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IMPORTANCE OF DIETARY PHOSPHOGLYCERIDES AND HUFA LEVELS FOR PIKEPERCH (*SANDER LUCERIOPERCA*) LARVAL DEVELOPMENT AND THEIR IN VIVO CAPABILITY TO METABOLIZE UNSATURATED FATTY ACIDS

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Pikeperch (*Sander lucioperca*) is a strong potential freshwater candidate for diversification of aquaculture farming in Europe. Though farming of this species takes place in freshwater, pikeperch have shown a high level of osmotic tolerance and degree of hypo-osmoregulatory capacity in a saline environment, which may suggest a growth potential for rearing in low saline waters or in water iso-osmotic to plasma. Studies have indicated that pikeperch larvae resemble most marine carnivorous fish larvae with a requirement for both phospholipids and HUFAs. This may stem from the evolutionary marine origin of the species and indicates a low FA elongation/desaturation capacity. Thus, diets low in HUFAs, especially DHA, during initial larval development have caused increased mortality, shock syndromes, and short- and long-term stress sensitivity and deficiency in neural development, which may affect swimming behavior and learning. Salinity may have variable positive effects on modulation and expression of Δ6-desaturase activity and fatty acid composition when exposed to low saline levels as shown in various freshwater fishes.

In two studies, we examined the importance of dietary phosphoglyceride levels (3-12% inclusion of soy lecithin) and possible additional effect of supplementation of HUFAs (DHA and EPA) until 30 day post hatch (dph), as well as the capability of pikeperch larvae to incorporate and metabolise in vivo unsaturated fatty acids (FA; i.e. using direct incubation, with 0.2µCi (0.3µM) of [1-14C] PUFAs, for 5h) when reared under various salinities (0, 5, 10ppt) and fed *Artemia* enriched with emulsions of different dietary FA origin (18:2n-6/18:3n-3 ratio). The studies were carried out in triplicate with three tanks per treatment. Studies included analyses on performance, deformities, hormonal prostaglandin

levels, proteomic and enzymatic development, stress resilience, respiration, and swimming behaviour.

Results revealed that dietary composition of phosphoglycerides was reflected in larval composition only to a certain extent, while FA and HUFA levels were. Supplementation of the highest dietary level of phospholipids (12% dietary inclusion) significantly improved growth performance, reduced deformities (especially cranium anomalies), and caused a higher alkaline phosphatase activity and lower trypsin activity, most likely related to differences in growth rate and ontogenetic development. As the liver is the major metabolic reactor of the body involved in protein and lipid metabolism, it was selected as site for protein gene expression. Results on changes in proteomic expression in liver underpinned results on growth and enzymatic activity and the larval expression of various proteins was in general down-regulated by high dietary PL/n-3 HUFA content. No differences were observed regarding larval respiration, stress resilience, or escape responses, but diets with the lowest inclusion of phospholipids and without HUFAs were not included in these tests due to markedly lower growth. Results on the salinity experiment showed no effect on salinity or dietary n-3/n-6 PUFA enrichment on growth performance. Incorporation of all the [1-14C] PUFA substrates, points to the suitability of this methodology to study in vivo lipid metabolism of fish larvae. The metabolism of FAs indicated that regardless of the environmental salinity or dietary regime pikeperch possess a marked specificity to incorporate and esterify FA into phospholipids. [1-14C]EPA was the most efficiently incorporated into total lipids (pmol.mg $prot^{-1}$.h), followed by [1-14C]ARA) and by [1-14C]DHA, [1-14C]C18:2n-6 and [1−14C]18:3n-3, which were the least and also similarly incorporated substrates preferably esterified into phosphatidylcholine. In contrast, [1-14C]ARA and [1-14C]EPA were mainly incorporated into phosphatidylinositol and phosphatidylethanolamine, respectively. Despite the results above, the majority of radioactivity (72-93%) was recovered as unmodified FAs with Elovls directly producing elongation/desaturation intermediate n-3, n-6 FAs. Δ6 enzyme activity was consistent with the significant production of 18:3n-6 from 18:2n-6 and 18:4n-3 from 18:3n-3, however no further desaturation from these products or any desaturation activity over EPA was evident, precluding the expression of a Δ5 or even a Δ4 in the larvae. The results provided compelling evidence to confirm that HUFA are essential nutrients, that must be supplied in diets of pikeperch larvae to guarantee survival and normal development

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