Aquaculture 404–405 (2013) 105–115

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The effects of dietary vitamin A in rotifers on the performance and skeletal abnormality of striped trumpeter *Latris lineata* larvae and post larvae

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ARTICLE INFO

Article history:
Received 20 January 2013
Received in revised form 11 April 2013
Accepted 11 April 2013
Available online 20 April 2013

Keywords:
Marine fish larvae
Vitamin A (retinol)
Rotifers
Dose–response
Malformations

ABSTRACT

Several nutritional studies have linked dietary vitamin A (VA) to skeletogenesis in marine fish larvae. In our study, rotifers were enriched with eight levels of retinyl palmitate. Striped trumpeter (*Latris lineata*) larvae were fed rotifers twice daily, VA-enriched rotifers (morning feed) and Algamac−3050 enriched rotifers (afternoon feed), in greenwater systems from 6 to 18 days post-hatch (dph). The VA-enriched rotifers had incorporated 0, 6, 14, 26, 57, 109, 215 and 388 ng retinyl palmitate mg^{-1} dry weight (DW) following 2 h enrichment with emulsions containing 0 (control), 68, 187, 532, 1402, 2670, 4808 and 9523 ng retinyl palmitate mg^{-1} emulsion. After the rotifer feeding phase (6 to 18 dph), the larvae were fed Algamac−3050 enriched *Artemia* until 43 dph. The pattern of increasing VA in the enriched rotifers was not reflected in the larvae. Larvae incorporated 11.08 ± 0.27 ng total retinol mg^{-1} DW (mean ± SD) when fed rotifers containing 0.93 to 2.32 ng total retinol mg^{-1} DW (6 to 14 ng retinyl palmitate mg^{-1} DW) and incorporated a lower level of 5.57 ± 0.30 ng total retinol mg^{-1} DW when fed rotifers with 0.52 (control) or ≥16.31 ng total retinol mg^{-1} DW (0 and ≥57 ng retinyl palmitate mg^{-1} DW). By 43 dph, neither larval growth in length (16.0 ± 0.1 mm) or dry weight (4.57 ± 0.20 mg), nor survival (34.8 ± 10.6%), were significantly affected by increasing dietary levels of retinyl palmitate. The prevalence of vertebral column malformations in 43 dph post larvae was positively correlated with total retinol content of larvae at 16 dph ($R^2 = 0.55$, $P < 0.001$). Unlike other studies on a range of marine fish species, retinyl palmitate enrichment in rotifers did not affect the type or severity of jaw malformations. By 43 dph, 81 ± 9% of post larvae displayed severe jaw malformations. Vitamin A daily inclusion of more than 123 ng total VA mg^{-1} DW rotifer, equivalent to more than 35 ng total retinol mg^{-1} DW rotifer (109 ng retinyl palmitate mg^{-1} DW rotifers), is recommended to reduce vertebral column malformations when *L. lineata* larvae are reared in greenwater.

1. Introduction

Reducing malformation is a continuous challenge in the production of marine finfish throughout the world (Boglione et al., 2001; Koumoundouros, 2010; Koumoundouros et al., 2002). The development of skeletal disorders in larvae and juvenile fish can be due to nutritional, environmental and genetic factors (Cahu et al., 2003; Divanach et al., 1997; Lall and Lewis-McCrea, 2007). Skeletal disorders are early indicators of low quality fish for farming (Roo et al., 2010). Although there is now a growing understanding of the factors that contribute to malformations, they remain an important issue for the international finfish hatchery industry and animal welfare (Koumoundouros, 2010; Sfakianakis et al., 2006).

One of the key parameters that affect skeletogenesis in larval fish is nutrition, especially at first feeding. Optimum forms and sufficient levels of lipids, amino acids and vitamins are essential for normal development (Cahu et al., 2003). Among the vitamins, A, D, E, K and C are all important (Hamre et al., 2010; Lall and Lewis-McCrea, 2007). Vitamin A (VA) or retinoids are the group of nutrients with compounds that are structurally similar or have the biological activity of retinol, where they can bind or activate a specific receptor or a group of receptors. Many retinoid forms are available in the market for dietary incorporation, retinol (parent compound, the alcohol form of VA), retinal (the aldehyde form), retinoic acid (the acid form) and retinyl acetate and retinyl palmitate (ester forms of VA) (Wolf, 1984). Only retinyl palmitate and acetate are recommended to fulfil the requirements of fish for retinoids. This is because they are less toxic than the other forms, and fish larvae at early stages have the required liver enzymes to metabolise them into the other VA forms (Fernández and Gisbert, 2011; Takeuchi et al., 1998). Due

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http://dx.doi.org/10.1016/j.aquaculture.2013.04.018

to the presence of different VA compounds, VA activity is expressed in international units (IU), where 1 IU of VA is equivalent to 0.3 μg retinol (Wolf, 1984).

