ATLANTIC BLUEFIN TUNA (*Thunnus thynnus,* L.) LARVAE ANTIOXIDANT MOLECULAR FUNCTIONS INDUCED BY DIETARY SELENIUM IN ROTIFER *Brachionus rotundiformis*

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Introduction

Recently the production cycle of Atlantic bluefin tuna (ABT) has been closed however with low survival at larval stages (Van Beijnen, 2017). Better knowledge of nutritional requirements could be one solution to boost ABT production. Selenium (Se) is an important micronutrient needed to produce selenoproteins involved in the antioxidant metabolism. The Se requirement in ABT remains unknown, but recent studies suggest that rotifers as starter feed do not provide sufficient Se for marine finfish species (Hamre et al., 2008). In general, fish exposed to high levels of stress have higher Se requirements (Küçükbay et al., 2009). Therefore, a supplementation at early stages, sensitive towards changing environmental conditions, might prove especially beneficial in ABT.

Material and Methods

The ABT eggs used in this study originate from ABT broodstock maintained in a floating net cage located at El Gorguel, off the Cartagena coast. From 2 days after hatching, larvae were fed with rotifer (*Brachionus rotundiformis*) grown on Algamac 3050 (Pacific Trading LTD, Kent, England) and enriched with different levels of Se (SelPlex®, Alltech, Meath, Ireland) for 18 h pre-harvest: 0.0 mg/10^6 rotifers (0 µg Se·L^{-1}, Se0), 3 mg/10^6 rotifers (3 µg Se·L^{-1}, Se3), 10 mg/10^6 rotifers (10 µg Se·L^{-1}, Se10), 30 mg/10^6 rotifers (30 µg Se·L^{-1}, Se30) and 100 mg/10^6 rotifers (100 µg Se·L^{-1}, Se100). The performance of ABT larvae was monitored throughout two weeks feeding period and antioxidant and selenoprotein expression was measured as well as Se contents on whole larvae sampled at the end of the trial.

Results

Rotifers accumulated up to 30.05 µg Se·g^{-1} dry weight in Se100, the highest supplementation level. Similarly, ABT larvae had maximum tissue Se concentration (194 ± 38 µg Se·g^{-1} dry mass) in Se100. In ABT larvae total length was highest in Se3, whereas maximum flexion index was reached in Se10. Selenium supplementation increased the expression of selenoproteins glutathione peroxidase 1 (*gpx1*) and methionine sulfoxide reductase 1 (*msrb1*) in Se supplemented treatments compared to the non-supplemented control (Figure 1). The expression of thioredoxin reductase 2 (*trxr2*) and selenoprotein P (*selenop*) also displayed differences between larvae fed supplemented and unsupplemented rotifers, albeit these were minor and varied with supplementation level (Figure 1). In contrast, the antioxidant enzymes catalase (*cat*) and superoxide dismutase (*sod*) showed lowest expression in Se100. The expression of other selenoproteins including iodothyronine deiodinases 1, 2 and 3 (*dio1*, *dio2*, *dio3*) was unaffected.

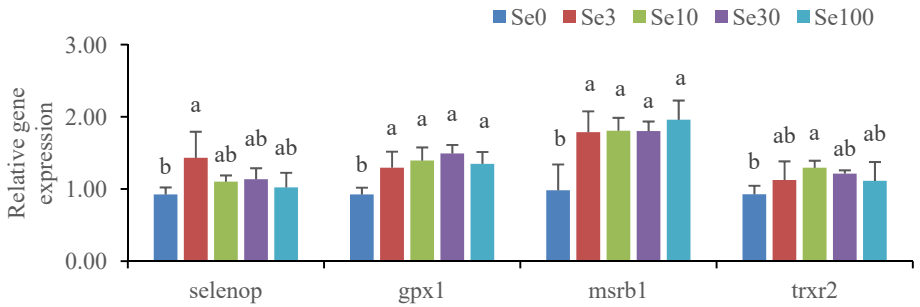

Fig 1. Expression of selenoproteins measured by real-time PCR. Data are normalized to a geometric mean of two housekeeping genes *ef1a* and *bactin* and expressed as fold-changes of mRNA abundance compared with Se0 (Pfaffl et al. 2002). Bars represent means ± SD (n=6). Means not sharing a common superscript letter are significantly different ($p < 0.05$) according to one-way ANOVA on ranks followed by Tukey's HSD. *Selenop*, selenoprotein P; *gpx*, glutathione peroxidase; *msrb*, methionine sulfoxide reductase; *trxr*, thioredoxin reductase.

Discussion and conclusion

Feeding Se enriched rotifers effectively increased Se in ABT larvae tissue. The improved growth observed in Se supplemented treatments might be related to an accelerated development as the flexion index was significantly higher in all Se enriched treatments compared to the non-supplemented control. A similar effect by Se supplementation has been previously described in Senegalese sole (Solea senegalensis) in relation to an enhanced thyroid hormone activity by Se supplementation (Ribeiro et al., 2012). The Se level of 0.10 µg g^{-1} dw measured in non-supplemented rotifers is below known requirements in fish (Antony Jesu Prabhu et al. 2016). In contrast, rotifers supplemented with the lowest Se level (Se3) contained 4.42 µg Se g^{-1}, which might be sufficient to cover requirements for this mineral as selenoproteins displayed maximum expression in ABT larvae fed this treatment. The increased seleno-enzyme production might have contributed towards an improved antioxidant status in ABT larvae, indicated by a transcriptional downregulation of redox sensitive antioxidant enzymes *cat* and *sod*. In conclusion, rotifers without Se enrichment are suboptimal for ABT larvae at first feeding. A dietary Se level of 4.42 µg g^{-1} dw is recommended as it boosted growth performance and improved the antioxidant status in ABT larvae.

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