Protein Metabolism and Amino Acid Requirements in Fish Larvae

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Abstract

Despite recent progress, knowledge on protein and amino acid (AA) requirements of fish larvae is limited. The major differences compared to larger fish seem to be a poorer capacity to digest and/or absorb complex proteins. In addition, the cumulative needs for protein deposition, protein turnover and AA catabolism necessary for rapid larval growth dictate a higher AA requirement during the larval stages. However, fish larvae seem to have an efficient control of AA catabolism, and use dispensable preferentially to indispensable AA as energy substrates. Still, larvae of most marine fish species hatch with a simple digestive tract and a poorly developed ability to digest proteins, and a fully mature protein digestion is only available weeks later. Therefore, fish larvae need diets rich in soluble molecular nitrogen, and avoiding complex proteins with low digestibility. The use of the indispensable AA profile of fish larvae as index of their requirements needs caution. Ontogenetic changes in the AA profile during larval stages need to be considered, and some AA are more efficiently absorbed and/or retained by fish larvae. In short, despite considerable progress in understanding protein utilisation in recent years, many questions remain open in relation to AA requirements of fish larvae.

Keywords: *amino acids, digestive capacity, growth*

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Introduction

If fish larvae are to meet their tremendous growth potential (e.g., Kamler 1992; Conceição, Grasdalen & Rønnestad 2003), sufficient dietary nitrogen of the right quality must be provided. In addition to the high requirement for amino acids (AA) as building blocks for protein deposition and growth, AA are a major energy source during the larval stage of most marine teleost species (e.g., Rønnestad, Tonheim, Fyhn, Rojas-Garcia, Kamisaka, Koven, Finn, Terjesen, Barr & Conceição 2003). However, knowledge on protein and AA requirements of fish larvae is limited, and often qualitative rather than quantitative (Conceição *et al.* 2003). The major differences in terms of larval nutritional physiology compared to larger fish, seems to be a poorer capacity to digest and/or absorb complex proteins and much higher AA requirements (Conceição *et al.* 2003; Rønnestad & Conceição 2005; Zambonino-Infante, Gisbert, Sarasquete, Navarro, Gutiérrez & Cahu 2008). The cumulative needs for protein deposition, protein turnover and AA catabolism necessary for rapid larval growth dictates a high AA requirement (see Fig. 1). There is an evident paradox when the potential fast growth of fish larvae is taken together with its poorly developed protein processing capacity of the digestive system.

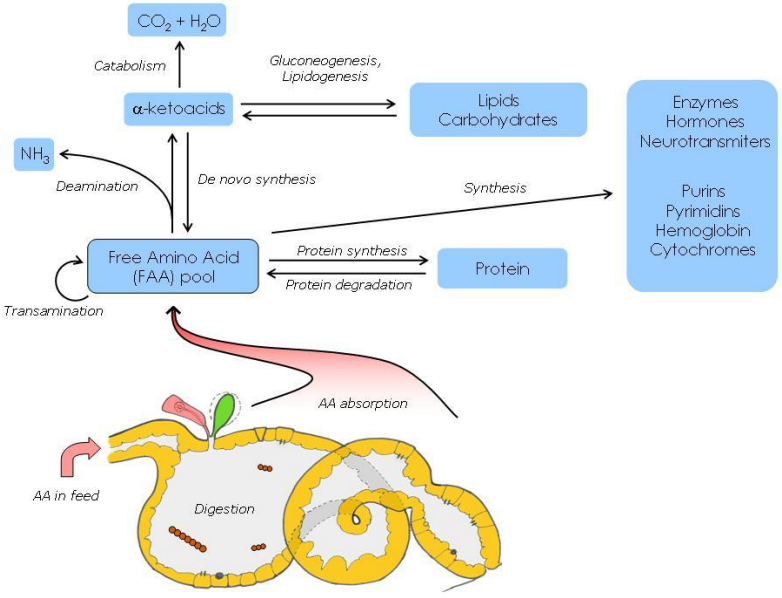

Figure 1. Fluxes of amino acids (AA) in fish larvae: from feed to utilisation

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Protein digestion

After onset of exogenous feeding the digestive tract is vital in supplying all the required dietary AA to the fast developing and growing larval tissues. However, larvae of most marine fish species hatch with a simple digestive tract and a poorly developed ability to digest proteins, and a fully mature protein digestion is only available weeks later (Rønnestad & Conceição 2005; Zambonino-Infante *et al.* 2008). To realize the growth potential fish larvae need diets rich in soluble molecular nitrogen, and avoiding complex proteins with low digestibility. This probably largely explains the difficulties in substituting live feeds by inert diets in culture systems for most species of fish larvae. Apparently, contrary to complex proteins, free AA (FAA) and peptides are rapidly and efficiently absorbed from the digestive tract.

