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Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*)

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Abstract

Highly unsaturated fatty acid (HUFA) synthesis in Atlantic salmon (*Salmo salar*) was known to be influenced by both nutritional and environmental factors. Here we aimed to test the hypothesis that both these effectors involved similar molecular mechanisms. Thus, HUFA biosynthetic activity and the expression of fatty acyl desaturase and elongase genes were determined at various points during an entire 2 year production cycle in salmon fed diets containing either 100% fish oil or diets in which a high proportion (75% and 100%) of fish oil was replaced by C_{18} polyunsaturated fatty acid-rich vegetable oil. The results showed that HUFA biosynthesis in Atlantic salmon varied during the growth cycle with peak activity around seawater transfer and subsequent low activities in seawater. Consistent with this, the gene expression of Δ6 desaturase, the rate-limiting step in the HUFA biosynthetic pathway, was highest around the point of seawater transfer and lowest during the seawater phase. In addition, the expression of both Δ6 and Δ5 desaturase genes was generally higher in fish fed the vegetable oil-substituted diets compared to fish fed fish oil, particularly in the seawater phase. Again, generally consistent with this, the activity of the HUFA biosynthetic pathway was invariably higher in fish fed diets in which fish oil was substituted by vegetable oil compared to fish fed only fish oil. In conclusion, these studies showed that both nutritional and environmental modulation of HUFA biosynthesis in Atlantic salmon involved the regulation of fatty acid desaturase gene expression.

Keywords: Polyunsaturated fatty acid; Gene; Desaturase; Elongase; Nutrition; Salmon

1. Introduction

Fish are the only major dietary source of n-3 highly unsaturated fatty acids (HUFA) for humans [1] and, with declining fisheries, aquaculture supplies an increasing proportion of the fish in the human diet [2–4]. However, the current high use of fish oils, derived from marine feed-grade fisheries, in aquaculture feeds is not sustainable in the longer term, and will constrain continued growth of aquaculture activities [5]. The only sustainable alternative to fish oils are vegetable oils, which can be rich in C_{18} polyunsaturated fatty acids (PUFA) such as linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, but devoid of the n-3HUFA, eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids that are abundant in fish oils [6]. The extent to which fish can convert C_{18} PUFA to $C_{20/22}$ HUFA varies with species, and is associated with their capacity for fatty acyl desaturation and elongation [7]. In this context, it is essential to determine what regulates HUFA biosynthesis, and how it can be

Abbreviations: FO, fish oil; HUFA, highly unsaturated fatty acids (carbon chain length $\geq C_{20}$ with ≥3 double bonds); VO, vegetable oil

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optimised to enable fish to make effective use of dietary vegetable oil.

Recently, a variety of fatty acid desaturases and elongases, critical enzymes in the pathways for the biosynthesis of the long-chain $C_{20/22}$ HUFA from shorter chain C_{18} PUFA, have been cloned from a range of freshwater and marine teleosts [8–10]. In particular, three cDNAs encoding enzymes in the HUFA biosynthetic pathway have been cloned from Atlantic salmon [11,12]. Heterologous expression in the yeast *Saccharomyces cerevisiae* showed that the cDNAs encoded a Δ6 desaturase responsible for the conversion of 18:3n-3 to 18:4n-3 [12], a Δ5 desaturase responsible for the conversion of 20:4n-3 to 20:5n-3 [11] and a PUFA elongase with high 18:4n-3 to 20:4n-3 activity, but also capable of elongating 20:5n-3 to 22:5n-3 and further to 24:5n-3 [11].

