two enzymes under saturation conditions. Section 1 is the pyloric caeca. Activities are expressed as a percentage of the maximum found in the intestine isolated from four trout. Average error in the determinations was below 20%. Data redrawn from Mommsen *et al.* (2003a)).

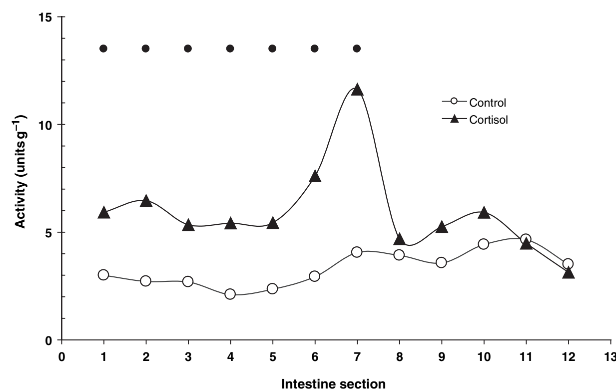

Figure 7 Zonation and hormone responsive of glucose 6-phosphate dehydrogenase in tilapia intestine. Tilapia (*Oreochromis niloticus*) intestine was cut into 12 parts of equal length and then assayed for the activity of G6PDH. Activities are expressed in units per gram of tissue fresh mass. Cortisol was administered as a single i.p. deposit in peanut oil. Animals were feeding and sampled 5 days after injection. The symbol on top of the figure indicates significant increases in enzyme activity after hormone exposure. Data redrawn from Mommsen *et al.* (2003a)).

idea (Fig. 6), as its main function is thought to be generation of NADPH (Mommsen *et al.* 2003a,b). Incidentally, we also noticed location-specific expression of proglucagon genes along the intestine (E.R. Busby and T.P. Mommsen, unpubl. results) hinting at zonation in gene expression and hormonal control along the fish intestine.

Carbohydrate digestibility

In accordance with the large diversity in intestinal structure and functions among fish species, and the great variation in sources of carbohydrates that have been investigated, reported digestibilities vary to a great extent both between and within species.

Low molecular carbohydrates

Mono- and di-saccharides show high digestibilities in all fish species fairly independent of dietary level (Singh & Nose 1967; Hilton *et al.* 1982; Storebakken *et al.* 1998). Rainbow trout, a carnivorous species considered to have limited capacity to modulate enzymes and transporters according to dietary carbohydrate levels, has been found to absorb more than 95% of dietary glucose, sucrose and lactose even when included at high levels. A study of plasma glucose in the white sturgeon (*Acipenser transmontanus*) after oral intubation of various carbohydrate sources also indicates high uptake rates of glucose (Deng *et al.* 2001). Considering the many routes of passive and active uptake mechanisms for these monosaccharides, the high absorbance rates should not come as a surprise.

Starch

The ability of various fish species to digest starch has been the focus of several investigations, but under highly varying conditions also regarding carbohydrate analysis. In the earlier studies and even in some of the newer, dietary and faecal content of starch has been estimated by difference as nitrogen-free extracts (NFE) with or without subtraction of content of crude fibre. Such estimates of starch content are rather inaccurate. In the present discussion regarding starch digestibility only results based on analysis of starch are considered, but in a few cases of particular interest. Table 2a–c present starch digestibility in fish as found in the literature. A wide range of species has been used in the experiments, spanning from herbivores to the strict carnivores. Only a few of the digestibility studies are true comparative studies.

Starch digestibility in common carp and rainbow trout has been compared in a study with diets containing 280 g kg^{-1} starch. The results demonstrate a higher starch digestibility in the carp, 90%, compared with 78% in the trout (Yamamoto *et al.* 2001).

Results of a recent comparative study of starch digestibility with rainbow trout and Atlantic salmon (Krogdahl *et al.* 2004) confirm the general impression given by various studies with one of the species, that rainbow trout digest starch better than Atlantic salmon. Both species digested starch fairly efficiently when included at 70 g kg^{-1} showing 98 and 83% starch digestibility, for the trout and the salmon, respectively. The rainbow trout were able to digest the starch efficiently also when included at 230 g kg^{-1} with a digestibility of 97%. In comparison, Atlantic salmon however, digested only 56% of the starch. A study involving rainbow trout and turbot (*Psetta maxima*) showed higher digestibilities in the trout than the turbot (Burel *et al.* 2000). However, different methods for faeces collection and higher inclusion of binder in the turbot diet prevent direct comparison.

