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Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae

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Abstract:

This study was carried out to evaluate the effects of dietary phospholipid on the development and rearing performance of pikeperch (Sander lucioperca) larvae. From day 10 post-hatching, fish larvae were weaned onto three isoproteic and isolipidic formulated diets with different phospholipid (PL) levels: 1.4 (PL1), 4.7 (PL5) and 9.5% (PL9) of dry matter, as soybean lecithin. Neutral lipid (NL) with inversed gradient was incorporated in diets. Survival, growth and deformities were monitored until day 34 post-hatching, as well as intestinal enzyme activities, leucine alanine peptidase (leu-ala), aminopeptidase N (AN) and alkaline phosphatase (AP), which were used as indicators of digestive tract maturation. This study showed that PL supplementation significantly improved growth but not survival. The increase in dietary PL from 1.4 to 9.5% led to a 50% increase in larval final weight suggesting that high PL levels are needed during larval stages of pikeperch. The incidence of deformities was not affected by dietary phospholipid level. The specific activity of brush border membrane enzymes (AN and AP) increased with dietary phospholipid levels, indicating an earlier or more efficient maturation of digestive structures. A gut maturation index based on the ratio of segmental activity of the brush border membrane enzyme AP related to segmental activity of a cytosolic enzyme, leu-ala, was significantly higher in PL5 and PL9 groups compared to PL1 group indicating that 1% phospholipid incorporation in diet was not sufficient to induce good enterocyte maturation. Diet fatty acid composition was affected by phospholipid incorporation, dietary n − 3 HUFA concentration decreasing with the incorporation of PL. Fatty acid composition in larvae reflected that of corresponding diet. The best results in growth and development obtained in the PL9 group seemed related to the PL entity, independently of its fatty acid composition. The results of this study indicate that pikeperch larvae have a relatively high PL requirement (at least 9.5% of the diet, dry weight).

Keywords: Digestive enzymes; Fatty acid; Larval development; Phospholipids; Pikeperch

1. Introduction

Pikeperch (*Sander lucioperca*) is a valuable species for aquaculture due to its rapid growth, flesh quality and high commercial value. In Europe, production of pikeperch fingerlings depends mainly on extensive and semi-intensive pond culture (Hilge and Steffens, 1996; Zakes, 1997). In spite of the small size and fragility of percid larvae which have limited the development of percid culture (Kestemont and Mélard, 2000), a growing interest in the development of pikeperch intensive rearing has developed in several countries (Klein Breteler, 1989; Ruuhijärvi et al., 1991; Zakes, 1999; Molnar et al., 2004; Ostaszewska et al., 2005; Hamza et al., 2007; Kestemont et al., 2007).
The initial studies on early weaning of pikeperch larvae reported poor results in terms of survival and growth (Ruuhijärvi et al., 1991; Schlumberger and Proteau, 1991; Proteau et al., 1993; Mani-Ponset et al., 1994). More recent studies using commercial diets have indicated significant improvements in survival and growth with appropriate larval diets and/or optimal weaning timing (Ostaszewska et al., 2005; Kestemont et al., 2007). The initial studies on the digestive ontogeny and lipid metabolism were essentially based on histological and cytochemical studies (Mani-Ponset et al., 1994; Diaz et al., 1997). A recent investigation using an enzymatic approach indicated that the digestive capacities of pikeperch larvae was affected by diet (live prey or compound diet) and weaning time (Hamza et al., 2007).
Until now, there is no feed specifically formulated for percid fishes (Kestemont and Mélard, 2000). Hilge and Steffens (1996) considered that the lack of an adequate formulated diet considerably limited further development of pikeperch fry intensive culture. Data concerning the nutritional requirements of percid fishes are scarce (Brown et al., 1996; Fiogbé et al., 1996; Kestemont et al., 1996; 2001) and, concerning pikeperch, are limited to the juvenile stage (Nyina-wamwiza et al., 2005; Schulz et al., 2005; 2006; Molnar et al., 2006). Knowledge of nutritional requirements and enzymatic capacities during the early life stages is thus needed.
It is well known that lipids constitute a major energy source for fish (Bell and Tocher, 1989) and play a critical role in larval development (Rainuzzo et al., 1997; Sargent et al., 1999). Also phospholipids (PL) have been demonstrated to significantly affect survival, growth, deformities and/or resistance to stress in several fish and crustacea (Kanazawa, 1985; Kanazawa et al., 1985; Geurden et al., 1995; 1998; Koven et al., 1998; Cahu et al., 2003; Gisbert et al., 2005). They play a major role in maintaining the structure and function of cellular membranes (Kanazawa, 1985; Tocher, 2003). They have been reported to act as emulsifiers in the gut (Koven et al., 1993) and to improve intestinal absorption of long chain fatty acids (Fontagné et al., 2000). Moreover, they stimulate lipoprotein synthesis in intestinal enterocytes (Fontagné et al., 1998; Geurden et al., 1998) and play an important role in the transport of dietary lipids (Kanazawa, 1991; Teshima et al., 1986). Few studies have demonstrated their effect on the maturation of digestive structures of fish larvae (Cahu et al., 2003; Gisbert et al., 2005; Morais et al., 2007).
Fatty acids are known to play a crucial role in membrane structure (Tocher, 2003). The n-3 (18:3; 20:5 and 22:6) and n-6 (18:2 and 20:4) fatty acids are essential for normal growth and survival of fish, which do not possess the capacity to synthesize them. Freshwater fish species have bioconversion capacity, allowing them to transform precursors (in particular 18:3n-3 and 18:2n-6) into highly unsaturated fatty acids (HUFA), as has been demonstrated in rainbow trout and Japanese eel by Kanazawa et al. (1979) and more recently for pike by Buzzi et al. (1997).
Considering the importance of PL for fish species, particularly during early life stages, when larvae are unable to synthesize them efficiently (Geurden et al., 1995; Coutteau et al., 1997; Fontagné et al., 1998), this study was carried out to evaluate the effects of dietary PL on survival, growth, deformities, digestive enzyme activities and fatty acid composition of pikeperch larvae.

