Control and Efficiency of Digestive Function of Marine Fish Larvae

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ABSTRACT

Recent downscaling and improvements of tube feeding techniques have allowed more detailed studies on the digestive and absorptive efficiency of larval fish, including the transfer kinetics of selected nutrients from the lumen of the digestive tract into the tissues of the body. Freely dissolved amino acids seem to be absorbed rapidly and with a high efficiency. There has also been some progress towards understanding how the digestive process is controlled in marine fish larvae. The peptide hormone cholecystokinin (CCK) has been targeted since it is believed to play an important role in controlling digestive function in vertebrates.

Key words: digestive function, cholecystokinin, absorption, amino acids, marine fish larvae

INTRODUCTION

When fish larvae commence exogenous feeding, the flow of nutrients formerly supplied only from yolk reserves becomes supplemented through the digestive tract. The majority of marine fish larvae currently targeted for cultivation hatch from pelagic eggs and their digestive system is still developing at the onset of exogenous feeding. A fully developed digestive tract, including gastric digestion, develops during metamorphosis. Although the larval gut is not completely developed at the onset of exogenous feeding, it is sufficiently efficient to support larval growth by digesting such prey as is available under natural conditions in the sea. The physiological constraints of the gut with respect to digestion of cultivated live prey and particularly formulated starter feeds still remain to be elucidated. This paper reviews some of our recent findings in the areas of control and efficiency of digestive function of marine fish larvae.

CHOLECYSTOKININ

The peptide hormone cholecystokinin (CCK) is a major regulatory hormone of digestion. CCK is produced in endocrine cells scattered among the epithelial cells lining the intestine (Liddle, 1995) which, when exposed to the appropriate stimuli in gut contents, release CCK into the circulation. The functions of CCK in vertebrates include (Figure 1): release of bile (1) and

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pancreatic digestive enzymes (2), regulation of stomach emptying (3), and influencing peristaltic and motor activity (4). Specific studies on fish have demonstrated that CCK stimulates gall bladder motility *in vitro* (Aldman & Holmgren, 1987; Rajjo *et al*., 1988; Andrews & Young, 1988) and *in vivo* (Aldman et al., 1992). CCK also stimulates trypsin and chymotrypsin secretion into the gut (Einarsson *et al*., 1997) and delays gastric emptying in rainbow trout (Olsson *et al*., 1999).

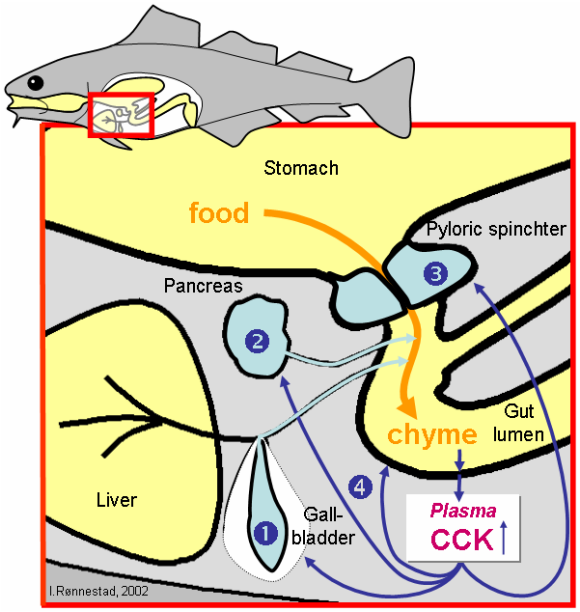


Figure 1. Roles of cholecystokinin (CCK) in digestion. Compiled from sources in the text.

The appearance and distribution of CCK-producing cells in the digestive tract of several developing larval fish were investigated by means of immunohistochemistry (Kamisaka *et al*., 2001a,b, 2002), employing a primary antiserum against CCK that had been cloned for Japanese flounder, *Paralichthys olivaceus*. The distribution of CCK-immunoreactive (CCK-IR) cells was limited to the anterior intestine and pyloric caeca in fish with a looped alimentary canal (Atlantic halibut, and bluefin tuna, *Thunnus thynnus*), while CCK-IR cells were distributed all along the intestine in fish with a straight gut (herring, *Clupea harengus* and Ayu, *Plecoglossus altivelis*), as well as in the rectum in herring larvae. The localization of CCK-IR cells in the anterior midgut, particularly adjacent to the pyloric caeca in Atlantic halibut and bluefin tuna, agrees with the results of studies on Japanese flounder (Yoshida *et al*., 1983; Kurokawa *et al*., 2000) and rainbow trout (Barrenechea *et al*., 1994).

