Comparative Biochemistry and Physiology, Part D 5 (2010) 130–137

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part D

journal homepage: www.elsevier.com/locate/cbpd

Differential protein expression profile in the liver of pikeperch (*Sander lucioperca*) larvae fed with increasing levels of phospholipids

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ARTICLE INFO

Article history:
Received 11 February 2010
Received in revised form 23 March 2010
Accepted 24 March 2010
Available online 2 April 2010

Keywords:
2D-DIGE
Metabolism
Phospholipid
Pikeperch
Proteomics

ABSTRACT

A comparative proteomic approach was used to assess the protein expression profile in the liver of 34 days old pikeperch larvae fed from day 10 post hatching, with three isoproteic and isolipidic formulated diets varying by their phospholipid (PL) contents (% dry diet weight): 1.4% (PL1), 4.7% (PL5) and 9.5% (PL9). Using 2D-DIGE minimal labelling of liver extracts, we were able to show 56 protein spots with a differential intensity ($p<0.05$) depending on the dietary PL content. Among these spots, 11 proteins were unambiguously identified using nanoLC-MS/MS tandem mass spectrometry. In the PL9 larvae, our results indicate that the glycolytic pathway could be down-regulated due to the under-expression of the fructose biphosphate aldolase B and the phosphoglucomutase 1. Meanwhile, propionyl coenzyme A carboxylase (a gluconeogenic enzyme) was under-expressed. In addition, another gluconeogenic and lipogenic enzyme, pyruvate carboxylase, was identified in 3 different spots as being under-expressed in fish fed with the intermediate PL level (PL5). A high PL content increased the expression of sarcosine dehydrogenase, an enzyme involved in methionine metabolism, along with vinculin, a structural protein. Moreover, several stress proteins (glutathione S-transferase M, glucose regulated protein 75 and peroxiredoxin-1) were modulated in response to the dietary PL level and fatty acid composition. In the larvae fed with the lowest dietary PL content (PL1), over-expression of both GSTM and GRP75 might indicate a cellular stress in this experimental treatment, while the under-expression of Prx1 might indicate a lower defence against oxidative stress. In conclusion, this nutriproteomic approach showed significant modifications of protein expression in the liver of pikeperch larvae fed different PL contents, highlighting the importance of these nutrients and their influence on metabolism processes and on stress response.

1. Introduction

Pikeperch (*Sander lucioperca*) is an economically important species, interesting for aquaculture and sport fishing in Europe and several Mediterranean countries where it was introduced. As for a large number of species, larval rearing remains a critical period particularly in terms of feeding and nutritional requirements (Barnabé, 1989). Feeding larvae with an artificial diet allows limiting or even avoiding the costly production of live preys (rotifers, *Artemia* nauplii). However, it poses a problem of satisfying the nutritional requirements and of providing the best performances.

It is well known that lipids constitute a major energy source for fish (Bell and Tocher, 1989), and play a critical role in larvae development (Rainuzzo et al., 1997; Sargent et al., 1999). Furthermore, phospholipids (PL) have been demonstrated to significantly affect survival, growth and deformities in several fish species (Kanazawa, 1985; Geurden et al., 1995; Cahu et al., 2003; Gisbert et al., 2005). They play a major role in maintaining the structure and function of cellular membranes (Kanazawa, 1985; Geurden et al., 1995; Cahu et al., 2003; Gisbert et al., 2005; Tocher, 2003). They also stimulate lipoprotein synthesis in intestinal enterocytes (Fontagné et al., 1998; Geurden et al., 1998), enhance the transport of dietary lipids (Kanazawa, 1991; Teshima et al., 1986), and improve the intestinal absorption of long-chain fatty acids (Fontagné et al., 2000). A previous

Abbreviations: AldoB, fructose biphosphate aldolase B; ATP, adenosin triphosphate; FDR, false discovery rate; GRP75, glucose regulated protein 75; HUFA, highly unsaturated fatty acids; NKEF, natural killer enhancement factor; Prx1, peroxiredoxin-1; PGM1, phosphoglucomutase 1; PUFA, polyunsaturated fatty acids; PCCA, propionyl coenzyme A carboxylase; PL, phospholipids; PC, pyruvate carboxylase; SrDH, sarcosine dehydrogenase; V, vinculin.

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doi:10.1016/j.cbd.2010.03.005

study demonstrated a positive effect of dietary PL on the pikeperch larvae development, especially regarding their growth rate and digestive capacities (Hamza et al., 2008). To date, little is known about their role in metabolic processes in fish species, even if several studies evidenced the effect of the nutritional status on some enzyme activities involved in the intermediary metabolism (Cowey et al., 1977; Moon and Johnston, 1980; Suarez et al., 1995; Metón et al., 1999; 2003; Caseras et al., 2002).