2. Materials and methods

2.1. Facilities and fish

Pikeperch (*Sander lucioperca*) larvae were obtained from a private hatchery (Viskweekcentrum Valkenswaard, The Netherlands) and transferred to INSTM (Institut National des Sciences et Technologies de la Mer, Tunisia) on day 2 post hatching (ph). Upon arrival, the larvae were acclimated in two 500 l tanks (20-22°C) supplied with U.V.-sterilized recycled fresh water. From mouth opening, on day 4 ph, they were fed *ad libitum* each hour from 0080 to 2000 h, on newly hatched small size

Artemia nauplii (AF, INVE Belgium). On day 10 ph, the larvae were transferred to the experimental unit in a recirculating system containing 12 cylindroconical tanks of 60 l each. Four tanks were randomly assigned to each experimental group. Initial stocking density was 20 larvae l^{-1}. Temperature and dissolved O_2, controlled daily, were maintained at 21-23°C and above 6 mg l^{-1}, respectively, with a water exchange of up to 100% h^{-1} (flow rate: 1 l min^{-1}). Nitrites and ammonia were determined twice a week and maintained at levels lower than 0.1 and 1 mg l^{-1}, respectively. The larvae were kept under low light intensity (30 lux maximum) except during cleaning and feeding. Tanks were cleaned by siphoning twice a day to remove the faeces and dead larvae.

2.2. Experimental diets

From day 10 to day 34 ph, larvae were fed one of three isoproteic and isolipidic microdiets (Table 1) formulated according to the patent WO0064273 (registered in World Intellectual Property Organization, November 2000) and containing modified levels of soybean lecithin (Etablissement Louis François) and cod liver oil to obtain three PL levels: 1.5 (PL1), 4.7 (PL5), and 9.5% (PL9). Microdiets were processed as follows: dietary ingredients were mechanically mixed with water, pelleted and dried at 50°C for 20 min. The pellets were sieved to obtain two sizes of particles: 200-400µm used during the first week, then 400-700µm until the end of the experiment. Feed was distributed manually every 30 min from 0080 to 2000 h.
The feeding levels were fixed at 25, 20, 15 and 10% of larval wet weight during the first, second, third and fourth week, respectively, corresponding to 1-5g $tank^{-1}day^{-1}$. A period of co-feeding (day 10-15) was applied to wean the larvae to the dry diet. During co-feeding, at least four meals were given prior any distribution of newly hatched *Artemia* nauplii (EG, INVE Belgium).

Table 1. Composition of the three experimental diets containing different levels of phospholipid (PL).

Diet Ingredients[a] (%)	PL1	PL5	PL9
Fish meal	60	60	60
Hydrolyzed fish meal CPSP G	14	14	14
Cod liver oil	13	7	0
Soybean lecithin[b]	0	6	13
Vitamin mixture[c]	8	8	8
Mineral mixture[d]	4	4	4
Betaine	1	1	1
Lipid composition(% DM)			
Neutral lipids	20.6	14.2	8.5
Phospholipids	1.4	4.7	9.5
Proximal composition (% DM)			
Crude protein	58	58	58
Crude lipids	22	20	20
Ash	12.0	12.5	13.2
% dry matter	96.2	95.3	90.5
Protein energy+lipid energy (kJ kg^{-1})[e]	18.0	17.2	17.2

[a] Dietary ingredients were commercially obtained. Fish meal, hydrolyzed fish meal (CPSP G, Concentré de Protéines Solubles de Poissons) and cod liver oil from La Lorientaise (Lorient, France). The soy lecithin was from Ets Louis François (St Maur des Fossés, France).
[b]Soybean lecithin, contains: phospholipids 620 g kg-1 including 260 g phosphatidylcholine, 200g phosphatidylethanolamine and 140g phosphatidylinositol
[c] Per kg of vitamin mix : choline chloride : 200g, retinyl acétate 0.34g, all-rac-α-tocopherol 10g, cholecalciferol 0.5 g, niacin 1g, D-calcium pantothenate 2g, thiamin 100mg, riboflavine 400 mg, pyridoxin 0.3g, ascorbic acid 20g, folic acid 0.1 g, cyanocobalamin 1g, biotine 1g, menadione 1g, meso-inositol 30g.