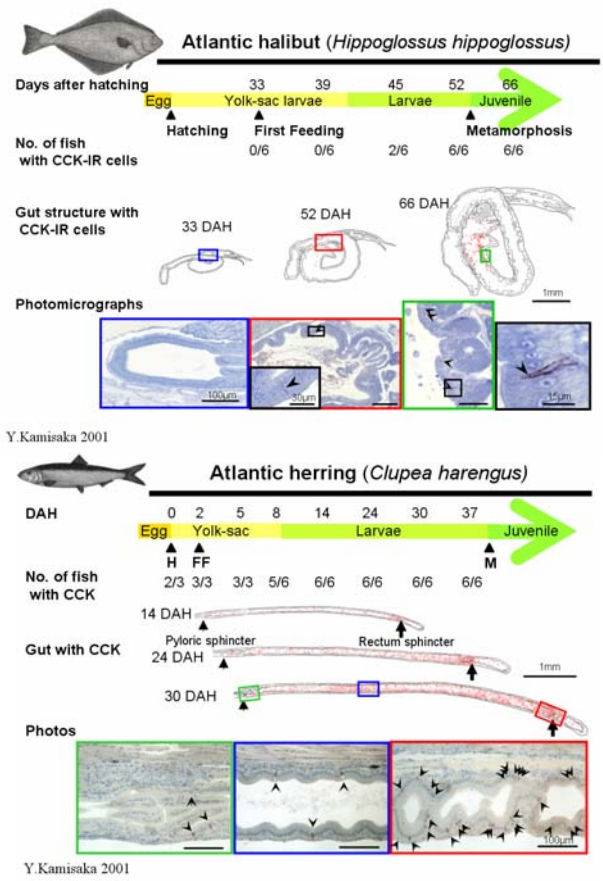


Figure 2. Ontogeny of CCK-IR cells in the digestive tract of larval Atlantic halibut (A) which has a rotated/ looped gut and Atlantic herring (B) which has a straight gut. Adapted from Kamisaka *et al*. 2002.

The stomachless teleost, *Barbus conchonius*, (Rombout & Taverne-Thiele, 1982), also showed a significant presence of CCK-IR cells adjacent to the entrance to the pyloric caeca. The distribution of CCK-IR cells is most likely linked to the requirement for close contact with the digesting food in order to properly regulate the release of digestive secretions. Such and arrangement would serve to optimize substrate-enzyme ratios in the intestinal lumen.

Rønnestad *et al*. (2000c) have proposed the participation of retrograde peristalsis in mixing chyme with digestive secretions in the region of the pyloric caeca. The retrograde contractions, which move in the opposite direction of normal propulsive waves, seem to be initiated from a specific location in the midgut curvature and spread anteriorly towards the pyloric sphincter (Figure 3).

