Aquaculture 428–429 (2014) 265–271

Contents lists available at ScienceDirect

Aquaculture

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

Dietary lipid levels in meagre (*Argyrosomus regius*): Effects on biochemical and molecular indicators of liver

Efthimia Antonopoulou [a,*], Evgenia Kousidou [a,1], Evangelia Tserga [a,1], Konstantinos Feidantsis [a], Stavros Chatzifotis [b]

[a] Laboratory of Animal Physiology, Department of Zoology, School of Biology, Faculty of Science, Aristotle University of Thessaloniki, GR-54124 Thessaloniki, Greece
[b] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, PO Box 2214, GR-71003 Heraklion, Crete, Greece

ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 17 March 2014
Accepted 20 March 2014
Available online 27 March 2014

Keywords:
Meagre
Lipid diets
Hsps
MAPKs
Metabolism
Antioxidant defences
TBARS

ABSTRACT

The present study provides characterization of cellular and metabolic responses and examines the antioxidant defence in the liver of meagre (*Argyrosomus regius*) in relation to dietary lipid consumed (13%, 17% and 21%). Molecular responses were addressed through the levels of Hsp70 and Hsp90 and the phosphorylation of mitogen activated protein kinases (p38-MAPK, the extracellular signal-regulated kinases — ERKs and c-Jun N-terminal kinases — JNKs). Metabolic capacities were assessed by studying the activities of lactate dehydrogenase (L-LDH), malate dehydrogenase (MDH), citrate synthase (CS), lipase (LP) and 3-hydroxyacyl CoA dehydrogenase (HOAD). For the determination of the possible activation of antioxidant defence, the activities of the antioxidant enzymes catalase (CAT) and glutathione reductase (GR) and the levels of thiobarbituric acid reactive substances (TBARS) were studied. The results showed that the 17% and 21%-lipid diets induced Hsp70 and activated the phosphorylation of p38-MAPK, while an induction of Hsp90 was found only in fish fed the 17%-lipid diet. Phosphorylation ratio of both ERKs and JNKs were mostly observed at 17%-lipid diet. Moreover, an increase was also observed on CAT activity levels in fish of 17% diet, while both 17% and 21%-lipid diets led to elevated GR levels. The lowest activity of the metabolic enzymes MDH and HOAD was observed in fish fed with the 17%-lipid diet, while fish under the 21%-lipid diet increased their activity levels compared to 17% group. The lowest activity levels of LP were observed in fish fed with the 21%-lipid diet. L-LDH, CS and TBARS were not affected by the dietary lipid level. The obtained results can be used in order to evaluate the possible effects of the lipid diets on the hepatic physiology of this species.

1. Introduction

In recent years a considerable amount of research is devoted to nutrient requirements of meagre, since it is a new species for the Mediterranean aquaculture. In a previous study by Chatzifotis et al. (2010), notable attention was given to the effect of dietary lipid levels on the growth and feed utilization in meagre. Overall, these results indicated that the best growth performance was observed in fish fed with the 17%-lipid diet, whereas the increase of dietary lipid level from 17% to 21% had a negative effect on growth. Additionally, the 21%-lipid diet compared to 17% and 13% diets showed significantly higher values of total lipids in whole body and muscle, but not in the liver, in which no effect was detected by the dietary regime. However, for the determination of dietary requirements, apart from growth in weight indexes, secondary effects on other biological functions should also be considered. To date, information regarding the effects of dietary lipids on cellular modulation, metabolic and antioxidant enzyme activity adjustments in fish and specifically in meagre is scarce. Therefore, understanding of cellular and metabolic responses of fish fed on different lipid levels is necessary in developing strategies to provide adequate fish health and stress resistance.

In mammals, it has been observed that high doses of fish oil increases susceptibility of cellular membranes to the induction of oxidative stress and thus higher amounts of antioxidants may be required (Garrido et al., 1989). Consequently, dietary lipids in fish may have an impact on the immune system and pathogenesis, causing micronutrient imbalances (e.g. Kiron, 2012; Lall and Lewis-McCrea, 2007). Furthermore, in Chinook salmon (*Oncorhynchus tshawytscha*), dietary lipids affect stress-induced physiological changes, such as plasma glucose and cortisol levels (Barton et al., 1988).