[d] Per kg of mineral mix : KCl 90 g, KL_4O 40 mg, $CaHPO_4 \cdot 2H2O$ 500g, NaCl 40g, $CuSO_4 \cdot 5H2O$ 3g, $ZnSO_4 \cdot 7H2O$ 4g, $CoSO_4 \cdot 7H2O$ 20mg, $FeSO_4 \cdot 7H2O$ 20g, $MnSO_4 \cdot H2O$ 3g, $CaCO_3$ 215g, $MgSO_4 \cdot 7H2O$ 124g, NaF 1g.
[e] Calculated as : lipid x 37.7 J kg^{-1} ; protein x 16.7 J kg^{-1}

2.3. Sampling procedures

Growth was monitored by sampling 30 larvae per tank on days 4 and 10 ph, and 10 larvae per tank on days 16, 22, 28 and 34 ph. The larvae were weighed collectively from day 0 to day 22, and individually on days 28 and 34. At the end of the experiment the weighed larvae were also examined to determine the rate of malformations (lordosis, scoliosis, jaw injury).
The number of sampled larvae was taken into account for survival calculation.
Growth, survival and deformity parameters were calculated as follows:
Instantaneous specific growth rate (SGR, % day^{-1}) = $100(LnWf - LnWi)\ \Delta T^{-1}$
Survival (S, %) =100 Nf/(Ni – Ns)
Deformities (D, %) = 100Nd/Ns
where Wf, Wi= final and initial weight of larvae (mg), T= time (days); Nf, Ni = final and initial number of larvae; Nd: Number of larvae presenting deformity and Ns: Number of sampled larvae.
To estimate the number of larvae which ingested the feed, in the first two days of co-feeding, 10 larvae per tank were collected in a glass becher to observe the presence (or not) of the dry diet in digestive tract.
To determine the pattern of enzyme activity, about 240 larvae were collected on day 10 and then 70, 30, 20, and 20 larvae per tank on days 16, 22, 28 and 34, respectively. On day 34, 20 larvae per tank were collected for analysis of total lipids, lipid class and fatty acids. Samples were taken before feed distribution and immediately stored at -80°C until analysis.

2.4. Enzyme assays

The heads and tails of ten day old larvae were removed to isolate the digestive segment. Older larvae were dissected as described by Cahu and Zambonino (1994), on a glass maintained on ice (0°C) under a binocular, to separate their intestinal segments. Pooled samples from each tank were homogenized in 10 volumes (v/w) of ice-cold distilled water. Leucine alanine peptidase (leu-ala), an enzyme of the cytosol, was assayed according to Nicholson and Kim (1975) using leucine-alanine (Sigma-Aldrich, St Louis, MO, USA) as substrate. Alkaline phosphatase (AP) and aminopeptidase N (AN), two enzymes of brush border membrane, were assayed according to Bessey et al. (1946) and Maroux et al. (1973) using p-nitrophenyl phosphate (Sigma-Aldrich) and L-leucine p-nitroanilide (Sigma-Aldrich) as substrates, respectively.
Enzyme activities are expressed as specific activities (U mg $protein^{-1}$) or as segmental activities (U per dissected intestinal segment). Protein was determined using the Bradford (1976) procedure.

2.5. Lipid and fatty acid analysis

Samples of 34 day old larvae were homogenized by ultraturax T10 (IKA). The lipid content in diets and larvae was analyzed after extraction with chloroform/methanol (2:1 v/v) according to the method of Folch et al. (1957). Whole body crude lipids were subsequently separated into polar and non polar fractions using Sep-Pak Silica cartridges (Waters, Milford, MA, USA). Chloroform and methanol were used as the mobile phases for neutral lipids (NL) and phospholipids, respectively (Juaneda and Rocquelin, 1985). The fatty acid methyl esters were prepared by transesterification with borontrifluoride in methanol as described by Metcalfe et al. (1966) and then separated by gas chromatography using an Agilent Technologies chromatograph 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID), a splitless injector and a polar INNOWAX 30M silica capillary column (0.25 mm i.d. and 0.25 µm film thickness). The temperature of the injector and detector were 220° and 275°C respectively. Helium was used as a carrier gas at a flow rate of 1.5ml min^{-1}.
The fatty acid composition of the three diets PL1, PL5 and PL9 is detailed in Table 2.

2.6. Statistical analyses

Results are given as mean values and standard deviations. Survival, malformations and fatty acid percentages were arcsin transformed. AP segmental activity was log10 transformed and AP/leu-ala (segmental activities) ratios were arcsin($x^{1/2}$) transformed (Zar, 1999). Data were compared by a one-way ANOVA followed by a LSD test when significant differences were found at $P<0.05$. Specific enzyme activities were log10 transformed and compared by a two-way ANOVA followed by the Tukey HSD test when significant differences were found at $P<0.05$. The two analyzed factors were age and phospholipid level. The homogeneity of variances was first checked using the Levene's test.

Table 2. Fatty acid composition (% of total fatty acids) of the phospholipid (PL) and neutral lipid (NL) fractions of the experimental diets PL1, PL5 and PL9.