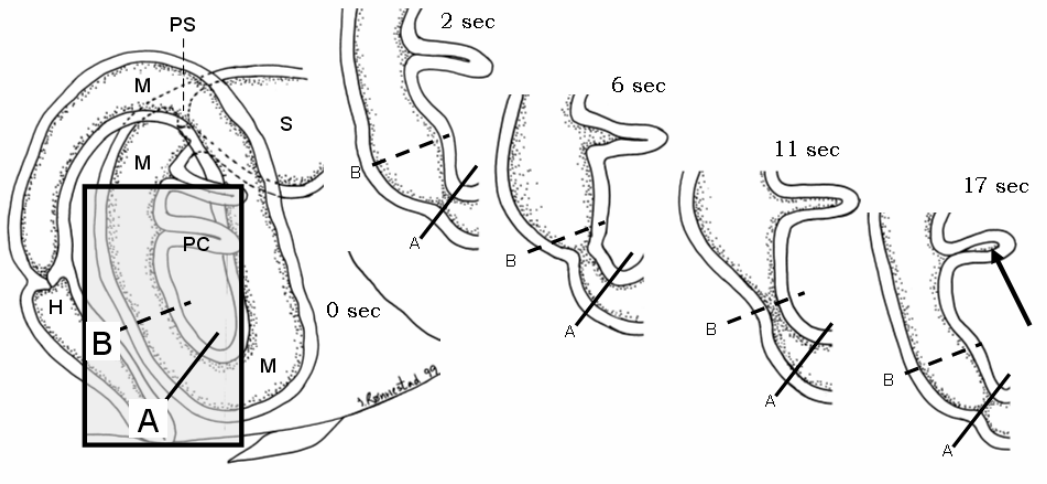


Figure 3. Retrograde peristaltsis in post-larval Atlantic halibut. The wave starts caudad to the pyloric caeca (line "A") and proceed cranially to become diminished in the region of the pyloric caeca (line "B"). The propulsion of one wave is shown in the sequence of figures (2 sec-17sec). S: stomach; M: midgut; PC: pyloric sphinchter, H: hindgut. Adapted from Rønnestad *et al*., 2000c.

This activity occurs in the area where the CCK-IR cells are located (Fig 2A; Kamisaka *et al*., 2001a), supporting the idea that the pattern of distribution of CCK-IR cells have functional relevance to the control of the digestive processes including enzyme secretions and possibly intestinal mixing movements. Retrograde peristalsis was also demonstrated as a mechanism for filling the pyloric caeca in Atlantic halibut (Fig 3, Arrow at 17 sec; Rønnestad *et al*., 2000c), a phenomenon that had formerly awaited confirmation for fish. A recent study has verified that retrograde peristalsis occurs in other larval fish as well (Holmgren *et al*., 2001), pointing to a common phenomenon in fish larvae. Further documentation of peristaltic movements and motor activity in fish larva is currently being done in our laboratory.

INDICATIONS OF A REGULATORY LOOP FOR PANCREATIC SECRETIONS

The control of digestive functions in vertebrates is highly complex and involves many factors including neural pathways and peptide hormones. Our current understanding of the factors implicated in its regulation highlight CCK as an important hormone in the regulation of exocrine pancreatic secretions. Trypsin has been implicated in the feedback control of CCK release from the CCK-producing cells in the intestinal brush border. Studies on mammalians have suggested that a luminal CCK-releasing factor is secreted into the gut where it stimulates the secretion of CCK, but only in the absence of trypsin, which proteolytically deactivates it (Figure 4; Lewis & Williams, 1990; Owyang, 1994; Liddle, 1995, 2000). Regulation is believed to occur when ingested protein out-competes CCK as substrates for trypsin thereby preventing the inactivation of the CCK-releasing factor (Owyang, 1994; Liddle, 1995, 2000).

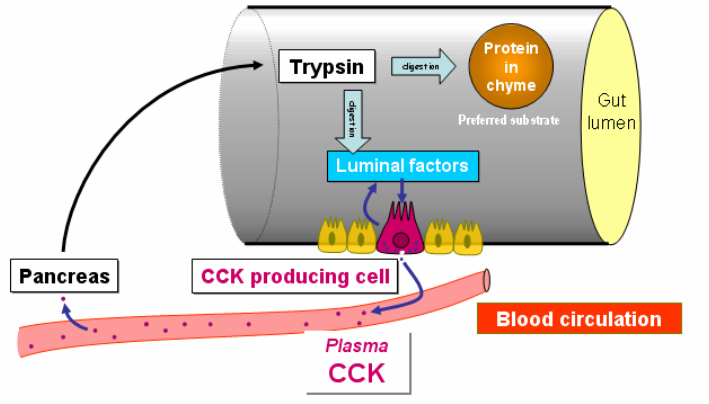


Figure 4. A proposed model for regulation of CCK release in mammals. Adapted from Lewis & Williams (1990) and Rojas-Garcia (2002).

Studies on humans have concluded that the most potent stimulants of CCK secretion are the partial digestion products of fat and protein, including di- and tri-peptides (Liddle, 2000). Studies on rats have shown that dietary stimulation of CCK release caused by intraduodenal administration of trypsin inhibitor, resulted in the transcription of new CCK mRNA (Liddle, 1994a). To complicate matters, the neuropeptide bombesin stimulates CCK secretion without modifying intestinal CCK mRNA levels (Liddle, 1994b). Thus, the secretion of CCK is not necessarily linked to its gene expression or its cellular content.