In marine organisms, including fish, a common metric to assess the cellular response to stress is the expression of heat shock proteins (Hsps) (e.g. Hsp70); these prevent the stress induced protein unfolding which might challenge protein homeostasis (Hofmann, 2005; Tomanek, 2010; Tomanek et al., 2011; Yamashita et al., 2010). In fish, Hsps are induced by various stress factors, like heat shock, toxic metal

* Corresponding author. Tel.: +30 2310998563.
E-mail address: eantono@bio.auth.gr (E. Antonopoulou).
[1] Have equally contributed.

http://dx.doi.org/10.1016/j.aquaculture.2014.03.024
0044-8486/

contamination, hypoxia/anoxia and chemical shocks (Currie and Tufts, 1997). They are expressed in different amounts in various cells and tissues, thus serving as indicators of cellular stress and health status (Cara et al., 2005; Iwama, 1999; Iwama et al., 1998; Sanders, 1993). Their expression seems to be closely related and regulated by members of the MAPK (mitogen-activated protein kinase) family, even in teleost fishes (Feidantsis et al., 2012). Specifically, all members of the MAPK family, ERK1/2 (p44/42 MAPK), JNK and p38-MAPK, have been identified in fish (Feidantsis et al., 2009; Fujii et al., 2000; Hashimoto et al., 1997, 1998; Kultz and Avila, 2001; MacDougal et al., 1984). MAPKs act as information messenger-molecules, transferring signals from the plasmatic membrane to the inner cell, resulting to immediate gene transcription and regulation (Gupta et al., 1996). Hsps, apart from their role under various stress effects; are induced in several species under different diet treatments such as feed deprivation and dietary lipid diets. Specifically, in Fisher F344 rats the effect of dietary lipids on the expression of Hsps showed that caloric restriction reversed the age-related decline in the induction of Hsp70 transcription in hepatocytes (Heydari et al., 1993). Probably, the high content of polyunsaturated fatty acids (PUFA) of high lipid diets can induce the Hsp response, since PUFA are more susceptible to reactive oxygen species (ROS) (Ibarz et al., 2010). On the contrary, hyperlipidemia caused by high cholesterol diet inhibited heat shock response in male Wistar rats (Csont et al., 2002). In teleosts, feed deprivation induces Hsp70 and Hsp90 protein expression in sea bream (*Sparus aurata*) and rainbow trout (*Oncorhychus mykiss*) according to Cara et al. (2005). This is similar to the results found in Rohu (*Labeo rohita*) and in sea bass (*Dicentrarchus labrax*) according to Yengkokpam et al. (2008) and Antonopoulou et al. (2013), respectively. Similarly, a life-long caloric restriction caused a significant induction of Hsp90 in the soleus muscle in Fisher 344 rats (Selsby et al., 2005). Food deprivation reduces energy expenditure and thereby oxygen consumption even in fishes (Cook et al., 2000; Rios et al., 2002) resulting to oxidative stress caused by hypoxia (Pörtner, 2001, 2002; Pörtner and Farrell, 2008). Under this condition, the enhanced ROS production can explain the induction of cell protection mechanisms.

Several data obtained from different fish species grown under different stimuli (Antonopoulou et al., 2013; Deng et al., 2009; Dias et al., 2005; Feidantsis et al., 2009) showed that liver is an early stress indicator compared to other tissues. Additionally, liver secretes digestive enzymes to the intestine where digestion and nutrient absorption is completed (Eshel et al., 1993; Glass et al., 1989). For these reasons, in the present study molecular and metabolic responses of meagre to different dietary lipid contents were investigated in the liver. Moreover, since meagre is a very lean fish (Chatzifotis et al., 2010) it may exhibit low tolerance to high dietary lipid diets.

Thus, molecular responses were addressed through the induction of Hsps, the phosphorylation of MAPK family members. Apart from these molecular and signalling mechanisms, metabolic responses to the different lipid diets were assessed from aerobic versus anaerobic fuel oxidation capacity, through quantifying the activities of various metabolic enzymes Moreover, the antioxidant capacity of meagre was assessed through determining the activities of antioxidant enzymes. Finally lipid peroxidation was assessed in the liver of meagre.

2. Materials and methods

2.1. Chemicals

All biochemicals were purchased from Sigma (Darmstadt, Germany), Cell Signaling (Beverly, MA, USA) and Biorad (Hercules, CA). All other chemicals were obtained from Sigma (Darmstadt, Germany), Merck (Darmstadt, Germany) and Applichem (Gatersleben, Germany) and were of analytical grade.

