Feeding and nutrition of pikeperch Sander lucioperca during early life stages – state of knowledge and perspectives

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Intensive culture of pikeperch Sander lucioperca has reached the commercial stage in several European countries, but reliable production of high quality fry still remains a major bottleneck. Although being a freshwater fish species, from a nutritional perspective, pikeperch in many ways resembles marine species. Based on histology and digestive enzyme activity, the development and functionality of pikeperch digestive system during early ontogeny have been finely described, providing evidence of a sequential chronology in its maturation.. The structure and functionality of the digestive tract are largely affected by dietary treatments, as evidenced by a retarded development of gastric glands in pikeperch larvae fed casein hydrolysates or the earlier maturation of the digestive structures and higher specific activities of brush border enzymes in larvae fed diets with high phospholipid (PL) levels. A recent study testing higher PL levels together with various levels of n-3 LC PUFAs confirmed the synergistic role of dietary PL and n-3 LC PUFAs in pikeperch larvae for growth, digestive enzymatic activity and skeleton morphogenesis. Separately or in interaction with PL and n-3 LC PUFA levels, other nutrients can influence the performance of pikeperch larval rearing. A fractional factorial experimental design including 8 nutritional variables (vitamins A, C, D, E; EPA + DHA, ARA, Ca/P ratio, Se) was conducted to identify the most influencing nutrients and their interactions, using digestive enzymatic activity, histology of the digestive tract and skeletal anomalies as endpoints. Results highlighted the major role of Ca/P and EPA+DHA/ARA ratios as well as their interactions with vitamin C during the early post-weaning period. Other variables may affect the success of pikeperch larval rearing. A multifactorial study investigated the optimal feeding strategy during the whole nursery period, by testing the relative influence of weaning time and duration, co-feeding and diet. The results suggested that a later onset and a longer duration of weaning improve larval survival and growth and reduce deformities in pikeperch larvae. Significant progress has been achieved so far regarding pikeperch feeding and nutrition during early life stages, but most results are largely affected by a huge cannibalism, which must be better controlled in the future.

Effect of increasing dietary levels of n-3 long-chain polyunsaturated fatty acids on liver composition and histopathology of meagre (Argyrosomus regius, Asso 1801) fingerlings

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Meagre (Argyrosomus regius) is a promising new aquaculture species, with great potential due to its high growth rate, feed efficiency and easy adaptation to captivity. Essential fatty acids (EFA) are required to sustain growth, development, immune status and survival (Watanabe, 1982; Izquierdo, 2005). EFA requirement of meagre fingerlings were previously estimated (Carvalho et al., 2018). A feeding trial was performed testing the effect of 5 increasing dietary n-3 LC-PUFA levels, below and above the requirement levels (0.8, 1.4, 2.0, 2.6 and 3.6% DM), on liver biochemical and FA composition and histopathology of meagre fingerlings.

Fingerlings with an initial body weight of 2.80 g ±0.23 (mean±SE) were allocated in 15 tanks of 200L supplied with filtered seawater. Temperature along the trial was 23ºC± 0.2 (mean±SE). Fish were fed the experimental diets 3 times a day until apparent satiety during 30 days. At the end of the trial, liver samples were collected for biochemical analysis and histological and bacteriological studies.

Fish fed the two lowest n-3 LC-PUFA diets (0.8 and 1.4%) showed a higher hepatosomatic index as well as a higher hepatic lipid infiltration. Consequently, these fish showed a more severe steatosis than fish fed 2.0-3.6% n-3 LC-PUFA. FA composition of liver total lipids reflected the dietary composition with the increase of dietary n-3 LC-PUFA increasing SFA, n-3 LC-PUFA (EPA, DHA) and n-6 LC-PUFA (ARA), and decreasing MUFA, LA and LNA. However, meagre FA composition of liver polar lipids was less affected by the diets, n-3 LC-PUFA being preserved. Furthermore, the lowest dietary content in n-3 LC-PUFA (0.8%) also led to a higher incidence of hepatic granulomas. The infectious origin of granulomas was discarded by the absence of symptoms of bacteria, parasites or fungi in visual observations, bacteriological cultures, or specific histological techniques. These results suggest that EFA-deficiency led to a higher incidence of granulomatosis in meagre. Therefore, a minimum of 2.0% to DM n-3 LC-PUFA is required to maintain the EFA profile of hepatic total lipids and normal histomorphology of liver, corroborating the requirement based on growth.