Where fish larvae are concerned, research is still in its infancy. A recent study on the first-feeding stages of Atlantic herring reported that soluble protein caused a more rapid and greater increase in CCK content (whole-body homogenate) than did free amino acids (FAA). In addition, tryptic activity increased in larvae fed protein, while no changes occurred in larvae fed FAA (Koven *et al*., 2002). However, another study on seabass (*Dicentrarchus labrax*) found that trypsin secretion was stimulated in larvae fed a mixture of FAA while a protein hydrolysate (casein) actually reduced secretion (Cahu & Infante, 1995 a,b). In support of these findings, it has been demonstrated that digestive end-products like L-lysine can act directly on the pancreatic acinar cells to stimulate enzyme secretion (Grendell & Rothman, 1981). Tryptophan and phenylalanine are also potent AA for stimulating CCK secretion (Liddle, 2000). The two latter studies support the notion that FAA are themselves stimulants for pancreatic exocrine secretions.

The Atlantic herring larvae studied by Koven *et al*. (2002), which were tube-fed physiological saline, did not display a CCK response despite the presence of peristaltic movements and the apparent fullness of the gut. This indicates that distension of the gut wall is not a trigger for CCK synthesis. As previously stated the values reported by Koven *et al*. (2002) represent whole-body homogenate CCK and therefore it is not possible to differentiate between neural transmitter CCK in the brain and hormonal CCK in the digestive tract. In mammals, CCK present in the central nervous system (CNS), has been demonstrated to contribute to regulation of food intake and satiety. Using immunohistochemistry we have recently documented CCK IR cells in the brain of Atlantic halibut and herring (Y. Kamisaka, unpublished; Figure 5).

Our recent studies on eviscerated guts and bodies of developing Atlantic halibut larvae have shown that in these early stages neural CCK in the CNS may be the quantitative dominant form (Rojas-García & Rønnestad, 2002a; Rojas-García, 2002.).

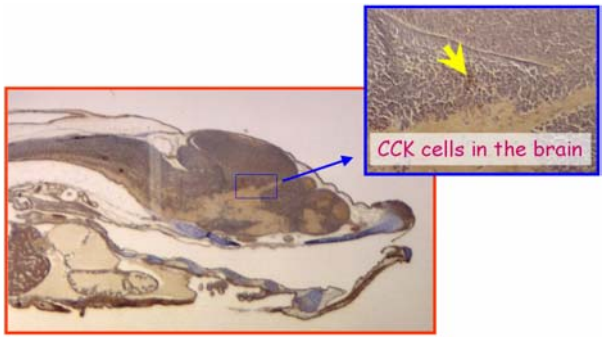


Figure 5. CCK-IR cells in the brain of Atlantic halibut larvae. Photo by Yuko Kamisaka, unpublished.

One week after the onset of exogenous feeding, the CCK content of the gut (analyzed by radio immunoassay RIA; Figure 6) represents 2% of the whole body CCK content, increasing to 62% at 4 weeks after first feeding (Rojas-García & Rønnestad, 2002a, Rojas-García, 2002.).

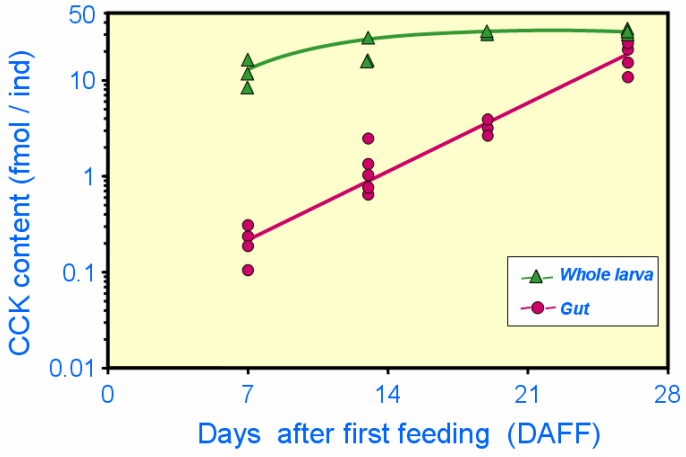


Figure 6. The CCK content of developing Atlantic halibut larvae (short-term fasted). Adapted from Rojas-García & Rønnestad, 2002a