	PL1		**PL5**		**PL9**	
Fatty acid	NL	PL	NL	PL	NL	PL
14:0	5.5	2.1	5.4	0.6	4.3	0.3
16:0	15.0	21.4	14.9	20.6	15.4	20.7
18:0	2.8	4.3	2.6	3.7	2.6	3.7
Σ Sat	23.3	27.8	22.9	24.9	22.3	24.7
16:1n-7	6.7	2.8	6.8	0.8	6.1	0.4
18:1n-7	3.5	3.8	3.6	1.9	4.0	1.7
20:1n-7	0.4	0.3	0.4	0.1	0.4	0.0
18:1n-9	14,6	13,8	14,7	11,3	15,3	10,9
20:1n-9	7.6	2.9	7.6	0.8	8.7	0.4
22:1n-11	7.8	1.3	7.7	0.4	9.1	0.2
Σ Monounsat	40.6	24.9	40.8	15.3	43.6	13.6
18:2n-6	2.4	1.6	2.5	44.0	3.5	50.1
20:2n-6	0,3	0,2	0,3	0,1	0,3	0,1
20:4n-6	0.6	1.7	0.6	0.4	0.6	0.2
Σ n-6	3.3	3.5	3.4	44.5	4.4	50.4
18:3n-3	1.0	0.5	1.1	4.0	1.1	4.5
18:4n-3	2.5	0.8	2.7	0.2	2.5	0.1
20:4n-3	1.1	0.6	1.1	0.2	0.7	0.1
20:5n-3	10.6	12.2	10.9	3.1	9.7	1.7
22:5n-3	2.0	1.4	1.7	0.3	0.9	0.2
22:6n-3	11.0	24.8	11.0	6.3	10.0	3.5
Σ n-3	28.2	40.3	28.5	14.1	24.9	10.1
n-3HUFA	27.2	39.8	27.4	10.1	23.8	5.6
n-3/n-6	8.55	11.51	8.38	0.32	5.66	0.20
Polyunsat/Sat	1.35	1.57	1.39	2.35	1.31	2.45
EPA+DHA (%diet dry weight)	3.99	0.28	2.37	0.25	1.01	0.24
Total EPA+DHA (%diet dry weight)	4.27		2.62		1.25	

Sat : Saturated, Monounsat: monounsaturated, Polyunsat : Polyunsaturated

3. Results

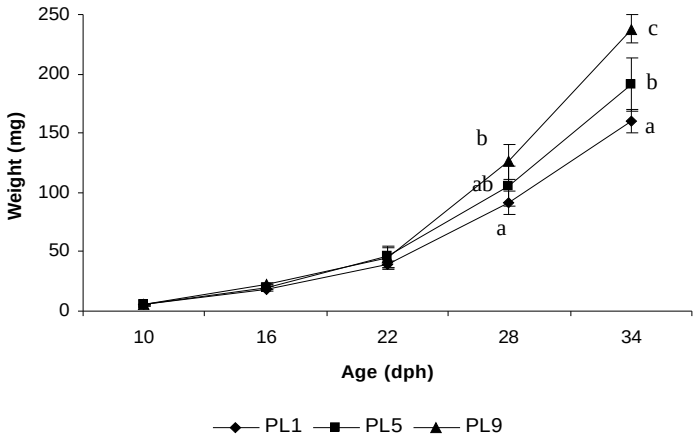
3.1. Larval zootechnical performance

The artificial diets were ingested by 80 to 100% of the larvae from the second day of co-feeding. The survival at the end of the experiment averaged 35% and no significant differences were observed among the experimental groups. The mortality reached a peak on day 12 independent of treatment. Between day 16 and 21, mortality remained relatively high (2 to 4% day^{-1}) and decreased from day 22 onwards.

From day 10 to day 34 larval weight increased 32-fold for the PL1 group to 48-fold for the PL9 group. A significant effect of dietary level of phospholipids on larval growth was detected from day 28 onwards, with PL9 larvae having significantly higher weights than PL1 larvae (P<0.01). The PL5 group was not different either from the PL1 or PL9 groups. At the end of the experiment, the growth (final weight and SGR) of PL9 larvae was significantly higher (P<0.01) than that of the two other groups, followed by the PL5 group, which grew significantly more (P<0.01) than the PL1 group (Fig. 1 and Table 3).

The deformities observed were almost exclusively limited to lordosis (the number of larvae suffering from scoliosis or incomplete jaw development was considered negligible). Malformations varied between 7 and 12% but no significant differences were found among treatments.

Fig. 1. Growth of pikeperch larvae fed different dietary phospholipid (PL) levels (PL1, PL5, PL9 = 1, 5



and 9% of phospholipids, respectively). Age is expressed in days post-hatching (dph).

Table 3. Survival, growth and percent deformities in the three experimental groups of pikeperch larvae fed different levels of dietary phospholipid, at the end of the experimental period.

	PL1	PL5	PL9
Survival (%)	34±1.5a	36.6±2.5a	33±3.1a
SGR (% day^{-1})	14.1±0.2a	14.8±0.5b	15.8c±0.2c
Total deformity (%)	12.5±12.5a	12.5±15.0a	7.5±9.6a

Means±SD (n=4) Values with different letters in the same line are significantly different (P<0.05)

3.2. Enzyme activities

Leu-ala specific activity decreased significantly between days 16 and 22 and then, increased significantly in all groups. No differences were observed among groups during the feeding trial regardless of dietary treatment (Fig. 2A). AP specific activity significantly increased from day 10 to day 16 and then, significantly decreased until day 34. On days 22 and 28, the PL5 and PL9 diets induced significantly higher AP activity than the PL1 diet. No more differences were observed among

treatments at the end of the experiment. (Fig 2B). AN specific activity significantly increased in all groups between days 10 and 16. It then significantly increased between day 16 and 22 exclusively for PL9 larvae and decreased for the PL1 group. On day 22, AN activity was at a significantly higher level in the PL9 group than in PL5 and PL1 groups. No more differences were noticed among the different larval groups from day 28 until the end of the experiment (Fig 2C).
AP segmental activity (mU fish^{-1}) was significantly affected by age and treatment (*P*=0.02). It was significantly higher in the PL5 and PL9 groups than in PL1 larvae on day 22 (Table 4). On days 28 and 34, AP segmental activity was significantly higher in PL9 larvae than in the two other groups.
The ratio of segmental activity of the brush border membrane enzyme AP related to the segmental activity of a cytosolic enzyme, leu-ala, was significantly higher in the PL5 and PL9 groups compared to the PL1 group on day 28 (Table 4).