Prior to the differentiation of a functional stomach, digestion of dietary protein larvae starts in the anterior midgut, where feed is mixed with pancreatic secretions and bile from the gall bladder. Alkaline pancreatic secretions contain a variety of proteolytic enzymes, with trypsin and chymotrypsin as the major components (Gildberg 2004), while elastase and carboxypeptidase A and B are also found. Trypsin is a key factor in the activation of the inactive pancreatic proenzymes, and trypsin is activated when in contact with enteropeptidase at the brush border. The combined effects of the trypsin and chymotrypsin activity yields protein fragments and small peptides in the gut lumen, and further hydrolysis is carried out by pancreatic and brush border exopeptidases (Buddington, Krogdahl & Bakke-McKellep 1997). Cytoplasmatic peptidases will perform additional breakdown of very small peptides towards FAA, but it is unknown whether small peptides are also transferred into the systemic circulation.

As stomach becomes gradually functional, typically a few weeks after first-feeding, acid digestion of proteins is initiated, with pepsin involved. The stomach is a highly efficient organ for degrading complex proteins, through the combined action of mechanical degradation by gastric motility, and HCl / pepsin secretion which enable acid denaturation

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and proteolysis (Gildberg 2004). The stomach also function as a storage reservoir for an ingested meal that can deliver small amounts of partly digested chyme into the midgut over an extended period thereby optimizing the substrate to enzyme ratio and optimize digestion better. Therefore, once the stomach is functional a wider range of feed ingredients may be used in fish diets. Protein hydrolysates are obvious ingredients for larval fish. In this respect it should be noted that peptide transport may also be an important route for absorption in teleost fishes, in particular in the young stages.

Understanding the digestibility of specific feed ingredients is essential for the formulation of optimised fish larval diets. However, little is known about the digestibility of various protein sources commonly used in formulation of inert microdiets for marine fish larvae. Available digestibility estimates for these ingredients are for juvenile fish, and other animal models, and need to be used with extreme caution as are likely grossly overestimating larval digestive capacity, in particular in the early ages.

Protein solubility has been suggested as an important determinant of digestibility in fish larvae (Carvalho, Sá, Oliva-Teles & Bergot, 2004). Protein solubility may, at least partly, explain the difficulties in using inert microdiets in marine fish larvae, since commonly used live feeds, unlike formulated feeds, contain a high proportion of water-soluble protein (e.g., Hamre, Opstad, Espe, Solbakken, Hemre & Pittman 2002; Conceição, Yúfera, Makridis, Morais & Dinis 2010).

Molecular form of dietary nitrogen has also been proposed to be a major sucess factor for inert microdiets. In particular the role of inclusion of protein hydrolysates has been the subject of several studies. However, while moderate levels of protein hydrolysates have been shown to improve growth and increase survival in young stages of different fish species, high levels seem to have negative effects (e.g., Carvalho, Escaffre, Teles & Bergot 1997; Cahu, Zambonino-Infante, Quazuguel & Le Gall 1999; Hamre, Naess, Espe, Holm & Lie 2001). One of the unanswered questions in these studies continues to be the loss of low molecular weight proteins, peptides and FAA from the formulated microdiets. One

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approach to overcome this experimentally is to use a tube-feeding methodology where the tested dietary compounds can be delivered directly into the lumen of the anterior part of the digestive tract. These studies have shown that the absorption efficiency of pre-hydrolysed protein preparations is high compared to intact protein, in particular at high doses (Tonheim, Espe, Hamre & Rønnestad 2005). These results support the view that at least in some species the larval capacity to digest and absorb dietary proteins is limited and may constitute a bottleneck for microdiet formulation.