A study with three species of the Indian major carp, *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*, comparing the species ability to digest carbohydrates, in this case analysed as NFE, showed very similar digestibilities by the three species (Erfanullah & Jafri 1998). We investigated 11 products of grains, potato, soybeans and lentil. On the other hand, large variations were observed among the feedstuffs.

In line with conclusions in earlier reviews (Wilson 1994; Pfeffer 1995) and further supported by the results presented in Table 2a–c, it is clear that many species show decreased starch digestibility with increasing dietary starch level (Singh & Nose 1967; Hemre *et al.* 1989, 1995; Buddington *et al.* 1987; Arnesen *et al.* 1995; Grisdale-Helland & Helland 1998; Burel *et al.* 2000; Hillestad *et al.* 2001; Krogdahl *et al.* 2003). Apparent digestibility coefficients (ADC) from 10 to 90% may be found, dependent on starch inclusion level (Hemre *et al.* 1989, 1995) and complexity of the carbohydrate source (Hemre *et al.* 1990; Buddington *et al.* 1997, Grisdale-Helland & Helland 1998). Greater variability dependent on starch source has been observed in cod (*Gadus morhua*) fed different wheat qualities (ADC from 40 to 60% at 200 g kg^{-1} starch inclusion), and gelatinized potato meal showed an ADC of 40% in cod (Hemre *et al.* 1990).

The dependence of starch digestibility on dietary inclusion level may be because of the limited capacity for starch digestion. Hence, starch digestibility may also depend on ration size. Experiments with rainbow trout and sea bream (*Sparus aurata*) show that this may be the case (Windell *et al.* 1978; Bergot & Breque 1983; Fernandez *et al.* 1998).

Dietary lipid level may affect starch digestibility as well. This is apparent from studies of Atlantic salmon (Grisdale-Helland & Helland 1997). Starch was less digestible in the high-fat diets (fat levels 240–300 g kg^{-1}) than the low fat diet (160 g kg^{-1}), and the difference seemed to increase with increasing dietary starch level. Effects of lipid level have also

Table 2 Starch digestibility in (**a**) cod and rainbow trout, (**b**) Atlantic salmon, Atlantic halibut, turbot and European sea bass and (**c**) carp and tilapia[1]

Fish species	Water	Fish weight (g)	Faecal collection method	Investigated carbohydrate	Diet process	Diet level of carbohydrate (g kg^{-1})	Diet gross energy (MJ kg^{-1} dry feed)	Starch digestibility (%)	Reference
(a) cod and rainbow trout									
Cod (*Gadus morhua*)	SW	400	Dissection	Dextrin of potato starch	P	100	26.8	26	Hemre *et al.* (1989)
						200	27.4	33	
						300	26.5	40	
	SW	150	Dissection	Soya NSP	P	200	20.0	0	Hemre *et al.* (1990)
				Raw wheat meal			23.0	45	
				Cooked wheat meal			22.9	59	
				Ex wheat			23.6	62	
				Ex potato			23.4	46	
	SW	200	Stripping	Extruded wheat	Ex	70	26.3	95	Hemre *et al.* (2003)
						180	22.6	92	
Rainbow trout (*Oncorhynchus mykiss*)	FW	10–25	Stripping	Potato starch	Moist pellet	200	22.4	69	Singh & Nose (1967)
						300	21.7	65	
						400	21.1	53	
						500	20.5	38	
						600	19.8	26	
				Dextrin		200	22.4	77	
						300	21.7	74	
						400	21.1	60	
						500	20.5	50	
						600	19.8	46	
	FW	120	Automatic collection from water	Native corn starch	P				Bergot & Breque (1983)
				50% feeding		310	19.1	55	
				100% feeding		310	19.1	38	
				Cooked corn starch					
				50% feeding		270	18.7	90	
				100% feeding		270	18.7	87	
	FW	100	Automatic collection from water	Potato starch	P	270	18.7	5	Bergot (1993)
				Manioc starch		270	18.7	19	
				Rice starch		260	18.7	43	
				Wheat starch		260	18.7	58	
				Maize starch		280	18.7	34	
				Amylomaize starch		260	18.7	24	
				Waxy maize starch		270	18.7	56	
				Gl. mize starch		280	18.7	96	
				Ex maize starch		260	18.7	96	
	FW	100	Stripping	Whole oat	P	180	19.0	68	Arnesen & Krogdahl (1995)
				Rolled oat		140	20.5	91	
				Oat meal		140	20.8	94	
	FW	100	Automatic	Wheat starch, gl.	P	140	22.1	98	Burel *et al.* (2000)
	FW	100	collection from water	Wheat starch, gl. + Peas starch, (1 : 3)	Ex	230	21.1	83	