The ontogeny and location of CCK in the brain of larval fish is currently being studied in our lab using *in situ* hybridization techniques. In contrast to the other species (Atlantic herring, Ayu, bluefin tuna) we have not been able to detect CCK in the digestive tract of Atlantic halibut at the stage when the larvae are offered live prey in aquaculture systems (Kamisaka *et al,* 2001; Rojas-García & Rønnestad, 2002a). The mechanisms controlling the release of bile

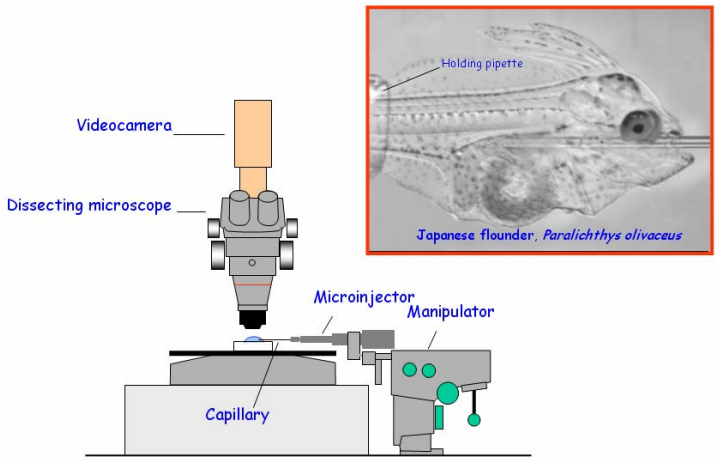
and pancreatic enzymes and peristalsis during the period between first feeding and the first detection of CCK in the gut remain to be clarified.

Clearly, more refined studies are required in order to understand the regulation of the digestive response in larval fish, in which the digestive system is still developing. These studies also demonstrate that it is necessary to separate neural and gastrointestinal sources of CCK in order to determine its alimentary role in fish larvae.

EFFICIENCY OF DIGESTIVE FUNCTION

Studies of digestive efficiency in marine fish larvae including absorption and nutrient assimilation are seriously limited by a number of factors. Fish larvae are very small at the onset of exogenous feeding with commercially important species such as turbot, *Scophthalmus maximus*, gilthead sea bream, *Sparus aurata* Senegal sole, *Solea senegalensis* and Japanese flounder have larvae that measure around 3 mm at the onset of exogenous feeding. Typical feed-particle sizes for these small stages range from 50 to 150 μm, which offers a major challenge to starter diet production technology. The acceptance of artificial diets by the larval fish is low and variable ingestion rates are obtained in feeding experiments. In some cases, first-feeding marine fish larvae ingest artificial feed but fail to grow, although recent advances have produced better results (Fernandez-Diaz & Yufera, 1997; Lazo *et al*., 2000; Cahu & Zambonino-Infante, 2001; Koven *et al*., 2001; Kolkovski, 2001). Nevertheless, in most species, problems arising from using formulated feeds from the onset of exogenous feeding persist.

A



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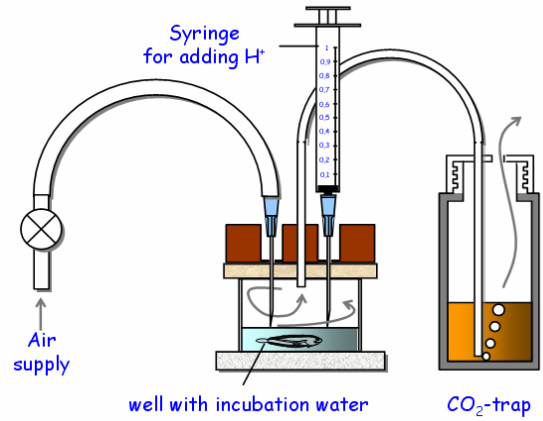

Figure 7. An *in vivo* method for controlled tube-feeding in fish larvae. A: the experimental set up. Insert shows first feeding Japanese flounder being tube-fed a colored solution. B: Metabolically produced 14CO2 is entrapped through aeration and manipulation of the pH of the incubation water. Drawing B by S. Tonheim and adapted from Rønnestad *et al*., 2001a.