Protein metabolism

Fish larvae have a tight control of AA metabolism. Indispensable (or essential) AA (IAA) must be provided through the diet, while dispensable (or non-essential) AA (DAA) can be synthesised *de novo* from α-keto acids or through transamination and other reactions from other AA. Studies using tube-feeding of single ^{14}C-labelled AA have demonstrated that larvae have a good capacity to discriminate between individual AA (Conceição *et al.* 2003). Larvae of different species use DAA preferentially to IAA as energy substrates, while catabolism rates vary among individual IAA (Fig. 3) (Rønnestad, Conceição, Aragão & Dinis 2001, Conceição, Rønnestad & Tonheim 2002, Applebaum & Rønnestad, 2004). In fact, fish are able to spare IAA from very early stages of development. Various enzymes are involved in catabolism and transamination of AA (Jürss & Bastrop 1995), allowing regulation for the differential use of individual AA.

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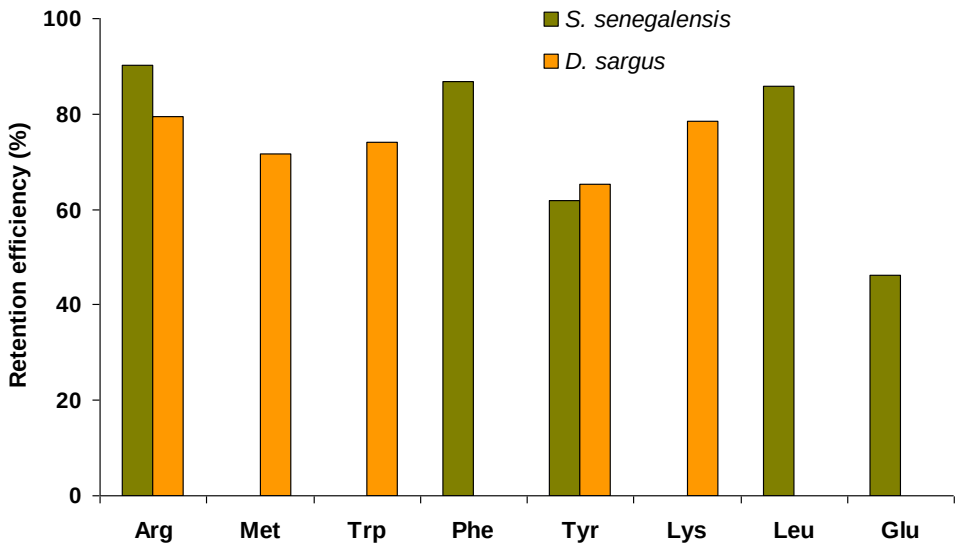

Figure 3. Retention efficiency (retained / absorbed) of individual amino acids in Senegalese sole (*Solea senegalensis*) and white seabream (*Diplodus sargus*) larvae, estimated by tude-feeding of the respective ^{14}C-isotopes. Please note that estimates were obtained with different larval batches and ages in both species. Data sources: Aragão *et al.* (2004a), Saavedra *et al.* (2008a,b), Pinto *et al.* (2009).

Dietary AA imbalances have been shown to lead to increased AA oxidation and lower retention efficiency (Aragão, Conceição, Martins, Rønnestad, Gomes & Dinis 2004a). Differences between dietary and fish larval AA profiles may cause catabolism of over 40% of the total absorbed AA (Conceição *et al.* 2003). Dietary AA are mostly absorbed as FAA or as small peptides (Rønnestad & Morais 2008). However, tissue concentrations of FAA are tightly regulated (Houlihan, McCarthy, Carter & Martin 1995), and absorbed AA are quickly polymerised into proteins, or irreversibly metabolised. Fish only store AA in the form of proteins that are synthesised based on functional and structural demands, so AA which are not polymerised into proteins due to AA imbalances will be catabolised for energy production, transaminated into another AA, used in gluconeogenesis or lipogenesis. Some AA are also used in the synthesis of other nitrogen-containing molecules (e.g., purines, several hormones), which may mean additional AA requirements in certain life

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stages. In addition, protein turnover keeps a dynamic relationship between the FAA and the protein pools, and may act as a temporary buffer of AA imbalances (Conceição *et al.* 2003).

