Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae

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Abstract

The effect of dietary taurine supplementation on growth performance, metamorphosis success and amino acid metabolism of Senegalese sole (*Solea senegalensis*) larvae was investigated. These parameters were assessed in larvae fed control and taurine supplemented microcapsules during the pelagic phase. Subsequently, a similar evaluation was carried out in newly-settled larvae fed upon *Artemia*, in order to verify the effect of earlier dietary taurine supplementation in larvae reared under improved feeding conditions. Results showed that dietary taurine supplementation did not affect larval growth performance and metamorphosis during the pelagic phase. However, by the end of the trial, Senegalese sole previously fed taurine supplemented microcapsules had a significantly higher growth performance and metamorphosis completion success than larvae fed control microcapsules. These differences were likely related to the improvement of feeding conditions upon settlement, which probably helped revealing the positive effects of earlier dietary taurine supplementation on Senegalese sole performance. Additionally, Senegalese sole may have benefited from taurine antioxidant properties during metamorphosis, since larval antioxidant defences may saturate at this stage. Furthermore, results from metabolic trials have shown that dietary taurine supplementation significantly increased amino acid retention in Senegalese sole larvae when a concomitant increase of taurine body levels was found. Therefore, an increase in larval growth potential and metamorphosis success was observed under dietary taurine supplementation and these results may help understanding why dietary taurine supplementation has been reported to simultaneously increase taurine body levels and growth performance in other fish species, leading to a better comprehension on the role of taurine during fish development.

Keywords: fish larvae, growth, metamorphosis, microdiets, *Solea senegalensis*, taurine
1. Introduction

Taurine is an amino sulphonlic acid present in high concentrations in the animal kingdom, including fish. Although usually classified as an amino acid (AA), taurine is not used for protein synthesis, since it lacks a carboxyl group. Instead, taurine is freely distributed throughout cytosol and particularly accumulated in excitable tissues. Moreover, taurine is involved in important biological functions, such as bile salt conjugation, osmoregulation, membrane stabilization, modulation of neurotransmitters, antioxidation and early development of visual, neural and muscular systems (Huxtable, 1992).

Several studies have indicated differences for the taurine biosynthesis capacity in mammals. For instance, cats are inherently deficient of cysteine sulfinate decarboxylase, the limiting enzyme for taurine biosynthesis, and kittens born of mothers fed taurine-deficient diets exhibit retinal degeneration (Hayes et al., 1975; Sturman, 1988). In addition, human neonates and certain monkey species are also unable to synthesize meaningful quantities of taurine and must rely on a dietary source (Huxtable, 1992). In fish, the ability to synthesise taurine also varies among species and during ontogenesis (Goto et al., 2001; Yokoyama et al., 2001; Goto et al., 2003; Kim et al., 2003; Kim et al., 2008). These findings suggest that taurine may be essential during the early life stages of fish development, leading some authors to recommend a dietary taurine supplementation (Takeuchi, 2001). Still, although several studies have demonstrated positive effects for dietary taurine supplementation in juvenile fish (Sakaguchi et al., 1988; Park et al., 2002; Kim et al., 2003; Martinez et al., 2004; Kim et al., 2005; Matsunari et al., 2005a; Takagi et al., 2006a; Takagi et al., 2006b; Kim et al., 2007; Matsunari et al., 2008; Takagi et al., 2008), much less work has been performed in fish larvae. However, taurine is expected to assume an important role during the larval stage. On one hand, the natural live prey of fish larvae (such as copepods) contain high taurine levels (Conceição et al., 1997; Helland et al., 2003; van der Meeren et al., 2008), suggesting that the physiological requirement for taurine may be high during this stage. In addition, it is during the larval stage that organ systems differentiate and develop, indicating that taurine may have important physiological functions at this point. Moreover, higher taurine levels in live feeds were suggested to result in higher growth rates of turbot larvae (Conceição et al., 1997). For these reasons, taurine has recently become one of the most promising candidates for growth promotion in fish larvae.