Requirements of omega-3 fatty acids in Atlantic salmon. Effects of graded feed additions of very long chain n-3 PUFAs on the fatty acid composition of intestinal phospholipid species.

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The intestines are at the same time an important barrier to the environment and the first organ to meet the different compounds in the feed. The aim of this study was to investigate how the different mid-intestinal phospholipid species in salmon changed their fatty acid composition with no or low levels of EPA and /or DHA in the feed. Atlantic salmon were kept in indoor seawater tanks and fed a commercial diet or experimental diets containing low levels of EPA or DHA or a mixture of EPA and DHA (1:1). The amount of the very long chain n-3 added varied from 0% to 2% in the diet, and the experiment lasted for 26 weeks, the salmon growing from about 50 to 400 grams. The percentages of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) were not affected by the different diets, while the fatty acid composition was markedly influenced. The fish fed the 0% diet had high levels of the n-6 fatty acids 18:2, 20:3 and 20:4. Upon gradually increasing percentages of EPA and/or DNA in the diet, the n-6 levels decreased, most markedly for 20:3. The high level of 20:4 n-6 in PI did, however, hardly change. When adding only EPA to the diet significant amounts of DHA were incorporated in the PLs, indicating a significant production of DHA from EPA. With increasing percentages of EPA and/or DHA the incorporation of DHA levelled off, even when only DHA was added.

Creeping up to freshwater: complementation of the DHA biosynthetic pathway in the lineage of freshwater sole (Pleuronectiformes: Achiridae)

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Marine teleosts are generally unable to synthesize docosahexaenoic acid (DHA; 22:6n-3) from α-linolenic acid (ALA; 18:3n-3) because they lack some fatty acid-metabolizing enzymes required in catalyzing the DHA biosynthetic pathway. Therefore, the marine teleosts require to uptake DHA concentrated in a marine diet. However, a lineage of Achiridae, including “freshwater soles,” expanded its habitat from marine to freshwater, which is very poor in DHA. To elucidate this nutritionally paradoxical migration, we examined the DHA biosynthetic pathways of the North American naked sole <i>Gymnachirus melas</i>, which is an ancestral marine Achiridae, and the freshwater sole <i>Hypoclinemus mentalis</i>, which is found only in freshwater even to the upper Amazon.

First, we performed metabolizing assay using short-term <i>in vitro</i> culture of brain and liver cells with radiolabeled ALA and docosapentaenoic acid (22:5n-3) as substrates. Next, we isolated desaturase and elongase genes and analyzed their functions by heterologous expression in yeast. Finally, we analyzed genomic loci of desaturase genes by long PCR.

There was no signal indicating the occurrence of DHA biosynthesis in any cell type of <i>G. melas</i> cultured with radiolabeled substrates. Its desaturase was deficient in Δ5 and Δ4 activities, which is seen in general marine teleost, whereas its elongase could convert C18–22 substrates. On the other hand, strong signals of DHA along with intermediate metabolites were markedly detected in the liver cell of <i>H. mentalis</i> cultured with ALA. From <i>H. mentalis</i>, we isolated two desaturase genes sharing 98% homology in amino acid sequences, one showing Δ5Δ6 activity and the other showing Δ4Δ5 activity. Notably, two desaturases catalyzed Δ6 desaturation of tetracosapentaenoic acid (24:5n-3). In addition, its elongase could convert C18–22 substrates. Therefore, the DHA synthesis of <i>H. mentalis</i> could be achieved through both Δ4 and Sprecher pathway by these three enzymes. Furthermore, long PCR and sequencing of its products revealed that the two desaturase genes were tandemly repeated. Our results suggest that the DHA biosynthetic pathway of <i>H. mentalis</i> is complemented by neofunctionalized paralogous desaturase generated by gene duplication and that DHA biosynthesis may be a limiting factor in expanding the habitat from marine to freshwater.

The hepatotoxicity of palmitic acid in zebrafish involves the intestinal microbiota

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Introduction: Palmitic acid (PA) is the main saturated fatty acid naturally occurring in animal fats and vegetable oil. Palm oil, an alternative lipid source containing high level of PA, has been widely used to replace fish oil in aquafeeds in recent decades.

Objective: We investigated the hepatotoxicity of PA in zebrafish and the underlying mechanism.