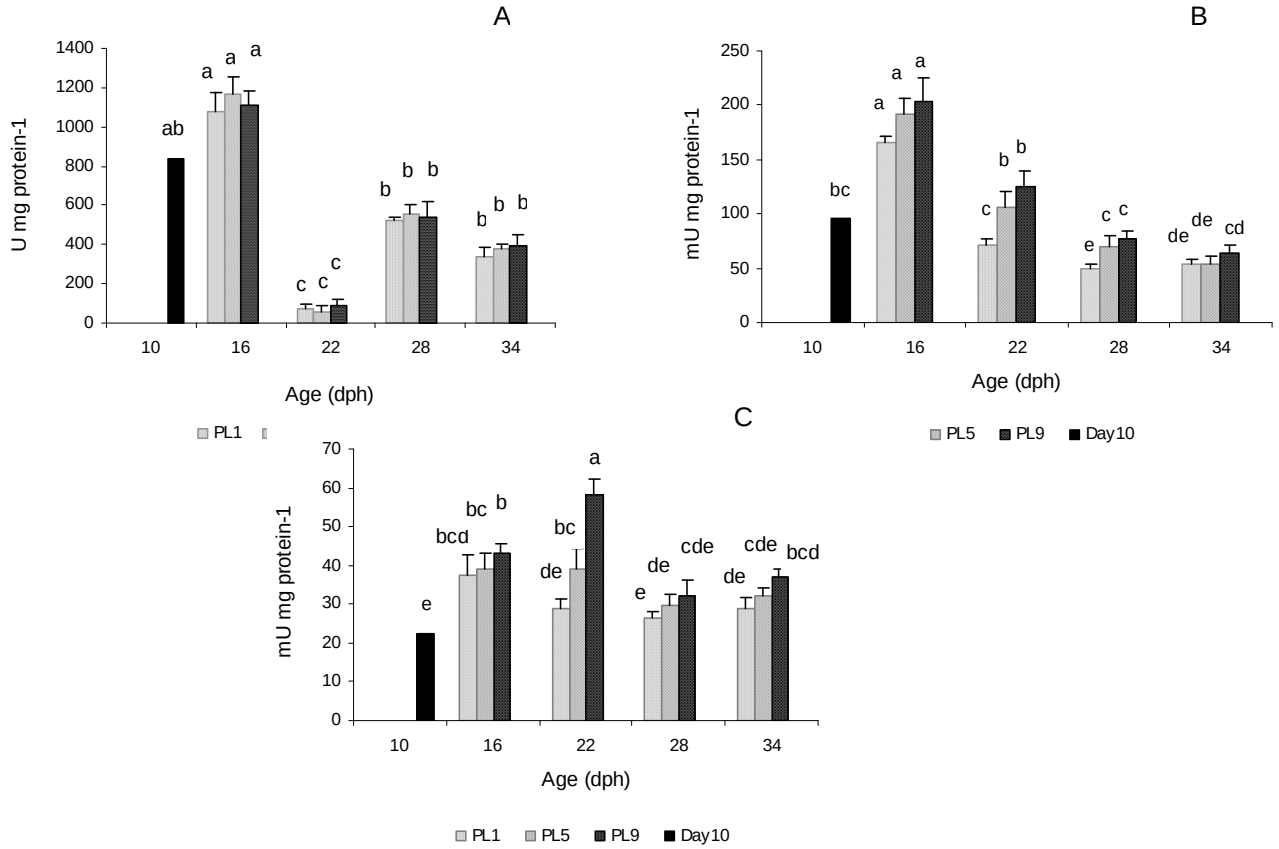


Fig. 2. Specific activity of (A) leucine alanine peptidase , (B) alkaline phosphatase and (C) aminopeptidase N during larval development of pikeperch fed with different dietary phospholipid levels (PL1, PL5, PL9 = 1, 5 and 9% of phospholipids, respectively). Data are means ± SD (n=4). Age is expressed in days post-hatching (dph). Bars with different superscript letters are significantly different (P <0.05; two way ANOVA followed by Tukey HSD).

Table 4. AP segmental activity on days 22, 28 and 34 and AP/leu-ala ratio (day 28) in pikeperch larvae fed different dietary phospholipid levels (PL1, PL5, PL9 = 1, 5 and 9% of phospholipids, respectively). Age is expressed in days post-hatching.