Senegalese sole (Solea senegalensis) is a flatfish species with ongrowing potential for the South-Eastern European aquaculture (Imsland et al., 2003; Conceição et al., 2007).
Although several attempts have been made to formulate inert microdiets for Senegalese sole larvae, results regarding larval performance (e.g., survival, growth and metamorphosis completion) are still far beyond those obtained with live feeds (Cañavate and Fernández-Díaz, 1999; Yúfera et al., 2005; Fernández-Díaz et al., 2006; Gamboa-Delgado et al., 2008). These findings indicate that microdiets for Senegalese sole larvae still need to be improved, and supplementation of specific nutrients, such as taurine, may be essential for meeting larval physiological requirements and achieve a successful transition from live feeds to inert microdiets.

This work aimed to evaluate the effect of dietary taurine supplementation on the performance of Senegalese sole larvae. For this purpose, Senegalese sole larvae were fed control and taurine supplemented microcapsules during the pelagic phase and the effect of dietary taurine supplementation on larval survival, growth, metamorphosis success and AA metabolism was assessed. In addition, these parameters were also evaluated in newly settled Senegalese sole larvae fed upon *Artemia*, in order to verify the effect of earlier dietary taurine supplementation in larvae reared under improved feeding conditions.

2. Materials and methods

2.1. Microcapsule diet preparation

Microcapsules were prepared by internal gelation following the method described by Yúfera et al. (2005) with some minor modifications. According to this procedure, two types of formulated microdiets were prepared – Taurine and Control. In the Taurine treatment, microcapsules were supplemented with taurine (3% of total composition), while in the Control treatment the microcapsules were not supplemented with taurine. The composition of both microcapsules is shown in Table 1.

2.2. Fish rearing

Newly hatched Senegalese sole (*S. senegalensis*) larvae were obtained from a Portuguese aquaculture hatchery (A. Coelho & Castro, Lda, Póvoa de Varzim, Portugal) and reared at CCMAR (Faro, Portugal) facilities. During the pelagic phase, larvae were reared at an initial density of 60 larvae L⁻¹ in six 100 L conical cylindrical sand-coloured tanks – 3 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 adjusted. Light intensity was 600 lx and a light/dark cycle of 12:12-h was adopted. Water temperature (20.6 ± 0.9 °C; mean ± standard deviation), oxygen saturation level (83.7 ± 9.2 %) and salinity (35.2 ± 0.4 g L⁻¹) were measured on a daily basis with commercial probes.

From the start of exogenous feeding (3 days after hatching; DAH) until 16 DAH Senegalese sole larvae were simultaneously fed a restricted ration of rotifers enriched with commercial products and Control or Taurine microcapsules provided in excess. From 16 to 25 DAH larvae fed exclusively on microcapsules. Microcapsules were distributed to the rearing tanks by automatic feeders in five cycles of 2 hours running and one hour break, from 8h00 to 22h00. Furthermore, an additional meal was offered to the larvae at 09h30, being distributed to the rearing tanks by hand.

At 25 DAH, newly settled Senegalese sole larvae from each treatment were pooled and transferred to two 21 L sand-coloured fibreglass raceways (0.21 m²; 10 cm water depth; initial larval density of 3,000 individuals m⁻²), where it were maintained until 32 DAH in a closed water recirculating system with two water renewals per hour. Light intensity was 200 lx and a 12:12-h light/dark cycle was adopted. Water temperature (20.3 ± 0.3 °C), oxygen saturation level (96.1 ± 1.3 %) and salinity (36.0 ± 0.0 g L⁻¹) were measured daily. During this stage, newly settled Senegalese sole larvae from both treatments were fed in excess with frozen Artemia metanauplii enriched with commercial products. Artemia was offered to the larvae three times per day, in the morning (10h00), early afternoon (14h00) and late afternoon (18h00).

2.3. Growth performance and metamorphosis

Senegalese sole larvae from both treatments were sampled regularly during the experimental period. Fish were analysed for total length (TL) and dry weight (DW) at 3, 14, 25 and 32 DAH. Relative growth rate (RGR) and survival were analysed from the beginning of the experiment until the end of the pelagic phase (25 DAH) and from this moment until the end of the trial (32 DAH).