Several studies have shown that the activity of the HUFA biosynthesis pathway in Atlantic salmon was increased in fish in which dietary fish oil was replaced with vegetable oils [13–16]. The underlying cause of the increase in activity in fish fed vegetable oil was unclear, but thought to be mainly due to a reduction in the suppression of enzyme activity by n-3HUFA abundant in fish oil [17]. The precise mechanism of the suppression of activity was not known but, subsequently, the expressions of both desaturase and elongase genes were demonstrated to be under nutritional regulation in salmon, being up regulated in a graded manner in the livers of fish fed diets in which graded increments of 18:3n-3-rich linseed oil replaced fish oil [18]. HUFA biosynthetic activity has also been shown to be under the environmental regulation in Atlantic salmon, being increased during the period of parr–smolt transformation with peak activities around seawater transfer [19,20]. Although it is known that smoltification is largely controlled by photoperiod with water temperature playing a secondary role, the mechanisms whereby these environmental triggers could regulate fatty acid metabolism are unknown [20]. However, feeding fish oil during the period of parr–smolt transformation was shown to attenuate the pre-adaptive rise in HUFA biosynthetic activity and reduce the peak activity at seawater transfer in comparison to fish fed vegetable oil [19,20], suggesting that similar biochemical or molecular mechanisms may be involved in both the nutritional and environmental regulation of HUFA biosynthesis in Atlantic salmon.

In the present study we aimed to test the hypothesis that both the nutritional and environmental regulation of HUFA biosynthesis in Atlantic salmon involved a similar molecular mechanism, specifically changes in the expression of key genes in the biosynthetic pathway. Thus, HUFA biosynthetic activity and the expression of fatty acyl desaturase and elongase genes were determined at various points during an entire 2 year production cycle in Atlantic salmon. In addition, the effects of diet on the natural cycle of activity was investigated by feeding the fish diets containing either 100% fish oil or diets in which a high proportion (75% and 100%) of the fish oil was replaced by a vegetable oil blend, formulated to mimic fish oil in saturated and monounsaturated fatty acid content, but with C_{18} PUFA replacing the n-3HUFA.

2. Materials and methods

2.1. Animals and diets

The effect of replacing fish oil with vegetable oil at two replacement levels (75 and 100%) was investigated in Atlantic salmon in a trial conducted over an entire 2 year production cycle. As the trial was both large scale and long term, it was carried out as a collaboration between the Institute of Aquaculture, University of Stirling, Scotland, and the National Institute of Nutrition and Seafood Research, Bergen, Norway, with the 75% replacement diet in Scotland and the 100% replacement tested in Norway, with the control FO diet replicated at each site. The diets were fed to triplicate tanks/cages and the experiments were performed using identical culture conditions other than the obvious environmental differences such as the slight differences in ambient water temperature at the two sites. In Scotland, the trial was carried out at Marine Harvest Ltd. facilities at Invergarry (freshwater) and Loch Duich, Lochalsh (seawater), and in Norway, the entire trial was conducted at the Nutreco Aquaculture Research Centre, Lerang Research Station, Stavanger. At each site, Atlantic salmon fry were distributed randomly into 6 tanks (3 m×3 m, depth 0.5 m) at a stocking level of 3000/tank, and weaned onto extruded feeds containing 20% added oil which was either fish oil (FO; capelin oil) or a vegetable oil blend (VO), containing rapeseed, palm and linseed oils in a 3.7:2:1 ratio, replacing 75% or 100% of the FO. Fish were fed the diets described above for around 1 year until sea water transfer, at which point the fish (average weight ~50 g) were transferred into 5 m×5 m net pens at 700 fish/pen. The fish were fed the same diet in seawater as in freshwater although the dietary oil levels were increased to 25% (3 mm pellet) rising to 32% (9 mm pellets) through the year long seawater phase. The diets aimed to be practical, and were formulated and manufactured by Nutreco ARC, Stavanger, Norway, according to current practices in the salmon feed industry. All diets were formulated to satisfy the nutritional requirements of salmonid fish [21]. The measured proximate and fatty acid compositions of the diets are given in Table 1. The only significant difference in final weights was that the fish fed 100% VO (2.69±0.07 kg) were slightly larger than the fish fed the FO diet (Norway) (2.36±0.13 kg) and 75% VO (2.37±0.13 kg). The final weight of the fish fed the FO diet in Scotland was not different to any other treatment (2.54±0.14 kg).