	PL1	**PL5**	**PL9**
AP segmental activity			
Day 22	52.1±1.0a	92.0±17.0b	87.4±17.0b
Day 28	88.4±21.4a	134.8±21.0b	189.7±34.7c
Day 34	189.5±8.5a	222.4±15.9b	287.2±33.1c
AP/Leu-ala (10exp^{-3})	0.09±0.01a	0.12±0.01b	0.14±0.02b

Means±SD (n=4) Values with different letters in the same line are significantly different (P<0.05)

3.3. Fatty acid composition

The main differences in fatty acid composition of the diets were noticeable in the PL fraction and concerned essentially n-6 and n-3 fatty acid series. The n-6 fatty acids varied from 3% (PL1) to 50% (PL9) and this change was mainly related to the increase of the 18:2n-6. All the n-3 fatty acids decreased except the 18:3n-3 which increased (from 0.5% in PL1 to 4.5% in PL9 diet) (Table 2).
The fatty acid composition of the larvae (Table 5) was globally influenced by the fatty acid composition of the corresponding diet in its NL and PL fractions. The PL (from soybean lecithin) supplementation resulted in a decrease of monounsaturated fatty acids and n-3 polyunsaturated fatty acids (PUFA) (except 18:3n-3), and in an increase of n-6 fatty acids (18:2n-6) in larval fatty acid composition. The main n-6 and n-3 polyunsaturated fatty acids, 20:4n-6 (ARA; arachidonic acid), 20:5n-3 (EPA; eicosapentaenoic acid), 22:5n-3 (DPA; docosapentaenoic acid) and 22:6n-3 (DHA; docosahexaenoic acid) presented a higher concentration in the PL fraction than in the NL fraction. The percentage of 18:2n-6 was two fold higher in the NL than in the PL fraction.

Table 5. Fatty acid composition (% of total fatty acids) in the phospholipid (PL) and neutral lipid (NL) fractions of pikeperch larvae (day 34) fed different dietary phospholipid levels (PL1, PL5, PL9 = 1, 5 and 9% of phospholipid respectively

		NL			PL	
Fatty acid	PL1	PL5	PL9	PL1	PL5	PL9
14:0	15.02±0.17a	12.23±0.54b	7.80±0.20c	4.78±0.35a	3.82±0.19b	2.54±0.51c
16:0	20.26a±0.91a	22.18±0.62b	25.46±0.86c	36.34±1.52a	39.75±0.49b	38.88±1.05b
18:0	0.55±0.05a	0.39±0.04b	0.17±0.01c	0.42±0.15a	0.67±0.55a	0.32±0.13a
Σ Sat.	35.83±0.97a	34.80±0.58a	33.43±0.90b	41.54±1.88a	44.24±0.46a	41.74±0.85a
16:1n-7	14.80±0.40a	11.90±0.37b	8.59±0.12c	3.64±2.03a	3.84±0.14b	1.80±0.41c
18:1n-7	2.43±0.28a	2.00±0.16a	1.92±0.37a	2.08±0.15a	1.98±0.10ab	1.76±0.11b
18:1n-9	15.25±0.59a	14.30±0.14b	13.72±0.21b	11.21±0.31a	10.95±0.38a	10.14±0.01b
20:1n-9	2.97±0.14a	2.67±0.10b	2.40±0.12c	1.59±0.10a	1.37±0.08b	1.24±0.03b
Σ Monounsat.	32.48±1.16a	28.19±0.39b	24.22±0.24c	18.52±1.72a	18.14±0.61b	15.28±1.01c
18:2n-6	2.72±0.09a	11.41±0.11b	22.45±0.71c	1.03±0.12a	5.12±0.04b	11.48±0.62c
20:4n-6	0.42±0.03a	0.30±0.03b	0.20±0.03c	1.33±0.04a	1.20±0.03b	0.92±0.05c
Σ n-6	3.14±0.11a	11.70±0.12b	22.64±0.71c	2.36±0.13a	6.32±0.07b	12.41±0.61c
18:3n-3	0.84±0.03a	1.30±0.04b	1.89±0.03c	0.23±0.02a	0.35±0.00b	0.59±0.05c
18:4n-3	1.83±0.09a	1.43±0.09b	1.03±0.05c	0.29±0.02a	0.25±0.01ab	0.22±0.04b
20:4n-3	0.51±0.02a	0.35±0.01b	0.18±0.01c	0.26±0.02a	0.21±0.01b	0.16±0.00c
20:5n-3	4.96±0.09a	3.23±0.07b	1.85±0.11c	5.69±0.16a	5.62±0.14a	4.94±0.19b
22:5n-3	0.96±0.05a	0.63±0.04b	0.34±0.03c	0.94±0.05a	0.79±0.02b	0.65±0.04c
22:6n-3	4.70±0.17a	3.22±0.23b	2.31±0.23c	13.14±0.45a	11.41±0.12b	10.01±0.89c
Σ n-3	13.80±0.39a	10.16±0.26b	7.61±0.29c	20.56±0.63a	18.64±0.13b	16.53±0.80c
Σ HUFA	11.13±0.29a	7.42±0.22b	4.68±0.34c	20.04±0.65a	18.03±0.13b	15.72±0.88c
n-3/ n-6	4.40±0.26a	0.87±0.03b	0.34±0.02c	8.74±0.75a	2.95±0.05b	1.34±0.11c
Polyunsat/sat	0.47±0.01a	0.62±0.01b	0.90±0.04c	0.55±0.03a	0.56±0.01a	0.69±0.02b

Sat : Saturated, Monounsat: monounsaturated, Polyunsat : Polyunsaturated
Means±SD (n=4). Values with different superscript letters in the same line per fraction are significantly different (P<0.05) HUFA Highly Unsaturated Fatty Acids;
DHA : Docosahexaenoic acid (22:6n-3); EPA : Eicosapentaenoic acid (C20:5n-3)

