Aquaculture 282 (2008) 130–137

Contents lists available at ScienceDirect

Aquaculture

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

Utilization of free amino acids, yolk protein and lipids in developing eggs and yolk-sac larvae of Japanese eel *Anguilla japonica*

Nobuyuki Ohkubo [a,*], Sayumi Sawaguchi [a], Kazuharu Nomura [b], Hideki Tanaka [b], Takahiro Matsubara [a]

[a] Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Hokkaido 085-0802, Japan
[b] National Fisheries Research Institute of Aquaculture, Fisheries Research Agency, Mie 516-0193, Japan

ARTICLE INFO

Article history:
Received 30 January 2008
Received in revised form 12 June 2008
Accepted 17 June 2008

Keywords:
Japanese eel
Free amino acids
Glutamine
Lipovitellin
Lipids

ABSTRACT

To elucidate the utilization of major yolk nutrients in eggs and yolk-sac larvae of Japanese eel *Anguilla japonica*, contents of free amino acids (FAA), 380 kDa lipovitellin (oLv; the major yolk protein in ovulated eggs), and lipids were measured. All larvae hatched by the 2nd day after fertilization at a water temperature of 23 °C, and the hatched larvae absorbed almost all of the yolk mass by the 10th day. The FAA composition in ovulated eggs is unique in that glutamine (Gln) is markedly high (32% of total FAA) in comparison to other pelagic eggs of marine teleosts suggesting the importance of Gln for early development of the Japanese eel. Total FAA content decreased to 45% of the initial level by the 4th day after fertilization. Gln content showed a rapid decrease to 5% of the initial level within 2 days after fertilization. oLv content, measured by enzyme-linked immunosorbent assay using an antiserum against lipovitellin, decreased until the 4th day and was, thereafter, undetected. Total content of triacylglycerol (TG) was stable until the 2nd day, and then decreased until the 8th day. On the other hand, the content of phospholipids (PL) gradually decreased until the end of yolk sac absorption. From these data, we divided the pattern of nutrient utilization in embryos and larvae of Japanese eel into two phases: first, utilization of FAA (especially Gln) and oLv from the 1st day, followed by TG utilization from the 2nd to 4th day by which time more than 30% of the FAA and the oLv stock have been utilized. In addition, PL was utilized as a subsidiary energy source throughout development. Almost all the yolk nutrient were absorbed by the 8th day after fertilization, and it is considered that the larvae need to start feeding before this period.

1. Introduction

The Japanese eel is one of the most important commercial food fish in Japan, and most of the consumed Japanese eel is produced by aquaculture. Juvenile eels are caught in river mouths or in coastal waters around south to mid Japan and used as seed for aquaculture and are reared to commercial size in an artificially controlled environment. However, the catch of glass eels has recently decreased probably due to over-fishing. Establishment of an artificial production system for glass eels has become urgent for supplementation of commercial aquaculture instead of relying on natural stocks. Recent research in small-scale glass eel production systems (Tanaka et al., 2003) has made notable progress, however, some important issues such as acquisition of high quality eggs, improvement of survival rate in early life stages, and development of suitable artificial diets for larvae and juveniles need to be further examined. Physiological information such as utilization of yolk nutrients and nutritional conditions during the early life stage of the Japanese eel is needed to solve these problems. Recent examination of the relationship between lipid or fatty acid composition of ovulated eggs with larval survival rate in Japanese eel have demonstrated that high quality eggs are characterized by low levels of total lipids especially in the polar lipids, and high contents of docosahexaenoic acid (DHC) in polar lipids when compared to low quality eggs (Furuita et al., 2003, 2006). Moreover, biochemical analysis of ovulated eggs with regard to egg buoyancy demonstrated a positive correlation between the egg water content or free amino acid (FAA) content and egg buoyancy (Seoka et al., 2003).