The low acceptance and growth resulting from the use of formulated starter diets complicate studies of nutrient absorption, since traditional methods cannot be used. One possible route for quantifying the digestive capacity and assimilation of marine fish larvae is an *in vivo* method for controlled tube-feeding (Figure 7A; Rust *et al*., 1993; Rust, 1995; Rønnestad *et al*., 2001a). In the refinement of this procedure, metabolically produced $^{14}CO_2$ is entrapped through aeration and manipulation of the pH of the incubation water (Figure 7B; Rønnestad *et al*., 2001a). This permits ^{14}C-labelled nutrients evacuated from the gut (or excreted elsewhere) to be distinguished from $^{14}CO_2$ originating from catabolism of the absorbed nutrients. In

combination with scintillation counting of isolated organs or body compartments (Figure 8), the system provides a useful framework for investigating features of gut absorption, oxidation and retention (assimilation) of nutrients.

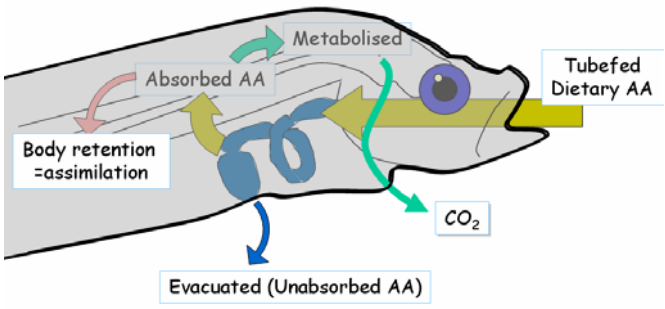

Figure 8. Using ^{14}C-labelled nutrients (eg amino acids) the fraction evacuated from the gut (or excreted elsewhere) may be distinguished from $^{14}CO_2$ originating from catabolism of the absorbed nutrients.

Rust (1995), using an early version of this tube-feeding setup and ^{35}S labelled AA, concluded that fish larvae without a stomach at first feeding, initially assimilated simple forms of AA more efficiently than more complex nutrients (assimilation order: FAA > peptides > protein). His results further revealed that differences between the assimilation efficiencies of the three sources of AA were reduced as the larvae approached metamorphosis.

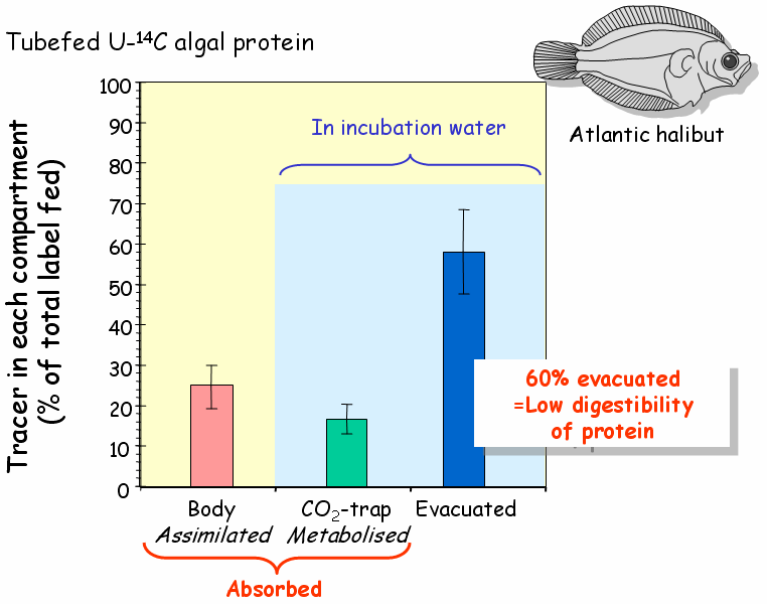

Figure 9. Atlantic halibut post larvae (46 Days post first feeding) tube-fed a ^{14}C-labelled algal protein. Adapted from Rønnestad *et al*. (2001a)

More recent data support the idea of low absorption of AA from protein. Using ^{14}C labelled algal protein, we demonstrated low absorptive capacity for protein from the digestive tract in

postlarval Atlantic halibut (Figure 9) supporting the data of Rønnestad *et al*. (2000a) and Rojas-García & Rønnestad (2002b), as well as previous data for juvenile and adult fish (Atlantic cod, Berge *et al*., 1994; Atlantic salmon, *Salmo salar*, Espe *et al*., 1993, 1999; rainbow trout, *Oncorhynchus mykiss*, Yamada *et al*., 1981) and other vertebrates including man (Metges *et al*., 2000).