4. Discussion

During the experiment, pikeperch larvae ingested readily the microparticulate diets as early as the 11th day (second day of co-feeding).
The survival of larvae fed the different compound diets varied between 33 and 36% and was not significantly affected by the dietary PL level. According to our morphometric observations, the peak of mortality observed on day 12 occurred concurrently with the depletion of exogenous nutritional reserves (yolk sac and oil globule). In a previous study, histological observations (Hamza et al., 2007) showed a resorption of reserves around day 9 p.h.. Between days 16 and 21, the relatively high mortality probably concerned the larvae that did not ingest enough feed and reached the point of no return, while in the last two weeks of the experiment the mortality was essentially due to cannibalism. In the same way, Szkudlarek et al. (2007) reported three phases of mortality in pikeperch larval rearing which were related to the beginning of the exogenous feeding (days 4-6), swimbladder inflation (days 8-14) and cannibalism (days 22-34). This last period coincides with an important cannibalism in our study.
The final mean weight of larvae ranged between 160 and 240 mg and was significantly influenced by dietary PL level (*P*=0.0002). The increase in dietary PL level from 1.5 to 9.5% (dry diet weight) led to a 50% increase in larval final weight suggesting that high PL levels are needed during larval stages of pikeperch. A similar conclusion was reached by Zambonino and Cahu (1999) for European sea bass.
The positive effect of PL on fish growth especially at the larval stage has been shown for freshwater species like common carp (Geurden et al., 1995; Fontagné et al., 2000) and marine species like European sea bass (Cahu et al., 2003; Gisbert et al., 2005), red drum, (Buchet et al., 2000) and gilthead sea bream, (Coutteau et al., 1997; Fontagné et al., 1999; Izquierdo et al., 2001).
At the end of the experiment, the occurrence of scoliosis or jaw deformity was very low. Lordosis was the main skeletal deformity observed among pikeperch larvae and was generally related to the non inflation of swimbladder. This is in agreement with Chatain (1994) who demonstrated that the non inflation of swimbladder induced lordosis in sea bass larvae. In this study, inflation began on day 7. On day 12, at least 80% of larvae displayed a well inflated swimbladder. As the inflation began before the introduction of the microdiet, the non-inflation phenomenon should not be attributed to the diets.
Our study failed to evidence an effect of dietary PL level in preventing deformities, as it was observed by Cahu et al. (2003) in European sea bass larvae. These authors observed that for the highest dietary PL level (12%) the sea bass larvae exhibited the lowest incidence (2%) of skeletal deformities. The decrease of malformations with the increasing level of dietary PL has been reported by other authors (Kanazawa et al., 1981; Geurden et al., 1998). In our study, the intra treatment variability was high and may have masked the effect of the diet composition. Nevertheless, malformations were lower than in other studies, even when larvae were fed live preys (Hamza et al., 2007; Kestemont et al., 2007). Scoliosis is generally more related to a nutritional deficiency like dietary ascorbic acid level (Kestemont et al., 2007). There are few data on vitamin C requirements of first feeding larvae (Gouillou-Coustans et al., 1998) but it could be assumed that the ascorbic acid level in this diet was sufficient when compared with commercial diets (Kestemont et al., 2007). This assumption was supported by the almost total absence of scoliosis in these larvae.
The general pattern of digestive enzyme activities observed during pikeperch development in our experiment was similar to that described for other species like European sea bass (Cahu and Zambonino, 1994) and perch (Cuvier-Péres and Kestemont, 2002). The increase in brush border membrane enzymes, such as alkaline phosphatase and aminopeptidase N, concurrently with the decrease of cytosolic enzyme, such as leu-ala peptidase reflected the normal maturation process of enterocytes (Cahu and Zambonino Infante, 1994). In this trial, leu-ala sharply decreased between days 16 and 22 when AP increased between day 10 and 16. We can assume that AP activity reached its maximal value between day 16 and 22 and then decreased because of somatic growth.
AP is an esterase whose activity is induced by phosphorylated substrates such as phospholipids and phosphoproteins. Its specific activity was significantly higher in the PL9 and PL5 groups on day 22 while the larvae of the three treatments still had similar weights, and on day 28. This higher AP activity

suggests a better development and intestinal maturation process in PL9 and PL5 groups compared to the PL1 group, as was suggested in European sea bass larvae by Cahu and Zambonino (1994) and in senegalese sole by Ribeiro et al. (2002).

In this study, we observed that AP segmental activity was closely influenced by both age and treatment. AP segmental activity significantly increased with dietary PL level. On day 22, it was higher in PL5 and PL9 larvae while all the larvae had similar weights. AP has often been considered as a sensitive indicator of the nutritional status of larvae (Cahu and Zambonino, 1994; Ribeiro et al., 2002).

The AN specific activity increased with the dietary PL level on day 22, even though PL is not a potential substrate for this enzyme. Again, this highlights the enhanced maturation of the intestine in larvae fed the high PL diet, as pointed out by Cahu et al. (2003) for European sea bass larvae. From day 28 onwards, no more differences were noticeable for AN specific activity. This can be explained by the fact that at that stage all larvae displayed a mature digestive tract. Indeed, Zambonino and Cahu (2001) associated an efficient brush border membrane digestion to the adult mode of digestion.

