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Is dietary taurine supplementation beneficial for gilthead seabream (*Sparus aurata*) larvae?

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ABSTRACT

This study evaluated the effect of dietary taurine supplementation on the growth performance and methionine metabolism of gilthead seabream larvae. For this purpose, a growth experiment was carried out in which seabream larvae were fed upon rotifers supplemented with "blank" or taurine-enriched liposomes. A complementary trial was also done in which seabream larvae fed with live prey were subsequently tube-fed a solution containing a L-[U-^{14}C] methionine with or without a taurine supplement. Results from the growth experiment showed that rotifers were successfully enriched with taurine, but no effects were observed on larval growth performance, survival or amino acid composition. Furthermore, dietary taurine supplementation did not result in an increase of larval taurine levels, a factor that may have been determinant for the absence of effects observed on growth performance. In the tube-feeding trial, results showed that dietary taurine supplementation led to an increase of methionine retention in larvae. These findings suggest the existence of an active taurine biosynthesis pathway for gilthead seabream during the larval stage. Hence, gilthead seabream may not be dependent on dietary taurine to maintain the taurine body pool, since it may convert taurine from methionine if required. Taken together, the results from this study indicate that dietary taurine supplementation does not seem to enhance the larval growth performance in fish species able to biosynthesise taurine during this stage, which seems to be the case of gilthead seabream. However, this study also showed that in these species, dietary taurine supplementation may ultimately affect larval metabolism by increasing methionine availability for several important physiological purposes, contributing to a better understanding on the role of taurine during the early life stages of fish development.

1. Introduction

Taurine is an amino sulphonic acid that is not incorporated into protein, being freely distributed throughout cytosol and particularly accumulated in excitable tissues, where it plays important biological functions (Huxtable, 1992). For instance, taurine is believed to be involved in key roles such as modulation of neurotransmitter and synaptic activity (Huxtable, 1989), mediation of calcium uptake for cardiac muscle contraction and antioxidant protection of retinal rod outer segments from light and chemicals (Pasantes-Morales et al., 1981).

The sulphurous amino acid (AA) cysteine is the key intervenient in the metabolic pathway for taurine biosynthesis in mammals. Cysteine is a semi-indispensable AA, since it can only be synthesised from methionine, an indispensable AA. In mammals, the major pathway for taurine biosynthesis from cysteine involves the oxidation of cysteine to cysteine sulphinate, following decarboxylation of this product to hypotaurine and then to taurine (Worden and Stipanuk, 1985). Cysteine sulphinate decarboxylase (EC 4.1.1.29; CSD) plays an important role in this pathway, not only because it catalyses the reaction that produces hypotaurine, but also because several species are inherently deficient on this enzyme. For instance, cats are unable to biosynthesise taurine due to a reduced CSD activity, and kittens born of mothers fed with taurine-deficient diets may develop a large number of neurological defects and retinal degeneration (Hayes et al., 1975; Sturman, 1988). In fish, the capacity to biosynthesise taurine also varies among species and throughout ontogenesis (Goto et al., 2001; Goto et al., 2003; Kim et al., 2008). For instance, the CSD activity in red seabream and Japanese flounder is approximately half the levels observed in rainbow trout (Yokoyama et al., 2001). In addition, a significant increase in the taurine content of juvenile Japanese flounder and red seabream was observed when fed with taurine supplemented diets (Kim et al., 2005a; Matsunari et al., 2008), suggesting that these species rely on dietary taurine for maintenance of the taurine body pool.

Among the positive effects observed in fish fed with taurine supplemented diets, an increase of feed intake and enhancement of

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growth performance have already been described (Matsunari et al., 2008). In fact, taurine has recently become one of the most promising candidates for growth promotion in fish, and this effect is expected to be even greater in fish larvae. On the one hand, because fish larval prey (e.g. copepods) in the natural environment contain high taurine levels (Conceição et al., 1997; Helland et al., 2003; van der Meeren et al., 2008), suggesting a high physiological requirement for taurine during the larval stage. In addition, taurine is present at a constant amount during embryo development in marine pelagic eggs, suggesting that taurine is incorporated into the free AA pool of the egg before spawning (Rønnestad and Fyhn, 1993). As it occurs for the mammalian embryo (Sturman, 1993), these findings suggest that taurine may have a significant physiological importance for the development of fish embryo. Therefore, dietary taurine supplementation has been recommended for fish larvae (Pinto et al., 2010a). However, fish larvae largely rely on live prey, and manipulating live prey AA composition to fulfil larval AA requirements is still challenging. To overcome this limitation, Barr and Helland (2007) developed a method to enrich live prey with liposomes that enables delivery of water soluble nutrients, such as taurine, to marine fish larvae. In addition, the tube-feeding technique described by Rust et al. (1993) and Rønnestad et al. (2001) has also been successfully used to study the effects of short-term supplementation of individual AA in terms of absorption (retention and catabolism) and evacuation (Conceição et al., 2007).

Gilthead seabream (*Sparus aurata*) is a dominant species in the Southern European aquaculture industry. Among the constraints found during the rearing of this species are the low survival rates and the incipient larval growth usually verified at the end of the first month of development (Parra and Yúfera, 2001). With this in mind, this study assessed the effect of dietary taurine supplementation on the performance of gilthead seabream larvae, by feeding larvae with rotifers supplemented with taurine-enriched liposomes. Moreover, a tube-feeding trial was performed to evaluate the effects of dietary taurine supplementation on the metabolism of methionine in gilthead seabream larvae, in order to obtain further insights on the ability of this species to biosynthesise taurine during the early developmental stages.

