Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 272 (2007) 502–513
Aquaculture
www.elsevier.com/locate/aqua-online

Phospholipids vs. neutral lipids: Effects on digestive enzymes in Atlantic cod (*Gadus morhua*) larvae

Per-Arvid Wold [a,*], Katja Hoehne-Reitan [a], Chantal L. Cahu [b], Jose Zambonino Infante [b], Jose Rainuzzo [c], Elin Kjørsvik [a]

[a] *The Norwegian University of Science and Technology, Department of Biology, N-7491 Trondheim, Norway*
[b] *UMR 1067 of Fish Nutrition, IFREMER BP 70, 29280 Plouzané, France*
[c] *SINTEF Fisheries and Aquaculture, N-7465 Trondheim, Norway*

Received 3 April 2007; received in revised form 18 June 2007; accepted 25 June 2007

Abstract

The aim of this study was to evaluate the effect of dietary lipid classes (phospholipid vs. neutral lipid) and level of n-3 highly unsaturated fatty acids (HUFAs) on growth, survival and digestive enzymatic activity in Atlantic cod (*Gadus morhua*) larvae. Larvae were fed enriched rotifers from mouth opening and were weaned to formulated diets during a co-feeding period lasting from 17 days post hatch (dph) to 24 dph. Larvae were fed exclusively compound diets from 24 dph until the end of the experiment. Three isoproteic and isolipidic compound diets with gradually decreasing levels of DHA and EPA in the dietary phospholipid fraction (diet PL3 > PL1 > NL1) and increasing levels of total dietary neutral lipids (PL3 < PL1 < NL1) were used. Larvae fed the PL3 or PL1 had a significant higher dry weight than larvae fed the NL1 diet at the end of the experiment (45 dph). Survival did not differ significantly between treatments. A gut maturation index based on the relation between the amount of the brush border enzyme alkaline phosphatase and the cytosolic enzyme leucine-alanine aminopeptidase showed that the enterocyte maturation on 35 and 45 dph was better in larvae fed the PL3 and PL1 diet than larvae fed the NL1 diet. Dietary composition did not significantly affect the activity of α-amylase, but an age specific decrease in activity was observed. The dietary composition did affect the specific activity of trypsin, although showing variation in pattern between different larval ages at sampling. The activity of neutral lipase showed high variability between dietary treatments, but at the end of the experiment the specific activity of neutral lipase was positively connected to the dietary neutral lipid levels. The results from this study showed that cod larvae use DHA and EPA in the phospholipid fraction more efficiently compared to those in the neutral lipid fraction.

Keywords: Atlantic cod larvae; Early weaning; Phospholipids; Digestive enzymes; Gut maturation

1. Introduction

The commercial juvenile production of Atlantic cod (*Gadus morhua*) is still a challenge, much due to sub-optimal nutrition during the first stages. Cod larval rearing is still largely depending on a live feed period with rotifers (*Brachionus sp.*) and *Artemia* during the early larval phase. The nutritional value of these commonly used live feed organisms is variable, and in particular the *Artemia* lipid composition after enrichment is rather unstable (Olsen et al., 2004). Due to high

* Corresponding author. Tel.: +47 73596346; fax: +47 73596311.
E-mail address: per-arvid.wold@bio.ntnu.no (P.-A. Wold).

0044-8486/$ - see front matter
doi:10.1016/j.aquaculture.2007.06.034

costs in live feed production, one main objective in cod larval rearing is to formulate a compound diet that can substitute live prey, in particular *Artemia*, as early as possible during larval development.

Cahu et al. (2003a) demonstrated that complete substitution of live feed by formulated diets in marine fish larval rearing is possible when larval sea bass (*Dicentrarchus labrax*) were reared only with compound diets from mouth opening. So far this is not achieved with cod larvae and cod larvae fed formulated diets before development of the stomach have shown less growth than larvae fed rotifers and *Artemia* (MacQueen Leifson, 2003). A shift from rotifers to *Artemia* or to a formulated diet 20–30 dph is usual in cod aquaculture industry (Hamre, 2006). Baskerville-Bridges and Kling (2000) and MacQueen Leifson (2003) demonstrated that cod larvae can be weaned directly from rotifers to a specialized microparticulate diet without incorporating an intermediate *Artemia* phase, although growth was poorer than in larvae fed *Artemia*. The present study is one of few early-weaning experiments performed with cod larvae where *Artemia* is entirely substituted by formulated diets as early as 17 days post hatching (dph) to investigate the effect of dietary lipid composition on larval growth and development.