The metamorphosis pattern of Senegalese sole larvae was assessed regularly after the onset of this process, according to Fernández-Díaz et al. (2001). These authors categorised five metamorphic stages, according to the eye migration status: 0 – pre-metamorphic (symmetric larvae); 1 – early metamorphic (beginning of left eye migration); 2 – middle
metamorphic (left eye touching the midline of the dorsal surface); 3 – middle metamorphic (changing in the swimming plane and left eye migration within the ocular side); and 4 – later metamorphic (completion of left eye migration and visibility of orbital arch).

2.4. Amino acid analysis

Senegalese sole larvae were sampled and analysed for total amino acid (TAA) content at 3, 10, 17, 21 and 32 DAH (results are only shown for taurine). Both microcapsules were also analysed for TAA composition. Fish and microcapsule samples were firstly hydrolysed (6 M HCl at 106 °C over 24 h in nitrogen-flushed glass vials) and then prepared and analysed using the PicoTag method (Waters, USA), according to the procedures described by Cohen et al. (1989). 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 in the microcapsules, since it is partially destroyed by acid hydrolysis. The resultant peaks were analysed with BREEZE software (Waters, USA).

2.5. Metabolic trials

Two metabolic trials were performed to assess the effect of dietary taurine supplementation on the AA metabolism of Senegalese sole larvae. In these trials, larvae from each treatment were fed $^{14}$C-labelled rotifers or *Artemia* metanauplii at 9 and 16 DAH, respectively. For this purpose, larvae were transferred to the room where trials were conducted, being acclimatized for 12 hours in white plastic trays previously prepared with clean seawater and aeration. Rotifers or *Artemia* were radionlabelled with L-[U-$^{14}$C] AA mixture (55 μCi mmol⁻¹; CFB25; Amersham Biosciences, U.K.), following the procedures described by Morais et al. (2004), respectively. Afterwards, radiolabelled rotifers or *Artemia* were added to the fish trays at densities of 15 and 5 individuals mL⁻¹, respectively.

Senegalese sole larvae from each treatment (n = 30) were allowed to feed upon radiolabelled prey for one hour. After this period, larvae were successively rinsed in two wells with clean seawater and individually transferred to incubation chambers filled with 7.5 mL of seawater. This water fraction was considered to contain all $^{14}$C-labelled AA resultant from fish evacuation (evacuated fraction). A connection through gentle airflow was provided between each incubation chamber and a KOH trap (5 mL, 0.5 M), allowing to collect $^{14}$CO$_2$
produced by larval labelled AA oxidation (catabolised fraction). Once the incubation period was over (24 h), larvae were sampled and the $^{14}$CO$_2$ remaining in water was collected as described by Rønnestad et al. (2001). Fish were solubilised with 500 µL Solvable (PerkinElmer, U.S.A) for 12 hours at 45 ºC for determination of retained $^{14}$C-labelled AA (retained fraction).

Disintegrations per minute (DPM) from all samples were determined by adding liquid scintillation cocktail (Ultima Gold XR, PerkinElmer, U.S.A) to the respective vials and counting in a Beckman LS 6000IC liquid scintillation counter (Fullerton, CA, U.S.A.). Metabolic budgets for Senegalese sole larvae were calculated after subtracting the blanks of each fraction (evacuated, catabolised and retained). All fractions were expressed as a percentage of total tracer fed (i.e. the sum of DPM in all compartments of a given larvae).

2.6. Data analysis

Results were expressed as means ± standard deviation (SD). Relative growth rate (RGR, % dry weight day$^{-1}$) was calculated as: RGR = $(e^g -1) \times 100$, where $g = (\ln W_i - \ln W_0) \times t$. $W_i$ and $W_0$ respectively correspond to final and initial dry weights, and $t$ is the duration of the chosen period.

All results were tested by Levene’s test for homogeneity of variances followed by Student’s t-test for detection of treatment mean differences. Data were analysed through Mann-Whitney U non parametric tests when mean variances were significantly different across treatments. Data regarding Senegalese sole metamorphosis pattern were analysed through Chi-square tests. The significance level was $P \leq 0.05$. All results expressed as a percentage were previously arcsine transformed (Ennos, 2007).