Proteomics aims at analysing theoretically all expressed proteins and their interaction in a given cell or tissue, including all protein isoforms and modifications. Proteomics and genomics have been extensively used, especially in the medical and clinical fields (Gharbi et al., 2002; Zhou et al., 2002), where they help in identifying proteins or genes involved in pathologies such as obesity, diabetes or cancer. In environmental sciences, proteomics focuses on the dynamics of the proteome of organisms in response to environmental changes. It offers a powerful tool to understand the effects of pollutants on protein expression in an organism (López-Barea and Gómez-Ariza, 2006; Monsinjon and Knigge, 2007; Nesatyy and Suter, 2007). More recently, nutritional genomics has also been the focus of much interest, aiming at understanding how diet influences gene transcription (nutritranscriptomics), protein expression (nutriproteomics) and metabolites synthesized by an organism (nutrimetabolomics) (Zhang et al., 2008). In reviews, Kussmann et al. (2006; 2008), considered that proteomics applied to nutritional research should contribute to the identification of bioactive food components, for assessing their biological efficacy, and to elucidate biomarkers for defining an individual's susceptibility to diet in nutritional interventions.

To date, only a limited number of studies have explored the dietary regulation of protein or gene expression (Cousins, 1999; Cheung et al., 2001; Schmid et al., 2003; Zeisel et al., 2005; De Roos et al., 2005), especially in fish species (Panserat et al., 2001; Martin et al., 2001; 2003; Vilhelmsson et al., 2004; Brunt et al., 2008; Murray et al., 2009; Sissener et al., 2009). In addition to understand the metabolic processes involved in the adaptive responses, these studies help to improve the animal health status and growth performances.

The present study aimed to assess changes in the protein expression profile in the liver of pikeperch larvae to address the metabolic processes at a cellular level. The liver was chosen because it is the principal site of protein, lipid and carbohydrate metabolism. We used the 2D-DIGE (two-dimensional differential in-gel electrophoresis) technique (Unlu et al., 1997) to analyze the effects of phospholipid supplementation in the diet on the liver proteome of 34 days old pikeperch larvae.

2. Materials and methods

2.1. Fish and diets

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). The larvae were acclimated in two 500 L tanks (20–22 °C) and fed from the mouth opening (day 4 post hatching, ph) *ad libitum* each hour from 8:00 to 20:00 h, with 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 (20 larvae L^{-1}). Four tanks were randomly assigned to each experimental group. Temperature and dissolved O_2 were maintained at 21–23 °C and above 6 mg L^{-1}, respectively, with water exchange of up to 100% h^{-1}.

From day 10 to day 34 ph, larvae were fed with one of three isoproteic and isolipidic microdiets (Table 1) formulated according to the patent WO0064273 and containing modified levels of soybean lecithin and cod liver oil to obtain three diets with increasing PL content: 1.5 (PL1), 4.7 (PL5), and 9.5% (PL9). The fatty acid composition of the three diets PL1, PL5 and PL9 are detailed in Table 2.

Table 1
Composition of the three experimental diets corresponding to different phospholipid (PL) contents.

	PL1	PL5	PL9
Diet ingredients (%)			
Fish meal	60	60	60
Hydrolyzed fish meal CPSP G	14	14	14
Cod liver oil	13	7	0
Soybean lecithin	0	6	13
Vitamin mixture	8	8	8
Mineral mixture	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 (DM)	96.2	95.3	90.5
Protein energy + lipid energy (kJ kg^{-1})	18.0	17.2	17.2

2.2. Sampling

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. Growth was estimated as follows: instantaneous specific growth rate (SGR, % day^{-1}) = 100(LnWf − LnWi) ΔT^{-1} where Wf and Wi = final and initial weight of larvae (mg), and T = time (days). For the proteomic analysis, 10 larvae per tank (40 per treatment) were collected on day 34 and immediately stored at −80 °C. Later, the larvae were dissected on a glass maintained at 0 °C and whole liver was extracted and immediately frozen in liquid nitrogen and kept at −80 °C until analysis. The individual liver weights were 2.3 ± 0.3, 2.8 ± 0.4 and 3.2 ± 0.5 mg, for PL1, PL5 and PL9 larvae, respectively.

Table 2
Fatty acid composition (% of total fatty acids) in the PL and 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 Mono	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 (PUFA)	28.2	40.3	28.5	14.1	24.9	10.1

Sat: saturated, Mono: monounsaturated, PUFA: polyunsaturated fatty acid.