As mentioned above, AA are absorbed at different rates in fish larvae and absorption efficiency vary between AA (Fig. 2), and may also change with species and developmental stage (Rønnestad *et al.* 2001; Conceição *et al.* 2002; Saavedra, Conceição, Helland, Pousão-Ferreira & Dinis 2008a; Saavedra, Conceição, Pousão-Ferreira & Dinis 2008b). These different efficiencies of absorption between individual AA may lead to transitory AA imbalances in the cellular FAA pool where the protein synthesis is carried out, leading to increased AA catabolism. Furthermore, although DAA can be in theory synthesised, it is unknown whether this *de novo* synthesis is of quantitative significance in adults, and whether fish larvae may have that capability. Transamination may be important in improving protein utilisation as it may compensate for DAA imbalances in dietary protein. However, it remains to be established whether in fast growing fish larvae transamination is sufficient to make such compensation.

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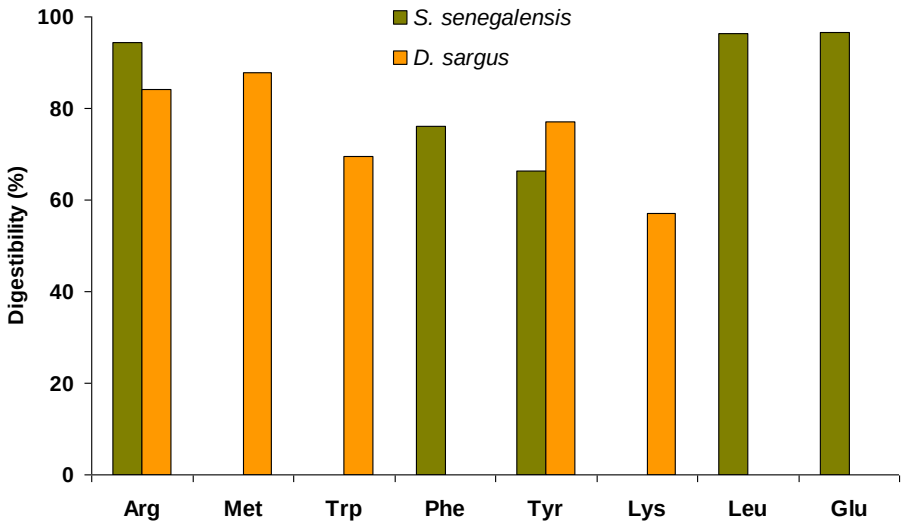

Figure 2. Digestibility (absorption/ total intake) of individual amino acids in Senegalese sole (*Solea senegalensis*) and white seabream (*Diplodus sargus*) larvae, estimated by tude-feeding of the respective ^{14}C-isotopes. Please note that estimates were obtained with different larval batches and ages in both species. Data sources: Aragão *et al.* (2004a), Saavedra *et al.* (2008a,b), Pinto *et al.* (2009).

Amino acid requirements

Amino acid requirements of fish larvae are not easily determined, due to difficulties in the use of formulated diets (Conceição *et al.* 2010) and on the manipulation of the live feed protein profile (Aragão, Conceição, Dinis & Fyhn 2004b). The IAA profile of larval fish is a reasonable index of their IAA requirements (Conceição *et al.* 2003). However, while the IAA profile of juveniles is rather constant both between and within species, some species present ontogenetic changes in the AA profile during larval stages. For instance, species with a marked metamorphosis, have more pronounced changes in the AA profile during ontogeny than species with a smoother metamorphosis (Aragão, Conceição, Fyhn & Dinis 2004c). Changes in AA profile during ontogeny are likely to be reflected in the IAA requirements. It should be noted that even what may seem small percentual changes in the

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IAA profile (Fig. 4) may have important implication in terms of IAA requirements. For instance, a 0.5% decrease in the contribution of methionine to the IAA profile of larval African catfish (*Clarias gariepinus* Burchell) was calculated to potentially result in an increase of up to 0.2 fold of the methionine requirement (Conceição, Ozório, Suurd & Verreth 1998).