During the last two decades ontogeny of digestive enzymes and developmental features of the digestive tract have been well documented in several species (Zambonino Infante and Cahu, 2001; Kjørsvik et al., 2004). The development of pancreatic enzymes follows a genetically programmed pattern which is subtly modified by the diet composition (Cahu and Zambonino Infante, 2001; Hoehne-Reitan and Kjørsvik, 2004). All digestive enzymes, except the stomach enzymes, seem to be present in pelagic marine fish larvae during the first period of feeding (Kjørsvik et al., 2004). This indicates that marine fish larvae are capable of digesting formulated diets from the beginning of start feeding. Recent work has described the activity of several key enzymes throughout the ontogeny of larvae cod (Perez-Casanova et al., 2006) in order to investigate the development of cod larval digestive capacity. The authors concluded that cod larvae are capable of digesting lipids, as also reported by Hoehne (1999) and protein at the time of mouth opening and that they have a limited capacity to digest carbohydrates. So far the dietary effects on digestive enzyme activity in cod larvae have not been shown.

Dietary lipids are the main source of energy for developing fish larvae (Sargent et al., 2002), and the n-3 highly unsaturated fatty acids (HUFA) have been identified as essential dietary components for marine fish since they cannot synthesize them de novo (Cahu and Zambonino Infante, 2001; Bell et al., 2003). The optimal fatty acid composition and lipid levels for cod larvae are not known. Generally, marine fish larvae require large amounts of docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) (Rainuzzo et al., 1997; Sargent et al., 2002; Izquierdo, 2004) and the optimal level of EPA+DHA in marine finfish larvae seem to be about 3% of dietary dry matter (Cahu and Zambonino Infante, 2001; Sargent et al., 2002). The use of diets with a high DHA/EPA ratio (>1.5–2.0) generally shows better larval survival and growth than a lower DHA/EPA ratio (Kjørsvik et al., 2004).

Marine phospholipids (PLs) are carriers of HUFAs and are considered as good lipid sources for starter feeds for marine fish larvae (Sargent et al., 2002). Marine fish larvae possess a high capacity to utilize phospholipids (Geurden et al., 1998; Salhi et al., 1999; Izquierdo et al., 2001) and micro diets containing more marine phospholipids than marine triacylglyceroles (TAG) resulted in better growth in larval sea bass (Cahu et al., 2003a; Gisbert et al., 2005). It is recommended that marine fish larvae are given 10% marine phospholipids of dietary dry matter (Sargent et al., 2002; Cahu et al., 2003b).

The aim of the present study was to evaluate the effects of dietary incorporation of n-3 HUFA in different lipid classes (phospholipids vs. neutral lipids) and the level of n-3 HUFA in formulated diets on growth, survival and activity of some digestive enzymes in early weaned cod larvae. Growth, survival and specific activity of pancreatic-and intestinal digestive enzymes were followed. The experimental design was based on isolipidic and isoproteic microdiets from 17 until 45 dph.

2. Material and methods

2.1. Experimental design

Atlantic cod eggs were obtained from Troms Marine Yngel A/S (Tromsø, Norway) two days before hatching. Eggs were disinfected in glutaraldehyde in seawater (0.4 g/l) for 10 min (Salvesen and Vadstein, 1995) and incubated at a stocking density of 150 eggs/l in nine 160 l cone bottomed black tanks in darkness at 7.5 °C (salinity 34‰). Each dietary treatment was run in three replicate tanks.

After hatching, light was turned on (24 h) and the temperature was gradually increased from 8 to 12 °C between 1 and 6 dph, and then kept constant at 12 °C±0.2 °C. Water exchange was gradually increased from 0.1 l/min at hatching to 0.9 l/min from 30 dph. Larval rearing lasted up to 45 dph. Dead larvae were removed every second day from 1–17 dph and were removed and counted every day from 17 dph onwards.