Methods: Liver PA level was compared between 1-month zebrafish fed a high fat diet (HFD) and 3 PA-incorporated HFDs (4%, 8% and 12% PA), namely 4PA, 8PA and 12PA diets, for 2 weeks. Further, Hepatic expression of endoplasmic reticulum (ER) stress markers (Grp78, Chop) and caspase-12 was evaluated in 1-month zebrafish fed a low fat diet (LFD), HFD, 4PA, 8PA and 12PA diets for 4 weeks. Caspase-12 activity was also assessed in germ-free (GF) zebrafish fed LF, HF and 12PA diets for 5 days. Moreover, caspase-12 was examined in GF-zebrafish colonized with HF or 12PA microbiota for 48h. Lastly, PA absorption was evaluated in GF-zebrafish colonized with HF or12PA microbiota.

Results: The proportion of PA in liver was linearly increased as its percentage in dietary lipid increased. The expression of (Grp78, Chop) was higher in 12PA group (2.2 and 2.7-fold, respectively, $P < 0.05$) than HF group. The activity of caspase-12 was increased by 61.1% in the 12PA versus HF group ($P < 0.05$). In GF zebrafish, caspase-12 activity was also higher in 12PA group compared with HF group ($P < 0.05$). Furthermore, GF-zebrafish colonized with PA-altered microbiota showed higher caspase-12 activity ($P < 0.05$) than those colonized by HFD microbiota. Lastly, the PA-altered microbiota promoted PA absorption ($P < 0.05$) and aggravated ER stress and liver damage in the context of high PA feeding.

Conclusions: Excessive dietary PA induces hepatotoxicity in zebrafish, which involves ER stress-mediated apoptosis. Besides the direct effect of PA accumulation in liver, the intestinal microbiota also contributes to the hepotatoxicity of PA, which involves two aspects: (i) the PA altered microbiota can directly induce ER stress and liver damage; (ii) the PA microbiota can promote the absorption of PA, leading to enhanced PA overflow to the liver and deteriorated lipotoxicity.

PERFORMANCE, FEED UTILIZATION AND HEPATIC MOLECULAR METABOLIC RESPONSE OF WEANED JUVENILE ATLANTIC BLUEFIN TUNA (Thunnus thynnus, L): EFFECT OF LIPID LEVEL AND SOURCE

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The development of formulated diets and feeds is essential to increase production of farmed tuna species. There is limited knowledge of this topic, mainly on Pacific Bluefin tuna (Thunnus orientalis) in Japan, whereas no major attempts have been made with Atlantic Bluefin tuna (Thunnus thynnus; ABT). In the present study, two trials were performed using inert formulated diets as on-growing feeds for weaned ABT juvenile in order to establish adequate dietary levels of both lipid and omega-3 long-chain polyunsaturated fatty acids (LC-PUFA). In a first trial, ABT (initial weight = 2.9±0.9g) were fed for 10 days with either a commercial (Magokoro®, MGK) or two experimental feeds with two different lipid levels (15 or 20%) using krill oil (KO) as the single lipid source in order to estimate the suitable lipid content. Fish fed MGK displayed the highest growth, followed by 15KO, with no differences in fish survival. Thus, a lipid content of 15% was considered better than 20% for ABT juveniles. In the second trial, fish (initial weight = 3.3 ± 0.6g) were fed either MGK, 15KO or a feed containing 15% lipid with a combination (1:1, v/v) KO and rapeseed oil (RO) (15KORO). Fish fed 15KO and 15KORO showed the highest growth in terms of weight and fork length (including weight gain and SGR). Increasing dietary lipid level or adding RO to the feeds did not increase liver lipid content. The liver fatty acid profile largely reflected dietary intake confirming very limited LC-PUFA biosynthetic activity for this teleost species. In this respect, liver of fish fed 15KO and 20KO displayed the highest contents of docosahexaenoic acid (DHA). The hepatic expression of genes of lipid and fatty acid metabolism, transcription factors, and antioxidant enzymes was investigated with many of the genes showing regulation by both dietary lipid and LC-PUFA contents. The present study showed promising results that suggested ABT juveniles can be on grown on inert dry feeds that supported good fish growth and the accumulation of the health-promoting fatty acid DHA. Further studies are required in order to fully elucidate lipid and fatty acid requirements of this iconic species