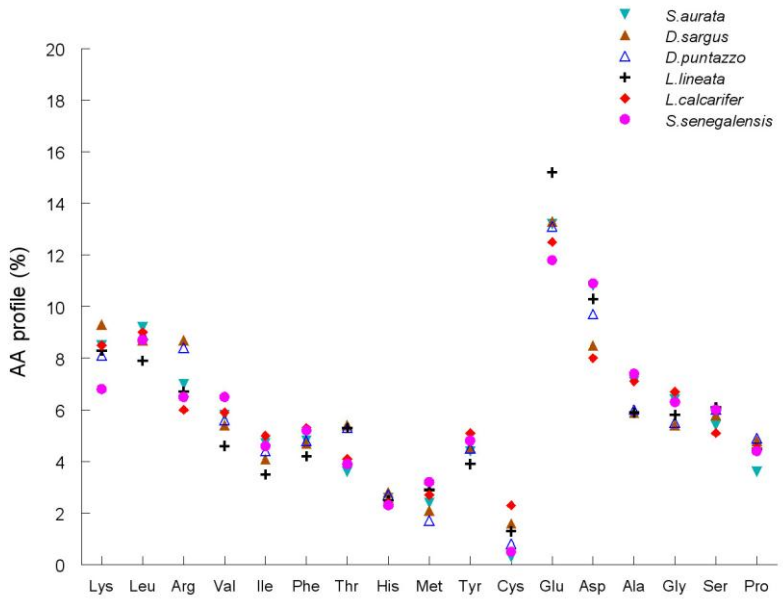

Figure 4. Mean amino acid (AA) profiles in fish larvae of different marine teleost species. Data sources: Asian seabass, *Lates calcarifer* (Syama Dayal *et al.* 2003), gilthead seabream and Senegalese sole (Aragão *et al.* 2004c), white seabream, *Diplodus sargus* (Saavedra *et al.* 2006), sharpsnout seabream, *Diplodus puntazzo* (Saavedra *et al.* 2007), striped trumpeter, *Latris lineata* (Brown *et al.* 2005).

Live prey commonly used in aquaculture, rotifers and *Artemia*, do not seem to meet the dietary requirements of larvae from several fish species (Conceição *et al.* 1998, Conceição *et al.* 2003; Aragão *et al.* 2004c; Saavedra, Conceição, Pousão-Ferreira & Dinis 2006), based on the use of whole-larval IAA profile as an estimator of IAA requirements.

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Nevertheless, this does not appear to be true for all species (Tulli & Tibaldi 1997; Brown, Battaglene, Morehead & Brock 2005).

However, AA profiles are only a rough estimator of fish larvae IAA requirements. This method does not take into account differences in the bioavailability of individual AA, due to selective absorption and/or catabolism (Conceição *et al.* 2003, Conceição, Morais & Rønnestad 2007). Absorption efficiencies and/or catabolism rates of individual AA have been shown (Figs. 2 and 3) to vary in larvae of several fish species (Rønnestad *et al.* 2001; Conceição *et al.* 2002; Aragão *et al.* 2004a, Saavedra *et al.* 2008a, b). In addition, the AA profile method takes only into account the AA requirements for protein synthesis, not considering the requirements for routine metabolic demands or for purposes other than protein synthesis. Methods using tracer studies have been proposed for determining relative biovailability of individual AA (Conceição *et al.* 2007). It is also important to realise that AA relative bioavailabilities, just as larval IAA profiles and thereby AA requirements, may change between species and also during development for a given species (Conceição *et al.* 2003). Using larval IAA profiles without correcting for bioavailability may both lead to overestimation or underestimation of IAA requirements, depending on bioavailabilities of the more imbalanced IAA in the diet.

The combination of larval protein AA profiles with bioavailability data can be instrumental in reducing the efforts needed to study AA requirements in fish larvae. Providing the requirement for a single AA is established for a given species by dose-response or oxidation studies (Conceição *et al.* 2007), IAA profiles corrected for bioavailability may be used to estimate requirements of other IAA.

Conclusions

Despite having a relatively simple digestive tract, the larval gut has the processing capacity to support a flow of nutrients that enables a very fast growth of fish larvae. However, this depends on providing a suitable feed where the dietary nitrogen is present in a form that can

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be easily digested. Fish larvae have a tight control over AA metabolism, using preferentially DAA for energy production and spare IAA for growth. Dietary AA utilisation depends on defining the larval AA requirements. Experimentally this is a challenge since traditional methods used in nutrition are difficult to apply to fish larvae. In short, despite considerable progress in recent years, many questions remain open in relation to AA requirements of fish larvae.

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