3. Results

3.1. Microcapsulated diet

The total AA composition of the microcapsules from the Control and Taurine treatments is shown in Table 2. Significant differences were found between both microcapsulated diets for histidine and taurine contents. The taurine content was significantly higher in microcapsules supplemented with taurine (7.7 ± 0.5 and 9.3 ± 0.7 mg AA g$^{-1}$ DW, for Control and Taurine treatments, respectively).
3.2 Fish larvae

No significant differences were found between treatments for growth and survival of Senegalese sole larvae until the end of the pelagic phase (25 DAH; Figure 1; Table 3). However, by the end of the trial (32 DAH), Senegalese sole larvae from the Taurine treatment presented a significantly higher growth than larvae from the Control treatment.

The eye migration pattern of Senegalese sole larvae was not significantly different between treatments during the first days, when most of larvae were still starting the metamorphic process (stages 1 and 2; Figure 2). However, by the end of the trial, the Control treatment had significantly more larvae at a middle metamorphic stage (stage 2) than the Taurine treatment. Conversely, the Taurine treatment had significantly more larvae at the later metamorphic stage (stage 4; about 20%), whereas in the Control treatment none of the larvae displayed these features.

No significant differences were found for the taurine content of Senegalese sole larvae from the Control and Taurine treatments at 3, 17, 21 and 32 DAH (Table 4). However, at 10 DAH, Senegalese sole larvae from the Taurine treatment had a significantly higher taurine content than larvae from the Control treatment. No differences were obtained for the content of other AA in larvae from the Control and Taurine treatments throughout the experimental period (data not shown).

3.3 Metabolic trials

At the end of the incubation period (24 h) in the metabolic chambers, survival rates for 9 DHA Senegalese sole larvae from the Control and Taurine treatments were 30 and 17 %, respectively. At 16 DAH, survival rates for larvae of each treatment were 80 and 70 %, respectively. Results showed no significant differences in evacuated and catabolised fractions at both larval stages evaluated (Figure 3). However, the $^{14}$C-AA retained fraction was significantly higher at 9 DAH in larvae from the Taurine treatment than in larvae from the Control treatment. No significant differences were found for the retained fraction at 16 DAH.

4. Discussion
The feeding plan used in the current experiment, combining the use of rotifers and microcapsules until 16 DAH and then microcapsules alone until 25 DAH, was able to sustain growth and survival of Senegalese sole larvae. The overall growth performance and survival of the larvae during this period were lower than what is usually observed for Senegalese sole exclusively fed upon live prey (rotifers and *Artemia*; Cañavate and Fernández-Díaz, 1999; Yúfera et al., 2005; Fernández-Díaz et al., 2006; Gamboa-Delgado et al., 2008; Engrola et al., 2009). Still, it was considered that larvae from the current experiment displayed a normal developmental pattern according to the adopted feeding regime. For instance, Yúfera et al. (2005) described similar growth performances and survival rates for Senegalese sole larvae fed upon rotifers the three first days of feeding and alginate microcapsules alone afterwards.

The AA analysis of microcapsules from the Control and Taurine treatments showed a similar composition. Although significant differences were also found for histidine, it was noteworthy that microcapsules of the Taurine treatment displayed about 20 % more taurine than microcapsules of the Control treatment, revealing that a successful taurine supplementation was attained.

When assessing the effect of dietary taurine supplementation in Senegalese sole larvae, distinct performances were found for the pelagic phase and after larval settlement. During the pelagic phase, Senegalese sole larvae from both treatments presented a similar growth performance and survival, as well as an identical pattern for starting metamorphosis. These results initially suggested that dietary taurine supplementation during the pelagic phase had no effect on larval performance. However, by the end of the trial, newly settled Senegalese sole larvae from the Taurine treatment displayed a significantly higher growth performance than larvae from the Control treatment. In addition, about 20 % of larvae from the Taurine treatment successfully completed the eye migration, whereas none from the Control treatment concluded this process. In other words, larvae of both treatments were able to start the metamorphosis at the same age, but only the larvae fed on microdiet supplemented with taurine were able to finish the process during the experimental period. These results indicate that the advantages of dietary taurine supplementation during the pelagic phase were only reflected on Senegalese sole larval performance after settlement. The reason why these benefits were only visible at a later stage is difficult to explain so far.

