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Modelling retinoid content in live prey: A tool for evaluating the nutritional requirements and development studies in fish larvae

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Abstract

This study was conducted to evaluate the accumulation of different levels of total vitamin A in live prey (*Brachionus plicatilis* and *Artemia* nauplii) enriched with a commercial emulsion (0.15 and 0.6 g l^{-1} for rotifers and *Artemia* nauplii, respectively) , which contained increasing levels of all-*trans* retinyl acetate. Emulsions used for rotifer enrichment contained 124, 138, 151, 165, 178, 192, 226, 259 and 327 μg total vitamin A l^{-1}, whereas those used for *Artemia* nauplii contained 494, 548, 602, 629, 710, 764, 899, 1034 and 1334 μg total vitamin A l^{-1}. Total vitamin A incorporation in rotifers was constant until a threshold comprised between 226 and 327 μg total vitamin A l^{-1}, above which the incorporation of total vitamin A from the emulsion was maximum (806 ng total vitamin A mg DW^{-1} in rotifers enriched with 327 μg total vitamin A l^{-1}). In *Artemia* nauplii, total vitamin A increased from 4.0 ng mg DW^{-1} up to 52 ng mg DW^{-1} in nauplii enriched with an emulsion containing 1334 μg total vitamin A l^{-1}. Retinoid levels in live prey increased as the content of all-*trans* retinyl acetate augmented in the emulsion, although they did not accumulate in a dose-dependent manner because retinoid incorporation in live prey was found to be not proportional to the content in the emulsion. Rotifers exhibited a higher retinoid incorporation pattern than *Artemia* nauplii, which seemed to be related to species-specific differences between both live prey. Both live prey were able to absorb and metabolize the vitamin A compounds administered through the emulsion, according to the results regarding retinol and retinoic acid content although the levels were higher in the rotifers than in the nauplii. The differential pattern of total vitamin A accumulation between rotifers and *Artemia* nauplii should be considered when designing nutritional studies dealing with this vitamin and first feeding marine larvae reared on live prey due to the difficulty in maintaining constant levels of total vitamin A especially during the transition feeding phase from rotifers to *Artemia* nauplii.

Keywords: Enrichment; Live prey; *Brachionus plicatilis*; *Artemia* nauplii; Retinoids

1. Introduction

Vitamin A is the generic name that describes compounds that possess the same biological activity as retinol. Retinoids are fat soluble compounds that function as highly bioactive molecules in a large variety of developmental processes. The biological activity of vitamin A is mediated mainly by its active metabolite, retinoic acid. There are two active forms of retinoic acid, 9-*cis* and all-*trans* retinoic acid, which are obtained by the dehydrogenation of retinol (Gouillou-Coustans et al., 1998).

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These compounds play a key role in morphogenesis, cellular differentiation and proliferation processes; such as normal growth and body patterning, central nervous system development, and differentiation of pigmentary cells, limbs and skeleton during vertebrate early development (see review in Ross et al., 2000). Fish are not able to synthesize retinoids de novo. Thus, both excess and deficiency of retinoids result in abnormal development during embryogenesis and larval development, and induce skeletal deformities (Herrmann, 1995; Haga et al., 2002a,b; Villenevue et al., 2005).

Morphogenesis is a rapid and complex process occurring during early ontogeny of fishes. Indeed, newly hatched larvae undergo dramatic changes in their body shape, morphology, metabolism, swimming abilities, and behaviour as they transform into a juvenile, which normally take place in a few weeks. Marine fish larvae hatch much earlier in their development than other vertebrates, suggesting that the spatiotemporal sequences of teleost development are quite different from those of higher vertebrates (Haga et al., 2002a). Consequently, marine species constitute an interesting and original biological model inasmuch as they allow us to study the influence of diet composition on the above-mentioned processes, especially skeletal development (Villeneuve et al., 2005). Despite the effort that has been put in recent years in the development of a compound diet for marine fish larvae, with the exception of European sea bass (Cahu et al., 2003), larvae of most marine fish cultured species still rely on live prey from the onset of exogenous feeding. Consequently, nutritional requirement studies and the effects of nutrients on early fish development should still be conducted using live feeds. The rotifer *Brachionus plicatilis* and the nauplii of the crustacean branchiopod *Artemia* have been extensively used as live prey in rearing marine fish larvae protocols due to their appropriate size, easy and rapid culture/production, and suitability for mass production under controlled conditions (Watanabe et al., 1983). However, generally, the use of rotifers and *Artemia* nauplii requires the improvement of their nutritional value to fit the nutritional requirement of fish larvae by means of enrichment procedures. Different studies have revealed that the rotifer and *Artemia* nauplii nutritional quality to larvae can be manipulated by the live prey feeding regime. The process by which beneficial substances are included inside the body of live prey is called bioencapsulation and consists of the incubation of live prey in a medium with the enrichment product. The non-selective feeding behaviour of live prey makes the dispersed particles of the enrichment product incorporate in the composition and digestive tract of the prey (Monroig et al., 2006). However, these procedures make it difficult to perform accurate nutritional studies because of the variability of the nutrient content, especially vitamins, in live prey (Villeneuve et al., 2005).

The objective of the present study was to evaluate the accumulation of different levels of vitamin A in live prey (rotifers and *Artemia* nauplii) using a commercial enrichment emulsion with increasing levels of total vitamin A. The accumulation pattern of this vitamin in live feeds would be of value when designing vitamin A nutritional studies with fish larvae fed on live prey.

2. Material and methods

Rotifers (*B. plicatilis*, S-1 strain, ICMAN-CSIC Spain, 323±42 μm length) were cultured with *Tetraselmis suecica* in a 100-ltank filled with filtered seawater (32 g l^{-1}) at a temperature that ranged between 19 and 20 °C. *Artemia* nauplii were obtained from the hatching of EG grade INVE NV (Ghent, Belgium) cysts. Both types of live prey were enriched with a commercial emulsion Easy Selco™ (ES, INVE) supplied with graded levels of all-trans retinyl acetate (1,500,000 IU g^{-1}; Acros Organics; 1 IU Vitamin A = 0.34 μg retinyl acetate). The lowest level of vitamin A in the enrichment emulsions was selected as that provided by the ES emulsion without all-trans retinyl acetate addition (control emulsion; 124 μg total vitamin A l^{-1} in rotifer enrichments and 494 μg total vitamin A l^{-1} in *Artemia* nauplii enrichments). Differences in basal retinoid levels between both types of live prey were due to differences in the amount of emulsion normally used for rotifer and *Artemia* nauplii enrichments (0.15 and 0.6 g ES l^{-1}, respectively; according to manufacturer instructions). Each experimental emulsion was tested by triplicate and at different dates. Emulsions used in rotifer enrichment contained 124, 138, 151, 165, 178, 192, 226, 259 and 327 μg total vitamin A l^{-1}, whereas those used for *Artemia* nauplii contained 494, 548, 602, 629, 710, 764, 899, 1034 and 1334 μg total vitamin A l^{-1}.

Just before each enrichment trial, the rotifer density was checked in the mass production tank and the quantity of rotifers needed for conducting enrichment trials was siphoned. Then, rotifers were filtered, gently rinsed in filtered seawater and inoculated at a density of 230 rotifer ml^{-1} into 30 l containers filled with filtered seawater and the enrichment emulsion. Rotifer enrichment lasted for 2 h at 20 °C and under continuous aeration and illumination. *Artemia* cysts were incubated and hatched following INVE instructions, and 6 h after hatching the nauplii were enriched in 10 l buckets filled with filtered seawater at 200 nauplii ml^{-1} for 24 h at