	SW	700	Stripping	Corn dextrin	P	200	21.7	44	Storebakken *et al.* (1998)
	FW							48	
	FW	38	Filtration from outlet water	Wheat starch + gl starch (3 : 1)	Ex	280	21	78	Yamamoto *et al.* (2001)
	FW	565	Stripping	Maize starch, gl.	Ex	70	24.0	99	Krogdahl *et al.* (2003)
						230	23.9	98	
	SW	565				70	24.0	98	
						230	23.9	97	
	FW	100	Automatic collection from water	Corm starch raw + gl.	P	38 + 4	20.1	80	Kim & Kaushik (1992)
						21 + 21	20.4	70	
						4 + 38	20.3	61	
(b) Atlantic salmon, Atlantic halibut, turbot and European sea bass									
Atlantic salmon (*Salmo salar*)	SW	600	Stripping	Raw corn + wheat grain[1] (1 : 1)	Ex	24	25.2	81	Aksnes (1995)
						98	25.2	71	
						165	23.9	61	
						230	24.2	43	
	SW	145	Stripping	Whole oat	P	70	20.7	61	Arnesen *et al.* (1995)
						90	19.4	50	
						120	19.4	43	
						140	19.2	30	
						160	18.7	33	
				Mixes of oat and corn					
				0 : 30		232	19.5	23	
				6 : 24		210	20.0	38	
				12 : 18		190	19.6	47	
				18 : 12		170	20.1	48	
				24 : 6		150	19.6	43	
				30 : 0		130	19.7	36	
	SW	450	Dissection	Cooked wheat	Micro-wave	50	23.8	91	Hemre *et al.* (1995)
						90	23.8	93	
						220	23.8	75	
						310	22.7	76	
		80	Stripping	Wheat starch	Ex	213	24.9	75[2]	Grisdale-Helland & Helland (1997)
						161	24.1	78[2]	
						145	24.0	82[2]	
						105	24.6	88[2]	
						221	22.3	82[3]	
						172	22.4	85[3]	
						131	22.4	88[3]	
						88	22.5	91[3]	
	SW	1900	Dissection	Precooked maize	Ex	60	23.3	80	Krogdahl *et al.* (1999)
						170	21.6	39	
	SW	500	Stripping	Rye flour	Ex	120	23.4	51	Thodesen & Storebakken (1998)
						150	22.9	53	
				Wheat flour		130	23.3	53	
						160	23.0	54	

Table 2 Continued

Fish species	Water	Fish weight (g)	Faecal collection method	Investigated carbohydrate	Diet process	Diet level of carbohydrate (g kg^{-1})	Diet gross energy (MJ kg^{-1} dry feed)	Starch digestibility (%)	Reference
	SW	180	Stripping	Whole wheat/fish oil		140	22.5	60	Nordrum *et al.* (2000b)
				Whole wheat/MCT	Ex	150	22.7	73	
	SW	940	Stripping	Wheat starch	Ex	120	24.1	72	Storebakken *et al.* (2000)
	SW	275	Stripping	Potato starch	Ex	180	23.2	46	Hillestad *et al.* (2001)
						120	23.3	54	
						80	22.9	62	
	FW	199	Stripping	Maize, gl.	Ex	70	24.0	90	Krogdahl *et al.* (2003)
						230	23.9	60	
	SW					70	24.0	83	
						230	23.9	56	
Atlantic Halibut (*Hippoglossus hippoglossus*)	SW	1000	Stripping	Extruded wheat	Ex	90	22.9	83[3]	Grisdale-Helland & Helland (1998)
						170	22.6	55[3]	
						90	24.5	85[2]	
						160	24.4	52[2]	
Turbot (*Psetta maxima*)	SW	110	Passive settling	Wheat, gl.	P	144	22.1	82	Burel *et al.* (2000)
				Wheat, gl. 120 g + peas ex, 300 g kg^{-1}		228	22.1	75	
European sea bass (*Dicentrarchus labrax*)	SW	28	Passive settling	Raw starch	P	250	20.3	66	Peres & Oliva-Teles (2002)
				Raw + gl. starch		125 + 125	20.9	85	
				Gl starch		250	20.7	98	
(c) carp and tilapia									
Common carp (*Cyprinus carpio*)	FW	13	Filtration of outlet water	Raw corn	P	320	20.1	60	Hernández *et al.* (1994)
				Raw and ex corn, (1 : 1)				84	
				Ex corn				92	
	FW	170–204	Filtration from outlet water	Flaked wheat	P	300	21.2	99	Medale *et al.* (1999)
	FW	45	Filtration from outlet water	Wheat starch + gl. starch (3 : 1)	Ex	280	21	90	Yamamoto *et al.* (2001)
Tilapia (*Oreochromis niloticus* × *Oreochromis aureus*)	FW	6	Siphoning two times a day	Corn starch	?	310	18.5	92	Shiau & Liang (1994)
						470	20.2	86	
				Corn starch + 100 g kg^{-1} agar		310	18.4	78	
						470	20.1	71	
	FW	6	Siphoning two times a day	Raw corn starch	?	340	18.8	95	Shiau & Liang (1994)
				D-glucose		340	18.8	93	