The late effect of dietary taurine supplementation may have been related to the change in the feeding conditions performed upon larval settlement. By the end of the pelagic phase, newly settled Senegalese sole larvae from both treatments were transferred to flat-bottomed tanks and started to feed upon *Artemia*. This change may have been important in determining
results obtained at the end of the trial. As previously mentioned, Senegalese sole larvae generally perform better when fed upon live prey. Therefore, under improved feeding conditions, the positive effect of earlier dietary taurine supplementation on growth performance of Senegalese sole larvae was finally visible.

Additionally to the improvement in feeding conditions, the late benefits of dietary taurine supplementation observed in the current experiment for Senegalese sole may have also been related to functions played by taurine during the metamorphosis of this species. During this critical stage of development, a saturation of larval antioxidant defences seems to occur (Solé et al., 2004), and the antioxidant properties of taurine may become especially important at this point. In fact, taurine has been indicated to affect cellular redox status, being crucial during oxidative stress conditions (Métayer et al., 2008). Moreover, in the current experiment dietary taurine supplementation significantly increased larval taurine levels before the onset of metamorphosis (10 DAH), but not after this process had started (from 17 DAH onwards). In addition, an accentuated decrease in the taurine content was found in larvae of both treatments from 17 to 21 DAH, which coincided with the beginning of the metamorphosis process. These findings suggest a high taurine expenditure at the onset of metamorphosis in Senegalese sole larvae. However, since larvae from the Taurine treatment presented a higher taurine content before the beginning of the metamorphosis process, they may have been more able to cope with the higher taurine physiological requirements that seemed to occur at this point. This probably contributed to the higher metamorphosis success in larvae of this treatment at the end of the trial. Like in the current study, the benefits of dietary taurine supplementation were also shown to positively influence the settlement of Japanese flounder (Takeuchi et al., 2001), suggesting that taurine may also act an important role during the metamorphosis of this species. Therefore, future research should address on clarifying the advantages of dietary taurine supplementation during the metamorphosis of Pleuronectiform fish, which is the case of Senegalese sole and Japanese flounder.

The two metabolic trials performed in the current study showed that dietary taurine supplementation significantly increased AA retention in Senegalese sole larvae at 9 DAH, but not at 16 DAH. These differences were most likely related to the taurine levels present in larval bodies at those ages. As mentioned before, larvae from the Taurine treatment had a significantly higher taurine content than larvae from the Control treatment at 10 DAH, while no differences were obtained between treatments at 17 DAH. Therefore, increased AA retention in Senegalese sole was only observed when larval bodies presented a higher taurine content. Since growth is essentially muscle protein deposition (Carter and Houlihan, 2001),
and AA are the building blocks for protein synthesis, these results indicate that dietary taurine supplementation may result in an enhancement of Senegalese sole larval growth potential. In addition, these results may help understanding why dietary taurine supplementation has often been mentioned to simultaneously increase taurine body levels and growth performance of other fish species. For instance, these findings were reported for larvae of cod (Matsunari et al., 2005b), red seabream (Chen et al., 2004) and Japanese flounder (Chen et al., 2005), and for juveniles of chum salmon (Sakaguchi et al., 1988), yellow tail (Matsunari et al., 2005a; Takagi et al., 2006a; Takagi et al., 2006b; Takagi et al., 2008), European seabass (Martinez et al., 2004), Japanese flounder (Park et al., 2002; Kim et al., 2003; Kim et al., 2005; Kim et al., 2007) and red seabream (Matsunari et al., 2008).