It is now generally recommended that retinoids be added to the broodstock diet and to the diet of all developmental larval fish stages (Alsop et al., 2008; Dedi et al., 1995; Zile, 2001). Determining optimal amounts and forms of VA for different developmental stages needs to be assessed for each species (Fernández et al., 2009; Moren et al., 2004b; Villeneuve et al., 2005). This is because excess or diminished VA affects normal growth and development (Dedi et al., 1995; Tarui et al., 2006; Villeneuve et al., 2005). The effects differ according to the species and there are often symptoms of hypervitaminosis with larger doses of VA where there is growth retardation and increased mortalities, malformation to different elements such as the brain, otoliths and otic placodes, skull, vertebral segments, caudal region, jaws and fins and abnormal pigmentation (Fernández et al., 2008, 2009; Haga et al., 2002; Takeuchi et al., 1998).

A dietary dose–response approach where the larvae are fed diets containing graded levels of VA is one of the best ways to study the effect of VA (or any other nutrient) on larval development. Determining the optimal amount of VA is complicated by the use of live prey. Rotifers and *Artemia* are able to metabolise different VA compounds and accumulate them in their body (Haga et al., 2006). In addition, due to species-specific differences between the two live preys, enrichment is not uniform and rotifers display a higher retinoid inclusion pattern than *Artemia* (Giménez et al., 2007). Thus, it is technically difficult to maintain the same VA levels during the whole live prey-feeding period of a larva. For that reason optimum VA levels are usually determined separately for the rotifer and *Artemia* feeding periods. To the best of our knowledge only one study has examined in detail the VA requirements of marine finfish using dose–response experiments in early larval stages (i.e., during rotifer feeding) (Fernández et al., 2008).

Striped trumpeter, *Latris lineata* (Bloch and Schneider, 1801), is native to south-eastern Australia and New Zealand and has been a candidate for sea cage aquaculture in the temperate regions of Australia to complement salmonid farming which is under threat from climate change (Battaglene and Cobcroft, 2007). Intensive culture of *L. lineata* larvae and post larvae has resulted in a high incidence of either jaw and/or spinal malformation in juveniles (Cobcroft and Battaglene, 2009; Cobcroft et al., 2001b; Trotter et al., 2001). Studies have examined the early requirements for the essential polyunsaturated fatty acids (PUFAs) and vitamins C and E, often in dose–response experiments (Battaglene et al., 2006; Bransden et al., 2005a, 2005b; Brown et al., 2005). *L. lineata* larvae are usually reared in greenwater, by adding the microalga *Nannochloropsis oculata*, which has a number of benefits in relation to improved prey intake, digestion, microbial management and larval performance (Cobcroft et al., 2001a; Makridis et al., 2010; Shaw, 2006). This microalga contains very small amounts of retinol (<0.25 ng mg^{-1}) and 290 ± 40 ng mg^{-1} β-carotene (Brown et al., 1999). β-carotene is a major dietary precursor of VA, with the nutritional equivalency accepted at 6 μg β-carotene equal to 1 μg retinol (Ross and Ternus, 1993).

The only published accounts of spinal malformation in *L. lineata* larvae or post larvae under intensive culture conditions describe kyphosis associated with swim bladder malformation, where the viscera is misplaced and pushes upwards on the vertebral column (Trotter et al., 2001, 2005). Jaw malformation in *L. lineata* is a significant problem and has been linked with a walling behaviour that is modified by tank colour, greenwater, live feed enrichment and the availability of live feed in the water column (Battaglene and Cobcroft, 2007; Cobcroft and Battaglene, 2009; Cobcroft et al., 2012).