Amino acids (FAA and protein constituent) have been suggested to be the main substrate for energy metabolism and for protein synthesis for embryonic body construction among yolk nutrient reserves in some marine fish species such as Atlantic cod *Gadus morhua* (Finn et al., 1995a, b) and Atlantic halibut *Hippoglossus hippoglossus* (Finn et al., 1995c), which spawn pelagic eggs with no visible oil droplets. Vitellogenin (Vg), a maternal serum glycophospholipoprotein, is the origin of the major nutrient components in the eggs of these species. As in other oviparous animals, Vg is a precursor of yolk proteins in fish and is synthesized in the liver under estradiol-17β stimulation. In maturing females, the synthesized Vg is transported to the ovary through the bloodstream then incorporated into the growing oocytes by receptor-mediated endocytosis, followed by a proteolysis into smaller yolk proteins (for reviews, see Opresko and Karpf, 1987; Stifani et al., 1990; Shen et al., 1993). In fish, the

* Corresponding author. Hokkaido National Fisheries Research Institute, 116, Katsurakoi, Kushiro, Hokkaido 085, Japan. Tel.: +81 154 91 9136; fax: +81 154 91 9355.
E-mail address: nobuyuki@fra.affrc.go.jp (N. Ohkubo).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.06.017

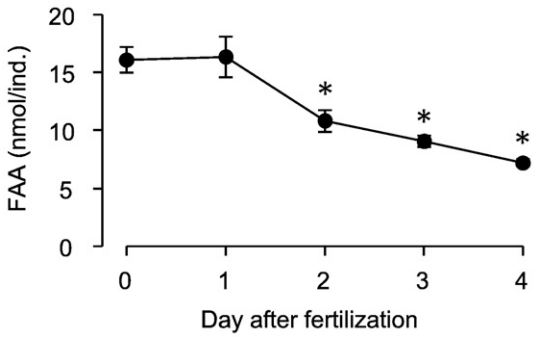

Fig. 1. Changes in the total contents of nineteen free amino acids (FAA) in developing eggs and larvae of Japanese eel. The total FAA contents are presented as the mean (±SE) of five samples. An asterisk (*) indicate that the values are significantly different from that of the control at $p<0.05$. Hatching occurred on day 2 after fertilization.

yolk proteins originating from Vg are classified into lipovitellin (Lv), phosvitin (Pv), β′-component (β′-c) and C-terminus component (C-t) (Hiramatsu et al., 2002a). After the initial processing of Vg, additional processing of the yolk proteins occurs during oocyte maturation in fishes spawning in brackish and marine waters (Wallace and Begovac, 1985; Wallace and Selman, 1985; Greeley et al., 1986; Matsubara et al., 1995). This second proteolysis provides the FAA pool, which is thought to be the osmotic effector for oocyte hydration and usable supply of nutrient for embryo (Craik and Harvey, 1987; Greeley et al., 1991; Thorsen et al., 1996; Matsubara and Koya, 1997; Matsubara et al., 1999). The yolk proteolysis during oocyte maturation and utilization of yolk nutrient has been studied in pelagic egg spawning fish of the superorder Acanthopterygii (Matsubara and Koya, 1997; Matsubara et al., 1999; Ohkubo and Matsubara, 2002) and Paracanthopterygii (Matsubara et al., 2000; Reith et al., 2001; Ohkubo et al., 2006). However, in Anguilliformes including Japanese eel, information about yolk proteolysis during oocyte maturation is limited (Seoka et al., 2004; Matsubara et al., 2006) and that about yolk nutrient utilization is not available.

To clarify utilization patterns of these Vg derived nutrient reserves in developing eggs and larvae, immunological assays using the antiserum of the major yolk protein, Lv in ovulated eggs (oLv), have been used as an effective tool (Hartling and Kunkel, 1999; Ohkubo and Matsubara, 2002; Ohkubo et al., 2006). In the present study, in order to analyze quantitative changes of the oLv in addition to FAA, we developed an enzyme-linked immunosorbent assay (ELISA) for the measurement of 380 kDa lipovitellin (oLv), the major yolk protein in ovulated Japanese eel eggs (Matsubara et al., 2006). Furthermore, we quantitatively examined the changes in the phospholipids (PL) and triacylglycerols (TG), that have been suggested to be the major lipid class catabolized by embryos and larvae in marine fish (Fraser et al., 1988; Rainuzzo et al., 1992; Finn et al., 1995b,c, 1996; Seoka et al., 1997; Ohkubo and Matsubara, 2002; Ohkubo et al., 2006).

2. Materials and methods

2.1. Samples

The adult male and female Japanese eels used in this study were kept in a 1 kL aquaria at the National Research Institute of Aquaculture, in Mie, Japan. The method of broodstock management and artificial induction of maturation are detailed in Tanaka et al. (2003).