The mechanisms through which taurine affects AA metabolism, protein synthesis and growth, both in fish and mammals, are still scarcely studied, not being fully understood. Since taurine is not incorporated into muscle protein, its effects on AA metabolism have been hypothesized to be related to indirect regulatory and/or metabolic functions. For instance, actions as stimulation of AA uptake into skeletal muscle cells (Huxtable et al., 1987) or production of growth hormone have been proposed for taurine (Huxtable, 1992). In species with an active taurine biosynthesis pathway, dietary taurine supplementation may also possibly increase the availability of its precursor AA (methionine and cysteine; Métayer et al., 2008), or even contribute for a better lipid digestion and absorption, since taurine has an important role in bile acid conjugation (Kim et al., 2007). These effects may spare dietary AA from being catabolised for energy production, leading to a higher AA availability for protein synthesis and growth purposes. Interestingly, the energy content of Senegalese sole larvae body tissues increase just before the onset of the metamorphosis, energy that is required during the transformation process (Parra and Yúfera, 2001). Taurine may play an important role during the pre-metamorphic period. Still, although several hypotheses have been proposed to ascertain the relationship between taurine and AA metabolism, it is clear that this largely unexplored subject still needs further clarification.

In conclusion, this study contributed to increase knowledge on the role of taurine during the early stages of fish development. Furthermore, it showed that dietary taurine supplementation may be important during these stages, contributing to increase larval growth potential and to enhance metamorphosis in the case of flatfish species, such as Senegalese sole. These findings may be important not only for Senegalese sole, but also for understanding the mechanisms through which taurine is involved on AA metabolism and
enhancement of growth performance usually observed for other fish species fed taurine supplemented diets.

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References


Figure captions

Fig. 1. Growth performance of *S. senegalensis* larvae reared under dietary taurine supplementation. Total length (TL; Control —○—; Taurine —●—) and dry weight (DW; Control —△--; Taurine —●—). DAH = days after hatching. Results are expressed as means ± standard deviation. For TL, n = 45 until 25 DAH and n = 30 at 32 DAH. For DW, n = 3 pooled samples until 25 DAH and n = 2 pooled samples at 32 DAH. Different letters indicate significant differences between treatments.

Fig. 2. Metamorphosis pattern of *S. senegalensis* larvae reared under dietary taurine supplementation. Results are expressed as percentage of each metamorphic stage (0 —■--; 1 —□--; 2 —△--; 3 —□□ and 4 —□□) found at a certain age. n = 45 for larvae with 14, 17 and 25 days after hatching (DAH) and n = 30 for larvae with 32 DAH. Different letters within metamorphic stages represent significant differences among treatments at a certain age.

Fig. 3. Metabolic budgets in *S. senegalensis* larvae fed with 14C-labelled live prey. Evacuated (■), catabolised (□□) and retained (□□) fractions. Results are expressed as means - standard deviation (n = 30). DAH – days after hatching. Different letters within fractions represent significant differences among treatments at a certain age.
Fig. 1, Pinto et al.
Fig. 2, Pinto et al.
Fig. 3, Pinto et al.
### Table 1. Diet ingredients in both experimental microcapsules

<table>
<thead>
<tr>
<th>Ingredient (g 100 g diet)</th>
<th>Type</th>
<th>Control</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial bread yeast¹</td>
<td>3.0</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Casein²</td>
<td>24.0</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>CPSP-90³</td>
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<tr>
<td>Cuttlefish meal⁴</td>
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</tr>
<tr>
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<tr>
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<tr>
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<tr>
<td>Vit C¹²</td>
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<td>1.6</td>
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<tr>
<td>Vit E¹³</td>
<td>1.6</td>
<td>1.6</td>
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</table>

¹ Commercial bread yeast
² ICN 901633
³ CPSP 90, Sopropêche, France
⁴ Squid Powder 0278, Rieber & Søn ASA, Bergen, Norway
⁵ ICN 101517
⁶ Aglonorse, Norway; Sigma H-8000
⁷ Cod liver oil, José M. Vaz Pereira, S.A., Sintra, Portugal
⁸ ICN 154724
⁹ PREMIX vitamin + mineral complex (Viana do Castelo, Portugal)
¹⁰ Lecithin Soy Refined, MP Biomedicals, LLC, Illkirch, France
¹¹ Sigma T0625.
¹² Sodium, calcium ascorbyl-2-phosphate, Rovimix STAY-C 35, DSM Nutritional Products, Inc.
¹³ DL-alpha-tocopherol acetate, MP Biomedicals, LLC, Eschwege, Germany.
Table 2. Total amino acid (TAA) composition of the experimental microcapsules.