In contrast to protein, FAA seem to be absorbed with a high efficiency. In postlarval Senegal sole, the AA tested were absorbed with similar efficiency (>97%; Figure 10). A small fraction (12 - 15%) of the indispensable AA lysine and arginine were catabolised and a high proportion was retained in the body (81-86%). For the dispensable AA tested, more were catabolised (ca 40 and 65% for glutamate and alanine, respectively) and less was retained (33% and 56%). Comparable values have also been found for Atlantic herring (Conceição *et al*., 2002)

A potential pitfall with the use of dietary FAA is overloading of the metabolic systems by a high AA flow from the gut. Absorbed dietary AA are either used for protein synthesis or are otherwise processed by being channelled to energy production, gluconeogenesis, lipogenesis (Berge *et al*., 1994), or may even be lost as intact molecules through the urine or gills. Urinary loss is not a major route of AA excretion in older fish (Ng *et al*., 1996). Kolkovski (2001) has suggested that most of the FAA and peptides in micro-diets will be flushed out of the digestive system. However, the data we have collected thus far suggest that only a small proportion (<3% in Senegal sole, see above) of the FAA supplied to fish larvae as a single pulse by tube feeding is lost in intact form. Whether differences in FAA loading to the gut or other experimental procedures may explain these differences is currently being studied in our lab. These experiments will also be targeted towards further describing and quantifying the roles and fates of amino acids in marine fish larvae as follow-ups to our previous work (e.g. Fyhn, 1989; 1990; Rønnestad & Fyhn 1993; Rønnestad *et al*., 1992a,b; 1994; 1999; 2000a,b,c; 2001a,b; 2002; Finn, 1995a,b,c; Terjesen *et al*., 2000, 2002, Conceicao *et al*., 2002a,b).

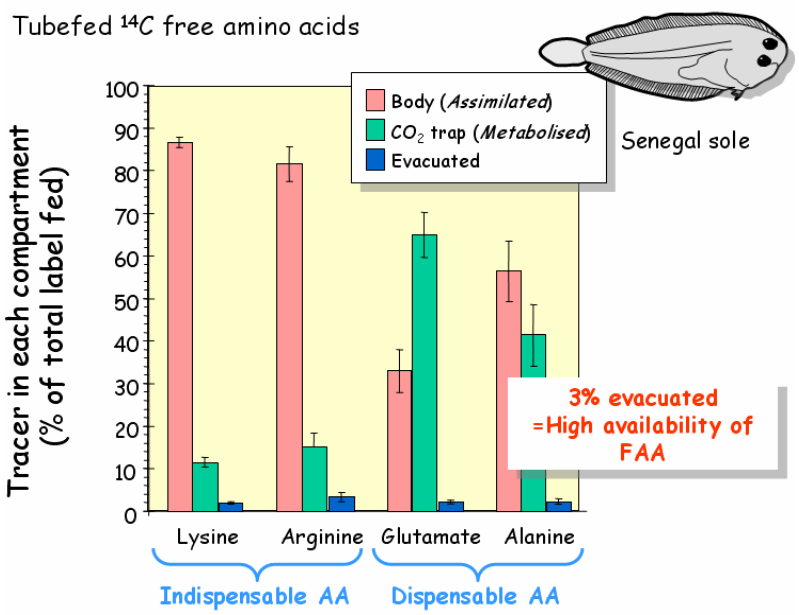

Figure 10. Senegal sole post larvae (30 days post first feeding) tube fed a mixture of free amino acids containing either ^{14}C labeled lysine, arginine, glutamate or alanine. (Adapted from Rønnestad *et al*., 2002)

CONCLUSIONS

Research is still in its infancy regarding the understanding of the control of digestion and the efficiency of digestive and absorptive process in larval fish. Such data will serve as the basis to developing dry formulated feeds which are easily accepted and digested by the larvae from onset of exogenous feeding.

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