2.1.1. Experiment 1: samples from just after fertilization to 4th day after fertilization (at the stage of mouth opening)

A total of five groups of fertilized eggs were obtained from different females in 2002 and 2006. For each group, about 5000–10,000 eggs were artificially fertilized and incubated in a flow-through hatching cylinder (500 L) at a temperature of 23 °C. Under these conditions, hatching occurred on the 2nd day after fertilization. Then, hatched larvae were kept in a flow-through aquaria (180 L) without feeding. Eggs and unfed larvae were sampled just after fertilization (day 0), and on the 1st (day 1), 2nd (day 2; hatched larva), 3rd (day 3), and 4th day (day 4) after fertilization. The samples were counted, 50 individuals were placed in each 1.5 ml microtube, surplus moisture removed, then stored at −80 °C until use. The FAA and oLv content were measured for these five groups.

2.1.2. Experiment 2: samples from just after fertilization to 10th day after fertilization (the end of yolk-sac absorption)

A total of five groups of fertilized eggs were obtained from different females in 2007. For each group, about 5000–10,000 eggs were artificially fertilized and incubated in a flow-through hatching cylinder (75 L) at a temperature of 23 °C. Then, about 2000–4000 hatched larvae were kept in a flow-through aquaria (5 L) without feeding. Under these conditions, hatching occurred on the 2nd day after fertilization, and almost all the larvae absorbed their yolk sacs within 10 days after fertilization. Eggs and unfed larvae were sampled just after fertilization (day 0), and on the 2nd (day 2; hatched larva), 4th (day 4), 6th (day 6), 8th (day 8), 10th (day 10) day after fertilization. The samples were treated and stored in the same procedure as stated above. Measurement of oLv and lipid content were carried out on these five groups.

2.2. Analysis of FAA

For analysis of FAA content, samples of 50 eggs or larvae were homogenized in 0.05 ml of 6% trichloroacetic acid. After centrifugation at 10,000 *g* for 10 min at 4 °C, supernatants were collected and volumes measured for calibration. Amino acids were analyzed using a Shimadzu amino acid analyzing system using a Shim-pack Amino-Li (6×100 mm) and a Shim-pack ISC-30/S0504 Li column (4×50 mm) (Shimadzu, Kyoto, Japan). The chromatograms were analyzed on a CLASS LC-10 (Shimadzu) calibrated from the chromatogram standard solution of amino acids, type AN-II, B with aspartic acid and glutamic acid (Wako, Osaka, Japan).

2.3. Purification of lipovitellin and preparation of the antiserum

A two-step chromatography procedure using a hydroxylapatite column (15×40 mm, Bio-Rad, CA, USA) and a gel filtration column of

Table 1
Content of free amino acids (FAA) in eggs of Japanese eel, barfin flounder[a] and walleye pollock[b]

	Japanese eel		Barfin flounder		Walleye pollock	
FAA	nmol/ind	% of total FAA	nmol/ind	% of total FAA	nmol/ind	% of total FAA
EAA	5.5	34.3	188.21	53.0	145.93	48.1
Leu	0.42	2.6	43.74	12.3	33.89	11.2
Ile	0.28	1.8	25.11	7.1	21.53	7.1
Lys	2.09	13.0	35.61	10.0	19.78	6.5
Val	0.53	3.3	25.30	7.1	19.83	6.5
Thr	0.29	1.8	15.54	4.4	15.09	5.0
Arg	0.65	4.1	16.88	4.8	10.01	3.3
Phe	0.13	0.8	9.89	2.8	8.24	2.7
Tyr	0.39	2.4	8.52	2.4	7.02	2.3
Met	0.08	0.5	–		6.49	2.1
His	0.63	4.0	7.64	2.2	4.04	1.3
NEAA	10.6	65.7	166.67	47.0	157.47	51.9
Ala	1.37	8.5	35.38	10.0	74.78	24.6
Ser	0.17	1.1	32.67	9.2	34.27	11.3
Gln	5.17	32.2	9.80	2.8	10.69	3.5
Gly	1.45	9.0	12.18	3.4	9.71	3.2
Pro	1.29	8.0	5.20	1.5	8.94	2.9
Asn	0.25	1.6	22.73	6.4	6.93	2.3
Glu	0.45	2.8	25.82	7.3	5.77	1.9
Asp	nd	0	7.97	2.2	nd	0
Tau	0.41	2.6	13.77	3.9	6.39	2.1
Total	16.06		353.75		303.69	

EAA: essential amino acids, NEAA: non-assential amino acids, nd: not detected, -: no data.
[a] Data derived from Matsubara and Koya (1997).
[b] Data derived from Ohkubo et al. (2006).