Results are expressed as means ± standard deviation (n = 3). Different superscript letters within rows indicate significant differences between the Control and Taurine treatments for a given AA. TAA – total amino acids; DW – dry weight; Arg – arginine; His – histidine; Ile – isoleucine; Leu – leucine; Lys – lysine; Met – methionine; Phe – phenylalanine; Thr – threonine; Val – valine; Ala – alanine; Asx – aspartate + asparagine; Glx – glutamate + glutamine; Gly – glycine; Pro – proline; Ser – serine; Tyr – tyrosine; Tau – taurine. Taurine is shown separately since it only exists in the free form.

<table>
<thead>
<tr>
<th>AA</th>
<th>Control</th>
<th>Taurine</th>
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<tr>
<td>Arg</td>
<td>38.4 ±18.0</td>
<td>50.7 ±8.2</td>
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<tr>
<td>His</td>
<td>16.1 ±1.2</td>
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<td>Ala</td>
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<td>33.7 ±1.3</td>
</tr>
<tr>
<td>Asx</td>
<td>60.3 ±9.3</td>
<td>56.3 ±12.3</td>
</tr>
<tr>
<td>Glx</td>
<td>107.3 ±17.6</td>
<td>107.1 ±2.9</td>
</tr>
<tr>
<td>Gly</td>
<td>20.9 ±1.8</td>
<td>20.0 ±1.1</td>
</tr>
<tr>
<td>Pro</td>
<td>51.0 ±2.7</td>
<td>45.5 ±2.2</td>
</tr>
<tr>
<td>Ser</td>
<td>43.4 ±2.9</td>
<td>37.7 ±3.4</td>
</tr>
<tr>
<td>Tyr</td>
<td>49.4 ±2.3</td>
<td>44.7 ±1.3</td>
</tr>
<tr>
<td>Tau</td>
<td>7.7 ±0.5</td>
<td>9.3 ±0.7</td>
</tr>
</tbody>
</table>

TAA (mg AA g\(^{-1}\) DW)
Table 3. Growth and survival for *S. senegalensis* reared under dietary taurine supplementation.

<table>
<thead>
<tr>
<th>Larvae</th>
<th>Treatments</th>
<th>RGR (% DW day(^{-1}))</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelagic (3 to 25 DAH)</td>
<td>Control</td>
<td>9.1 ± 0.6</td>
<td>28.4 ± 5.8</td>
</tr>
<tr>
<td></td>
<td>Taurine</td>
<td>8.6 ± 0.8</td>
<td>36.3 ± 18.0</td>
</tr>
<tr>
<td>Newly-settled (25 to 32 DAH)</td>
<td>Control</td>
<td>4.9 ± 0.3 (^a)</td>
<td>50.3 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Taurine</td>
<td>10.5 ± 1.3 (^b)</td>
<td>56.0 ± 0.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation. \(n = 3\) pooled samples for pelagic larvae and \(n = 2\) pooled samples for newly settled larvae. Different superscript letters within columns indicate significant differences between treatments. DW – dry weight; RGR – relative growth rate; DAH – days after hatching.
Table 4. Taurine content of *S. senegalensis* larvae reared under dietary taurine supplementation.

<table>
<thead>
<tr>
<th>DAH</th>
<th>Control</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>9.1 ± 0.9</td>
<td>9.1 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>3.4 ± 0.2</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>a</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>17</td>
<td>4.1 ± 1.0</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>21</td>
<td>2.4 ± 0.3</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>32</td>
<td>8.7 ± 0.1</td>
<td>7.5 ± 0.6</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation (n=3). DW = dry weight; DAH = days after hatching. Different superscript letters within rows indicate significant differences between treatments at a certain age.