[1] Water quality: FW = fresh water; SW = salt water; P = pelleted; Ex = extruded; NFE = nitrogen free extract; gl = gelatinized.
[2] High fat diet.
[3] Low fat diet.

been studied in Atlantic halibut (Grisdale-Helland & Helland 1998). In the latter species no lipid effect on starch digestibility was observed. An explanation for the effect observed in Atlantic salmon is difficult to suggest as the opposite effect might have been expected. Lipids are known to influence the velocity of all nutrients passing through the gastrointestinal tract. Indications exist that increased dietary lipid contents reduce passage-velocity giving the enzymes more time for hydrolysis (Buddington *et al.* 1987).

Also lipid quality has been observed to modify starch digestibility. In a study with Atlantic salmon, medium chain triglycerides (MCT) elevated starch digestibility from 60 to 73% compared with fish oil. The test diets were extruded, contained 100 g kg^{-1} MCT and 150 g kg^{-1} starch, and were pair fed (Nordrum *et al.* 2000b). In a dose–response experiment MCT showed corresponding results (Nordrum *et al.* 2003).

Processing conditions also have great impact on starch digestibility (Hemre *et al.* 1990; Jeong *et al.* 1992; Kim & Kaushik 1992; Arnesen *et al.* 1993; Bergot 1993; Wilson 1994; Erfanullah & Jafri 1998; Zemke-White & Clements 1999; Allan *et al.* 2000; Booth *et al.* 2001). Beneficial effects of heat treatment are apparent even for the herbivorous fish (Jeong *et al.* 1992; Erfanullah & Jafri 1998). The variety of starch sources tested range from purified corn and potato starch to starch in complex algae. Processing conditions also vary widely, and comprise dehulling, concentration, heat treatment in pellet press or extrusion, heating under wet or dry conditions, fermentation, etc. From these studies it may be concluded that most starches need heat treatment to be digestible for both carnivorous and herbivorous species. On the other hand, a study of various oat products showed oat starch to be highly digestible without heat treatment in rainbow trout (Arnesen & Krogdahl 1995). Moreover, Atlantic salmon appears to digest raw oat starch to a certain degree (Arnesen *et al.* 1995). When 100 g kg^{-1} raw oat starch was included in salmon diets 61% was digested, whereas, the digestibility at 300 g kg^{-1} inclusion was 33%. The latter study also showed positive effects of combining starch sources. When a 50/50 mix of oat and maize starch was included at a total level of 330 g kg^{-1} in the diet, substantially higher digestibilities were found than when each were fed as the sole starch source. The capacity of the digestive tract for carbohydrate digestion and absorption appeared to be utilized to a greater extent when fed the mixed starch sources than when fed only a single source. Hence, starch digestibility also varies depending on the combination of starch sources in the diet.

For anadromous fish such as salmonids, water salinity may affect starch digestibility. In the salmon digestibility of starch was 6% lower in saltwater than freshwater, and the effect was independent of inclusion level (60 and 220 g kg^{-1} in the diet) (Krogdahl *et al.* 2003). In rainbow trout the effect appeared less pronounced than in Atlantic salmon, with the observed effect varying between 1 and 2.5%-units (Storebakken *et al.* 1998; Krogdahl *et al.* 2003). These results are in line with the lower glucose transport rates observed in both Atlantic salmon and rainbow trout using the everted sleeve technique (Nordrum *et al.* 2000a).