The aim of the current study was to determine the effect of VA on early larval development of *L. lineata*, particularly on jaw and spinal malformations. Larvae were reared using standard practices in greenwater and fed from 6 to 18 days post hatch (dph) with rotifers enriched with eight graduated concentrations of retinyl palmitate. The larvae were then fed on *Artemia* until 43 dph to allow them to fully develop jaw and skeletal elements. Growth, survival and development were assessed in relation to rotifer enrichment with VA.

2. Materials and methods

2.1. Eggs and stocking of larvae

Gametes were obtained from wild-caught acclimated broodstock held at the Institute for Marine and Antarctic Studies, Fisheries Aquaculture and Coasts Centre (IMAS-FACC), Hobart under controlled light and ambient water temperature. Eggs stripped from one female were fertilised by mixing with sperm from three males. Immediately after fertilisation the embryos were disinfected in ozonated seawater 1.05 ppm for 57 s, in order to prevent the transmission of pathogens (Battaglene and Morehead, 2006). The seawater used in the egg incubation and larval rearing systems was filtered to 1 μm and ozonated to 700 mV ORP for ≥10 min, treated with UV at 254 nm and filtered with carbon before distribution to the tanks at 300–350 mV ORP.

The embryos were incubated in 250 l conical tanks at 14.2 ± 0.2 °C (mean ± SD, here and throughout). Incubators were maintained on flow-through at approximately 150 l h^{-1}, photoperiod 14 h L:10 h D, salinity ranged from 33.6 to 34.5 ppt, pH 8.13 to 8.26 and DO 95.8 to 106.3%. The central screens were 250 μm. Paper towels were applied to the surface for the removal of any oil or debris. The fertilised eggs hatched after 5 days. From 1 dph the temperature was slowly increased at 0.1 °C h^{-1} to 16.2 ± 0.2 °C. Yolk−sac larvae were stocked to larval rearing tanks on 1 dph at 10 larvae l^{-1}.

2.2. Experimental design

The effect of dietary VA on the performance, growth, survival and skeleton malformation was determined in early *L. lineata* larvae using emulsions containing graded levels of retinyl palmitate (1,600,000 IU g^{-1}, MP Biomedicals, Australia) prepared by Nutrakol, WA; 0 (control), 100, 300, 750, 1500, 3000, 5000 and 10,000 mg retinyl palmitate l^{-1} WW (wet weight) emulsion. The emulsion basic constituents were lipids 57%, vitamin E 4% and vitamin C 4% (Nutrakol, WA). Rotifers enriched with these graded levels of retinyl palmitate were fed to larvae from 6 to 18 dph in three replicate tanks per treatment.

2.3. Larval rearing conditions

Larvae were reared in black 300 l hemispherical tanks, from 1 dph until 43 dph. The temperature ranged from 15.5 to 16.5 °C (16.1 ± 0.2), salinity from 32.1 to 33.5 ppt (32.9 ± 0.3), pH from 8.04 to 8.63 (8.18 ± 0.07) and dissolved oxygen >84.7% (101.6 ± 5.5). Photoperiod was 16 h L:8 h D (lights on at 09:00 and off at 01:00) from stocking until 18 dph. The light period increased gradually to 18 h L:6 h D on 19 dph, 22 h L:2 h D on 20 dph and 24 h L from 21 dph onward to reduce downward nocturnal migration and associated mortality (Bransden et al., 2005a). The light intensity was 10.56 ± 1.71 μmol s^{-1} m^{-2} at the water surface, provided by a 50 W halogen globe.

Tanks were static from 1 dph (stocking) until 5 dph. On 6 dph (first feeding) the incoming flow of seawater exchange was 103 ± 4 l h^{-1} for 9 h from 23:00 to 08:00. Additional recirculation flow started on 19 dph and duration of incoming flow was increased to 24 h, and both continued until the end of the experiment, increasing the total flow to 200 ± 33 l h^{-1} for 24 h. Each tank had a surface skimmer to remove the oily film from the water surface in order to facilitate initial swim bladder inflation (Trotter et al., 2005). The skimmers were applied from 8 dph until 13 dph, and occasionally thereafter until 19 dph (<2 h day^{-1}) to ensure a clean water surface.

Larvae were reared in greenwater, from 6 dph until 20 dph; live *N. oculata* was added to each tank to achieve a turbidity of 3 NTU