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Rotifers enriched with iodine and selenium increase survival in Atlantic cod (*Gadus morhua*) larvae

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ABSTRACT

In the present study, rotifers were enriched with iodine and selenium up to the levels found in the most abundant organisms in the natural diet of fish larvae, i.e. copepods. Cod larvae fed iodine and selenium enriched rotifers had slightly lower growth than control larvae, but the survival increased by 32%. There was an increase in selenium concentration, but not in iodine concentration, in the larvae in response to feeding the selenium and iodine enriched rotifers. The glutathione peroxidase (GPx) activity in the larvae was not significantly different between the groups, but an insignificant trend of increase of 19% was observed (P=0.11). Outer ring deiodinase (ORD) activity was not affected by the treatments. An increase in thyroid hormones was indicated but not statistically proved. In conclusion, it is recommended to enrich rotifers used for marine fish larval culture with iodine and selenium. The data are too week to conclude whether iodine, selenium or both were deficient in the control rotifers used in the present study.

1. Introduction

Production of Atlantic cod has increased sharply in recent years, but production of juveniles is still a bottleneck in cod culture. Intensively reared cod larvae grow at a lower rate (10 compared to 13–>27% per day; McQueen Leifsson, 2003; Otterlei et al., 1999) and develop more deformities (Imsland et al., 2006) than larvae reared semi-extensively on their natural prey, copepods, filtered from pond systems. Sub-optimal nutrition in larvae fed rotifers is one possible reason for the inferior performance of intensively, compared to semi-extensively cultured larvae. The nutrient profile of both rotifers and copepods has recently been analysed (Srivastava et al., 2006, unpublished data; Hamre et al., 2008; van der Meeren et al., 2008). The data show that there are numerous differences in nutrient composition between the two food items. Copepods generally contain higher levels of the micronutrients than rotifers and the levels are also most often considerably higher than the requirements reported for fish by the NRC (1993) (Hamre et al., 2008).

Mineral nutrition of marine fish larvae has been given little attention, both in research and commercial culture. The mineral and trace element levels, with exception of calcium, magnesium and iron, were lower in rotifers than in copepods. Magnesium and selenium levels were also lower in rotifers than the requirements of fish reported in the NRC (1993) (Hamre et al., 2008). In the present experiment, we have concentrated on selenium and iodine requirements of cod larvae, because of the very low level of selenium found in rotifers and it's interaction with iodine in thyroid hormone metabolism.

Thyroid hormones are synthesised in the thyroid gland epithelium by combining iodine with residues of the amino acid tyrosine, and stored in thyroglobulins in the colloid of the gland (Yen, 2001). They are excreted mainly as T_4 (thyroxine) into the circulation, taken up by peripheral organs and converted to the more potent form, T_3 (triiodothyronine), by type I or type II deiodinases. The dominant organs for T_3 synthesis are the liver and head kidney where deiodinase I is the principle factor (Eales and Brown, 1993; Klaren et al., 2005). Deiodinase II is more important for conversion of T_4 to T_3 in the target tissues (Bianco et al., 2002). Deiodinase III catalyzes inner ring deiodification resulting in deactivation of either T_4 or T_3, this takes place in tissues that need to be protected from thyroid hormone actions, e.g. parts of the brain during development (Mol et al., 1997). Type I and II deiodinases have been demonstrated to be selenoproteins (Yen, 2001). Thyroid hormones are necessary for completion of metamorphosis in flatfish (Schreiber and Specker, 1998; Yoo et al., 2000) and stimulates gastric gland formation and pepsinogen synthesis in summer flounder larvae (Huang et al., 1998). T_3, together with retinoic acid, also increases transcription of growth hormone in carp (Stenberg and Moav, 1999).

Selenium is an integral part of several enzymes with antioxidant activity, glutathione peroxidases (GPx), which reduces hydroperoxides at the expense of reduced glutathione (GSH) (Brigelius-Flohe,

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1999; Arteel and Sies, 2001). Four GPx have been described in mammals; a cytosolic cGPx, which is the classical enzyme that neutralises water-soluble and fatty acid hydroperoxides, plasma pGPx, which has similar properties as cGPx, gastro-intestinal GI-GPx, which is exclusively expressed in the gastro-intestinal tract, and phospholipid hydroperoxide glutathione peroxidase (PHGPx), which is active in biological membranes and reduces lipid hydroperoxides (Brigelius-Flohe, 1999; Arteel and Sies, 2001). The active site of these enzymes contains selenocysteine residues. In addition, selenoprotein P (SeP), present in the plasma of mammals and expressed in cellular membranes, contains 10 selenocysteine residues and is regarded as a transport protein for selenium but also has antioxidative properties (Steinbrenner et al., 2006). A total of approximately 30 selenoproteins have been detected in mammals by feeding radio-labelled selenium, but the sequence and function are only known for 12 (Brigelius-Flohe, 1999).

In fish, studies of the interactions between selenium and vitamin E have shown that deficiency of selenium may lead to reduced levels of tissue α-tocopherol. Combined selenium and vitamin E deficiency signs are muscular dystrophy, muscle specific proteins in plasma and anaemia (Poston et al., 1976; Bell et al., 1985, 1986, 1987; Gatlin et al., 1986). The signs of selenium deficiency alone were reported to be disintegration of membranes and condensed nuclei in liver cells, pathological changes in nerve cells and anaemia, while no signs were visible at the macroscopic scale (Bell et al., 1986). Selenium and iodine deficiency in rats affect thyroid hormone metabolism and deiodinase activities while selenium deficiency affects both deiodinase and GPx activities (Beckett et al., 1993; Meinhold et al., 1993: Brigelius-Flohe, 1999; Arteel and Sies, 2001).