Non-starch polysaccharides

Non-starch polysaccharides (NSP) comprise cellulose, hemicellulose, β-glucans, pectins and gums and belong to the natural diet of herbivorous and omnivorous fish, but not carnivorous species. However, NSP are often found in diets for cultivated carnivorous fish as constituents of grains, soybean meal, specific binders, etc. No experiments have supplied information indicating that carnivorous fish are able to utilise NSP. Information on NSP digestion is scarce also for herbivorous and omnivorous species, and the available information is unreliable not at least because of the analytical difficulties associated with NSP analysis (Saunders & Hautala 1979). In the herbivore *Labeo rohita* feeding on macrophytes carbohydrate digestibilities vary between species of macrophytes (Ray & Das 1994) supposedly because of the variation in carbohydrate composition. Variation in contents of pectins in macrophytes, seagrass, and algae may account for the observed variation in NSP-utilization. A comparative study between an herbivorous and an omnivorous species fed diets with algae showed comparable and low digestibilities for algae fibre and algae cell walls (Galetto & Bellwood 1994). Efforts to evaluate nutritive value of NSP in the omnivorous pinfish (*Lagodon rhomboides*) indicate some ability to utilize soluble low-molecular carbohydrates from seagrass, but little or no utilization of the complex carbohydrates (Lindsay & Harris 1980).

Rather than supplying nutrients, NSP may reduce utilization of other nutrients and thereby act as anti-nutrients. This is true for domesticated, terrestrial animals (Krogdahl 1986) and appears to be true for fish both carnivores and herbivores (McGoogan & Reigh 1996; Erfanullah & Jafri 1998). Within plants NSP form structural networks and have important functions, i.e. they bind water, exchange cations, bind minerals and adsorb organic compounds like sterols and acids. These characteristics are expressed also when they enter the gastrointestinal tract of animals. Under certain

circumstances these characteristics may be beneficial for the animal, but mostly they will exert negative impact on nutrient utilization.

Non-starch polysaccharides may be classified according to their water solubility; insoluble NSP include cellulose and many hemicelluloses, and soluble forms include pectins, gums and mucilages. In mammals and poultry insoluble fibres tend to increase transit rate whereas soluble fibres slow the rate of passage for the digesta. Increased transit rate decreases the time available for nutrient digestion and would be expected to restrict nutrient utilization. In pigs and calves this seem to be valid (Krogdahl 1986). The tendency of soluble fibre to slow down transit rate does not seem to be of much benefit. The gelling, adsorption and ion exchange characteristics of such fibres tend to retard hydrolysis and absorption.

Information on antinutritional effects of NSP in fish is incomplete. However, some NSP seem to increase the transit rate through the gut (Montgomery & Targett 1992), which is possibly related to the decreased nutrient concentration in the intestine. For instance, undigested starch seems to increase transit rate in rainbow trout (Spannhof & Plantikow 1983). As pointed out above, other NSPs slow the transit rate (Storebakken 1985). There is also experimental evidence that increased inclusion of ingredients high in NSP decreases dry matter digestibility in many species (Omoregie & Ogbemudia 1993; Shiau & Liang 1994; Sullivan & Reigh 1995; Wen-Zhang *et al.* 1995; Schwarz & Kirchgessner 1995; McGoogan & Reigh 1996; Refstie *et al.* 1999). Moreover, the NSP reduce utilization of digestible carbohydrates, proteins and lipids *in vivo* (Schwarz & Kirchgessner 1995) as well as the *in vitro* protein digestibility (Ryu *et al.* 1992). An *in vitro* study of effects of pectin, gum karaya, alginate and cellulose showed that all these NSP may inhibit protein hydrolysis. Including alginate and guar gum in diets for rainbow trout reduce protein and lipid digestibility to a great extent, with the effect more pronounced for guar gum than alginate (Storebakken 1985).

Concluding remarks

Carbohydrates are at present important ingredients in fish diets because of their attractive physical characteristics. They are also considered to supply energy at low cost. However, the digestible carbohydrates are found in ingredients that also contain other compounds, including NSP that are indigestible and may inhibit digestion and utilization of all nutrients. The net gain in energy from carbohydrate ingredients may therefore be marginal or even negative in some species. From the present knowledge on digestive processes it is not possible to understand the effects of starch sources and the resulting digestibilities and nutrient utilization. The events taking place in the intestine are undoubtedly of utmost importance for the overall result. Most studies are of applied character of little explicatory substance. Basic and comparative studies are clearly needed.

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