It seems that high dietary PL level improved gut maturation and larval development of pikeperch larvae as has been shown for red drum and European sea bass by Buchet et al. (2000) and Cahu et al. (2003), respectively.

Zambonino et al. (1997) considered AP/leu-ala and AN/leu-ala ratios as enterocyte maturation indices. In our work, the ratio of segmental activity of the brush border membrane enzyme AP related to the segmental activity of a cytosolic enzyme, leu-ala, which was significantly higher on day 28 in the PL5 and PL9 groups, compared to PL1, indicating that 1% PL incorporation in the diet was not sufficient to induce good enterocyte maturation. Earlier enterocyte maturation, leading to better nutrient absorption and incorporation, has been associated with the best growth obtained with diet PL9. The role of PL level in improving lipid absorption can be partly related with its effects on enzyme activity but more likely attributed to the enhancement of lipoprotein synthesis. Indeed, PL are known to be a structural component of lipoproteins, which play an essential role in the transport of NL (Coutteau et al., 1997; Fontagné et al., 1998).

The incorporation of a gradient level of PL from vegetable source (soybean lecithin), at the expense of marine NL (fish oil), increased the PL concentration of the diet and modified its fatty acid composition. Indeed, the fatty acid composition of the larvae was globally influenced by the fatty acid composition of the corresponding NL and PL fractions in the diet. The major effect of the dietary PL supplementation on larval fatty acid composition was an increase in the percentage of total n-6 (particularly 18:2n-6) in both NL and PL fractions. This increase was accompanied by a decrease in the percentage of n-3 fatty acids (especially 20:5n-3 and 22:6n-3). These changes in FA composition were similar to those observed in Atlantic salmon fed diets containing vegetable oils (Ruyter et al., 2006). A preferential deposition of certain fatty acids (18:2n-6) in the NL fraction was also observed in this study, as was shown by Rinchard et al. (2007) in rainbow trout juveniles fed different vegetable oils and lecithin. On the contrary, n-6 and n-3 HUFA seemed to be preferentially retained in the PL fraction of the larvae, as reported by Ruyter et al. (2006). These HUFA also seem to be conserved in larval tissues whatever their concentrations in the diet. Indeed, the magnitude of the differences in HUFA levels is attenuated in the fish compared to the diets. The relatively high levels of n-3 HUFA in PL9 larvae contrasting with their low level in PL9 diet could be attributed to an elongation and desaturation process of C18 fatty acids. Indeed, it has been shown that freshwater fishes like trout and salmon fed vegetable oils have the capacity to convert C18 to C20 and C22 by elongation and desaturation (Bell et al., 1993; Tocher et al., 1997; Buzzi et al., 1997). Schulz et al. (2005) demonstrated that juvenile pikeperch have the capacity for n-3 PUFA elongation and desaturation. This process could be more important when fish are fed low levels of n-3 HUFA (Buzzi et al., 1996; Caballero et al., 2002). Best results of growth in PL9 larvae could hence be due to higher levels of PUFA, particularly of 18:2n-6 and 18:3n-3, which are essential fatty acids for freshwater species. Moreover, a higher polyunsaturated/saturated fatty acid ratio was observed in the PL fraction of the PL9 diet, which resulted in the best growth and development in this group. This was also observed by Caballero et al (2002) who showed that an increase of this ratio in the diet improved apparent fatty acid digestibility.

In a review, Morais et al. (2007) reported several studies which attributed the growth depressing effect to a high dietary NL level which decreased the activity of digestive enzymes and the absorption efficiency. In this study, it may have been the case of the PL1 diet which contained the highest level of NL. Indeed, on day 22 there was a correlation between PL level and brush border membrane enzyme activities. Furthermore, PL are known to play an essential role in lipid transport, particularly of triacylglycerol (Coutteau et al., 1997; Fontagné et al., 1998), thus affecting energy supply (Coutteau et al., 1997). In the same way, our results could indicate a better utilization of the available energy from dietary NL in larvae fed high dietary PL, as growth in the PL9 group was higher, even though larvae in the 3 groups were fed isoenergetic diets (17.2 to 18.0 kJ kg^{-1}).

It is believed that fish larvae use more efficiently n-3 HUFA from the PL fraction than those from the NL fraction (Cahu et al., 2003; Gisbert et al., 2005; Villeneuve et al., 2005). The best growth and development (maturation of the digestive tract) were observed in PL9 larvae, although the levels of EPA+DHA in the PL fraction were similar (around 0.25% diet dry weight) in the different diets. Hence, the best growth observed in PL9 larvae was not due to a higher level of EPA+DHA in the dietary PL fraction. It is generally admitted that NL is not a very efficient way to supply EPA and DHA. Nevertheless, a negative effect of high EPA and DHA level in NL could be also suspected with our results, this level reaching 4% of dry matter in diet PL1.

Conclusion

The results of this study showed that pikeperch can be successfully weaned from day 10 with a compound diet, with satisfactory growth and survival. To our knowledge, this work is the first study that analysed a nutritional requirement of pikeperch at the larval stage. It appeared that a diet containing 9% PL can support good growth and development in this species. The possibility of using a compound diet, with a formulation based on that of the PL9 diet, would allow conducting more detailed experiments to determine the specific nutritional requirements of pikeperch young developmental stages. In particular, HUFA dietary requirements, and their form of supply as NL or PL remains to be established.

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