2.2. Animals and liver sampling

The feeding experiment was conducted at the Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research as described thoroughly by Chatzifotis et al. (2010). Specifically, three isonitrogenous experimental diets were formulated. The total dietary protein content of all diets was 44%. However, the lipid content differed among the diets (13%, 17% and 21%) and described in Table 1. At the end of the feeding trial, five fish from each tank (15 biological replicates per experimental diet) were sacrificed and liver samples were taken. Immediately after the dissection, the liver samples were frozen in liquid nitrogen and stored at −80 °C for later analysis of a) Hsp70 and Hsp90 activation, as well as MAPK (p38-MAPK, ERKs and JNKs) phosphorylation b) activities of lactate dehydrogenase, L-LDH (E.C. 1.1.1.27.), malate dehydrogenase, MDH (E.C. 1.1.1.37), citrate synthase, CS (E.C. 4.1.3.7.), lipase, LP (E.C. 3.1.1.-), 3-hydroxyacyl CoA dehydrogenase, HOAD (E.C. 1.1.1.35), catalase, CAT (E.C. 1.11.1.6), glutathione reductase, GR (E.C. 1.8.1.7) and c) lipid peroxidation by determining the levels of thiobarbituric acid reactive substances (TBARS).

2.3. SDS-PAGE and immunoblot analysis

Frozen tissues were homogenized in 3 mL g^{-1} of cold lysis buffer (20 mM β-glycerophosphate, 50 mM NaF, 2 mM EDTA, 20 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 0.2 mM Na_3VO_4, 10 mM benzamidine, pH 7, containing 200 μM leupeptin, 10 μM trans-epoxy succinyl-L-leucylamido-(4-guanidino) butane, 5 mM DTT (dithiothreitol), 300 μM phenyl methyl sulfonyl fluoride (PMSF) and 1% v/v Triton X-100), and extracted on ice for 30 min. Samples were centrifuged (10,000 *g*, 10 min, 4 °C) and the supernatants were boiled with 0.33 volumes of SDS/PAGE sample buffer (330 mM Tris–HCl, 13% v/v glycerol, 133 mM DTT, 10% w/v SDS (Sodium Dodecyl Sulfate), 0.2% w/v bromophenol blue). Protein concentration was determined using the BioRad protein assay.

Equivalent amounts of protein (50 μg) were separated on 10% (w/v) acrylamide, 0.275% (w/v) bisacrylamide slab gels and transferred electrophoretically onto nitrocellulose membranes (0.45 μm, Schleicher

Table 1
Ingredients and calculated chemical composition (% dry weight) of the experimental diets (13, 17 and 21% crude lipid content per diet).
Based on Chatzifotis et al. (2010).

Ingredients	Dietary lipid content		
	13%	17%	21%
Fish meal	34.5	34.5	34.5
Fish oil	7.9	11.9	15.9
Soya meal (43% protein)	13.5	14.5	15.5
Gluten meal	16.5	16.5	16.5
Wheat	23.4	18.4	13.4
Soyabean oil	1.75	1.75	1.75
Vitamins and trace minerals[a]	2.45	2.45	2.45
Proximate composition			
Crude protein	43.2	43.1	43.4
Crude lipid	12.9	17.3	23.1
Ash	8.3	8.1	8
Moisture	8.7	8	8.4
Carbohydrate[b]	26.9	23.5	17.1
Gross energy (kJ g^{-1})[c]	19.9	21.1	22.3

[a] The mixture supplied (mg kg^{-1} diet) vitamin A: 18.000 IU, vitamin D3: 2.500 IU, vitamin E: 250 mg, vitamin K: 10 mg, vitamin B1: 25 mg, vitamin B2: 30 mg, vitamin B12: 0.100 mg, vitamin B6: 10 mg, vitamin C (as 2-monophospate): 62.500 mg, iodine (as calcium iodate): 4 mg, selinium (as sodium selenite): 0.150 mg, iron: 45 mg, BHT: 50 mg, folic acid: 3.250 mg, biotin: 0.300 mg, nicotinic acid: 230 mg, pantothenic acid: 60 mg, cobalt (as cobalt carbonate): 0.325 mg, zinc (ass zinc sulphate): 60 mg, manganese (as manganese oxide): 35 mg, inositol: 150.000 mg.
[b] Carbohydrates% = 100 − (crude protein% + crude lipid% + moisture% + ash%).
[c] Energy (kJ/g diet) = (%crude protein × 23.6) + (%crude lipids × 39.5) + (%carbohydrates × 17.3).