2. Materials and methods

2.1. Fish rearing

Gilthead seabream (*S. aurata*) eggs were obtained from a Portuguese aquaculture hatchery. Larvae were reared at University of Algarve (Faro, Portugal) facilities in eight 100 L cylindroconical sand-coloured tanks — 4 tanks per treatment according to the feeding regime (Control or Taurine). Each tank was individually equipped with a closed water recirculating system and 4 to 5 daily water renewals were initially used. Initial larval density was 180 larvae L^{-1}. Light intensity was 900 lx and a light/dark cycle of 10:14 h was used. Water temperature (20.0 ± 0.7 °C; mean ± standard deviation), oxygen saturation level (83.8 ± 8.0%) and salinity (35.7 ± 0.9 g L^{-1}) were measured on a daily basis with commercial probes.

Gilthead seabream larvae were fed with rotifers (*Brachionus plicatilis*) from the onset of exogenous feeding (3 days after hatching; DAH) until 19 DAH. Rotifers were enriched with commercial products to cover the known nutritional requirements of seabream larvae and "blank" (Control) or taurine-enriched liposomes (Taurine) during 1 h previously to larval feeding. Freeze dried empty liposome, produced with hydrogenated soy phospholipids (Epikuron 200 SH™, Degussa, Germany) were hydrated and extruded with sterilised seawater (Control) or taurine solution (Taurine) before the enrichment. The taurine solution was prepared by dissolving crystalline taurine (1.5% of rotifer dry weight — DW; Sigma-Aldrich, Germany) in seawater. Liposome hydration and rotifer enrichment were performed according to the procedures described by Barr and Helland (2007). From 14 to 29 DAH, gilthead seabream larvae were also fed with *Artemia* nauplii, while *Artemia* metanauplii enriched with commercial products were offered to the larvae from 25 DAH until the end of the experiment (31 DAH). Gilthead seabream larvae were always fed in excess, three times a day.

2.2. Fish sampling and analysis

Gilthead seabream larvae were sampled regularly during the experimental period for total length (TL), dry weight (DW) and AA content. Relative growth rate (RGR) and survival were assessed at the end of the experimental period. Rotifers from both treatments were also sampled for AA analysis. Fish and rotifer samples were firstly hydrolysed (6 M HCl at 106 °C over 24 h in nitrogen-flushed glass vials) and then processed and analysed by the PicoTag method (Waters, USA), according to the procedures described by Cohen et al. (1989). Amino acid analyses were done by high-performance liquid chromatography (HPLC) in a Waters Reversed-Phase Amino Acid Analysis System, using norleucine as an internal standard. Tryptophan was not determined, since it is partially destroyed by acid hydrolysis. The resultant peaks were analysed with BREEZE software (Waters, USA).

2.3. Tube-feeding trial

To conduct the tube-feeding trial, gilthead seabream were reared separately from the larvae of the growth experiment. Gilthead seabream eggs were obtained from a local aquaculture hatchery. Larvae were reared at the University of Algarve facilities in a 200 L sand coloured conical–cylindrical fibreglass tank, using similar conditions to those described in Section 2.1. Gilthead seabream larvae were fed with rotifers and *Artemia* enriched with commercial products according to the feeding plan described in Section 2.1.

On the day prior to the tube-feeding trial, gilthead seabream larvae were acclimatised to the room where the experiment was done. Fish were fasted for 12 h and freely allowed to swim in small white trays previously prepared with clean seawater and aeration. On the day of the trial (38 DAH), fish were fed upon *Artemia* metanauplii enriched with commercial products for half an hour before being tube-fed with a radiolabelled solution. Two types of solutions were prepared: control and taurine supplemented. The control solution was a physiological solution. The taurine supplemented solution was a physiological solution containing taurine. This solution was prepared such that the pulse delivered by tube-feeding represented a taurine supplement equivalent to 3% of the dietary protein from a normal *Artemia* meal. To determine the amount of taurine to supplement fish gut content, data was gathered from Rocha et al. (2008) and L. Ribeiro (personal communication) regarding the ingestion of *Artemia* metanauplii by gilthead seabream during a half hour period. The amount of supplement was then calculated taking into account *Artemia* protein ingested during that period, according to procedures described by Aragão et al. (2004b). Both solutions (control and taurine supplemented) were freeze-dried and added with L-[U-^{14}C] methionine (American Radiolabelled Chemicals). Therefore, two solutions were obtained and used for tube-feeding: control solution with ^{14}C-methionine and taurine supplemented solution with ^{14}C-methionine. It should be noted that the amount of ^{14}C-methionine tube-fed has no nutritional significance.

The *in vivo* method of controlled tube-feeding described by Rust et al. (1993) and modified by Rønnestad et al. (2001) for marine fish larvae was used to evaluate the effect of dietary taurine supplementation on the metabolism of methionine in gilthead seabream larvae. After feeding for half an hour on *Artemia* metanauplii, fish were anaesthetised with 33 μM MS-222 and tube-fed the ^{14}C-methionine solution with or without the taurine supplement. Each solution was