The purpose of the present study was to investigate whether cod larvae fed rotifers without supplementation of extra iodine and selenium, become deficient in these nutrients. Rotifers were enriched with iodine and selenium up to levels found in copepods and cod larvae were fed control or enriched rotifers. Larval growth, survival, thyroid hormone levels, deiodinase and GPx activities were analysed in the larvae at the end of the rotifer feeding period (26 days post hatch (dph)). In addition, the nutritional status for a number of other nutrients was assessed to identify possible interactions between iodine and selenium enrichment and nutrient metabolism in the rotifers.

2. Material and methods

2.1. Larvae

The experiment was performed at the experimental facility of Marine Harvest Cod AS, in Rong, Norway. Atlantic cod (*Gadus morhua*) larvae were hatched from eggs of wild caught broodstock (Gulen, Sogn, Norway). Fertilized eggs (74%) were incubated in an industrial scale incubator, at 7 °C with aeration, and after 112 day degrees the hatching percent was 98%. The larvae were transferred randomly by volume, from the incubator to 8 experimental tanks the following day (day 2), at an estimated density of 100 larvae L^{-1}. This estimate was calculated from the total amount of larvae in the incubator ((stocked amount of eggs)−mortality) divided by the water volume). The larvae were collected after having assured a homogenous distribution of larvae in the water by aeration. However, the results on survival at 26 dph, showed that the density at transfer of larvae in the experimental tanks must have been at least 150 ind L^{-1}. The experimental tanks (100 L) were circular and flat bottomed with dark walls and light grey bottom and with water inlet above the surface. Temperature (increasing from 8.0 to 11.5 °C during the first two weeks, thereafter 11.7±0.3) and oxygen (>90% saturation) were measured daily in each tank. pH (7.5±0.2) and total gas saturation (94±4% saturation) was monitored at the inlet water, after filtration, UV-treatment and heating. The experimental tanks and outlet mesh were cleaned 2 times per week. Water flow was initially set to 2 L min^{-1} and the tanks were gently aerated to avoid larval clustering at the start of experiment. Green algae, *Nannochloropsis* (Reed Mariculture Inc. Campbell, CA 95008, USA) were added to the inlet water of the experimental unit.

2.2. Rotifer rearing and enrichment

As start feed for the larvae, the rotifer *Brachionus* "Cayman" was grown as batch cultures in 4 m^3 circular tanks at 23 °C with aeration and oxygenation. The cultures received a continuously supply of bakers' yeast containing a vitamin mixture. They were also batch fed an oil enrichment, Rich (Catvis BV, 5222 AE 's-Hertogenbosch, The Netherlands) once a day. Each morning, the required amount of rotifers was washed in a 500 L tank with a 63 μM outlet mesh prior to enrichment. Half of the rotifers were enriched for 1.5 h with a 4:1 algae mixture of *Pavlova* and *Isochrysis*. The rotifer density was 3500–4500 ind mL^{-1} and the oxygen level was kept above 5 mg L^{-1} in the enrichment tank. The rest of the rotifers were divided and enriched with two different enrichments, representing the two experimental treatments. Control diet: 80% Multigain (Danafeed, DK-8700 Horsens, Denmark) and 20% Omegalec® (Aker Biomarine ASA, 0115 Oslo, Norway) at a total concentration of 1 g L^{-1}. The experimental rotifers received 7 mg L^{-1} sodium selenite and 400 mg L^{-1} sodium iodide in the culture water in addition to the control diet. Enrichment time was 1.5 h and rotifer density was 3500–4500 ind mL^{-1}.

After the enrichment, the rotifers were washed separately before the algae enriched rotifers were divided and pooled with the two enrichment treatments. The water temperature was kept at 23 °C throughout the whole enrichment, and after mixing, the rotifers were transferred to a cooler and rapidly cooled to 4 °C. The immobilised rotifers were then stored at this temperature at a density of 4000 ind mL^{-1} with aeration until use. Rotifers were sampled for analyses at start, 15 dph and 26 dph, subsamples for the different analyses were immediately frozen on dry ice and stored at −80 °C until analysis.

2.3. Feeding trial

The feeding trial started at 3 days post hatch (dph) and the two diets were fed to larvae in four experimental tanks each. The rotifers were added to the inlet water of each tank by a dosage pump, at four batches each day. The initial batch size was 2.5 mill rotifers, and this was increased to 5 mill at day 4 and to 6.25 mill at day 17, giving a total of 10, 20 and 25 mill rotifers per day to each tank, respectively. Samples of larvae for nutritional, enzyme and thyroid hormone analyses were taken on day 26 and immediately frozen on dry ice and stored at −80 °C until analysis.

2.4. Analytical methods

The standard length (SL, from the tip of the nose to the end of the notochord) was measured on 30 anaesthetised (MS 222) larvae per tank, using a binocular microscope with a measuring scale. Thirty pooled larvae per tank were dried overnight at 105 °C, and weighed dry. Larval density in the rearing tanks was estimated by sampling of the water column on three places in the tank, using a tube of 5 cm diameter. Near to homogenous density of larvae was obtained by aeration prior to sampling. The sampled volume of water was measured and number of larvae in the sample counted to obtain the number of larvae per L. Unfortunately this was not done at the start of the study.

Dry weight of the samples was determined gravimetrically after drying the samples at 105 °C over night. For trace element analyses, samples of diet ingredients and rotifers were wet digested by a microwave technique in nitric acid with 30% hydrogen peroxide (Julshamn et al., 2000). The samples were then analysed for Mn, Cu, Zn