Biochimica et Biophysica Acta 1861 (2016) 227–238

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Assessment of a land-locked Atlantic salmon (*Salmo salar* L.) population as a potential genetic resource with a focus on long-chain polyunsaturated fatty acid biosynthesis

M.B. Betancor [a,*], R.E. Olsen [b,c], D. Solstorm [b], O.F. Skulstad [b], D.R. Tocher [a]

[a] Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK
[b] Institute of Marine Research, Matre 5984, Matredal, Norway
[c] Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway

ARTICLE INFO

Article history:
Received 23 September 2015
Received in revised form 15 December 2015
Accepted 18 December 2015
Available online 28 December 2015

Keywords:
Omega−3 long chain polyunsaturated fatty acids
Atlantic salmon
Land-locked
Desaturases
Elongases

ABSTRACT

The natural food for Atlantic salmon (*Salmo salar*) in freshwater has relatively lower levels of omega−3 (n−3) long-chain polyunsaturated fatty acids (LC-PUFA) than found in prey for post-smolt salmon in seawater. Land-locked salmon such as the Gullspång population feed exclusively on freshwater type lipids during its entire life cycle, a successful adaptation derived from divergent evolution. Studying land-locked populations may provide insights into the molecular and genetic control mechanisms that determine and regulate n−3 LC-PUFA biosynthesis and retention in Atlantic salmon. A two factorial study was performed comparing land-locked and farmed salmon parr fed diets formulated with fish or rapeseed oil for 8 weeks. The land-locked parr had higher capacity to synthesise n−3 LC-PUFA as indicated by higher expression and activity of desaturase and elongase enzymes. The data suggested that the land-locked salmon had reduced sensitivity to dietary fatty acid composition and that dietary docosahexaenoic acid (DHA) did not appear to suppress expression of LC-PUFA biosynthetic genes or activity of the biosynthesis pathway, probably an evolutionary adaptation to a natural diet lower in DHA. Increased biosynthetic activity did not translate to enhanced n−3 LC-PUFA contents in the flesh and diet was the only factor affecting this parameter. Additionally, high lipogenic and glycolytic potentials were found in land-locked salmon, together with decreased lipolysis which in turn could indicate increased use of carbohydrates as an energy source and a sparing of lipid.

1. Introduction

Omega 3 (n−3) long chain polyunsaturated fatty acids (LC-PUFA), eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3), are essential dietary nutrients with well-known health benefits in humans [1]. Fish and seafood are the main dietary source of n−3 LC-PUFA for humans, with aquaculture providing close to half (47%) of all the products on the market [2], although almost all commercially available Atlantic salmon (*Salmo salar* L.) is farmed. Feeds for farmed fish have traditionally relied on the use of fishmeal and fish oil derived from marine fisheries as the main protein and lipid sources. The resulting demand for marine resources is such that requirements for aquaculture feeds now exceed global supplies of fish oil [3]. Continued expansion of aquaculture to supply the global demand for fish is only possible by replacing fish oil and, currently the main sustainable alternatives are vegetable oils, which can be rich in C_{18} PUFA such as linoleic (LNA; 18:2n−6) and α-linolenic (ALA; 18:3n−3) acids, but devoid of EPA and DHA [4]. Thus, fish fed diets formulated with vegetable oil are characterised by increased levels of C_{18} PUFA and decreased levels of n−3 LC-PUFA compared to fish fed fish oil, reducing their nutritional value to human consumers [5]. Therefore, flesh n−3 LC-PUFA content and composition is a key trait determining the nutritional quality of farmed salmon [6].

A recent study comparing 50 families from a salmon breeding programme fed vegetable oil showed high variability in flesh n−3 LC-PUFA levels, with the trait showing a high level of heritability estimated at $h^2 = 0.77$ [7], indicating that the trait is affected by environment to only a very limited degree and that differences observed between populations have most likely a genetic origin. This demonstrated the potential of selective breeding to improve this trait, and has prompted a focus on the genetic architecture of LC-PUFA biosynthesis and metabolism in salmon. Recent studies have indicated that tissue fatty acid compositions are determined by the relative activities of a range of metabolic pathways including transport and uptake, oxidation and deposition, and endogenous biosynthesis [8–11]. However, precisely what the most critical pathways and genes in determining tissue LC-PUFA compositions are, and how diet interacts with and affects these molecular pathways are poorly understood in all animals, including salmon.

* Corresponding author.
E-mail address: m.b.betancor@stir.ac.uk (M.B. Betancor).

http://dx.doi.org/10.1016/j.bbalip.2015.12.015
1388-1981/

Atlantic salmon start life in freshwater followed by smoltification and migration to the sea before they return to their native river to spawn [12]. However, after the last glacial period, some Atlantic salmon stocks were isolated from the sea in both North America and Europe and these land-locked populations complete their life-cycle in freshwater, spending their adult life mainly in large lakes instead of the sea. This means that land-locked salmon are exposed to lower levels of EPA and DHA, during their lifespan than their anadromous counterparts, which asks the question of whether land-locked populations may have potentially higher capacity for LC-PUFA biosynthesis than anadromous populations [5,13]. In an earlier study, the fatty acid compositions of land-locked and farmed Atlantic salmon fed diets formulated with vegetable oils were compared [14]. The authors interpreted the data to suggest that land-locked salmon may have a higher conversion capacity for n−3 and n−6 PUFA than anadromous counterparts, and that there was a genetic influence on DHA content in phospholipids [14]. This suggested that these wild populations could provide a highly valuable genetic resource for enhancing farmed Atlantic salmon stocks but, as few studies have assessed farmed and land-locked salmon populations in detail, they represent an under-exploited resource.

In this context, the overarching aim of this work was to characterise the n−3 LC-PUFA trait in land-locked salmon and to determine the molecular and biochemical basis for any differences observed in the trait between farmed and land-locked Atlantic salmon populations. To achieve this, a 2 × 2 factorial nutritional trial employing two feeds containing fish oil or vegetable oil as the added lipid was tested over 8 weeks in two populations of Atlantic salmon, the Norwegian national farmed stock of the Aquagen strain (F) and the Swedish Gullspång land-locked stock (W). Effects of dietary fatty acid composition on gene expression in liver by microarray (transcriptome) analysis, hepatocyte fatty n−3 LC-PUFA biosynthesis activities by incubation with radiolabelled 18:3n−3, and resultant muscle (flesh) fatty acid compositions were determined.

Table 1
Formulation (percentage of the feed ingredients indicated), proximate and fatty acid compositions (percentage of fatty acids) of the experimental feeds.

	FO	RO
Fish meal	33.5	33.5
Soy protein concentrate	20.0	20.0
Fish oil	17.0	–
Rapeseed oil	–	17.0
Wheat	10.0	10.0
Wheat gluten	15.0	15.0
Vitamin mix	2.0	2.0
Mineral mix	0.5	0.5
Monosodium phosphate	2.0	2.0
Analysed composition		
Dry matter (%)	91.1	91.3
Protein (%)	50.0	49.4
Fat (%)	15.1	15.2
Ash	8.1	8.0
Fatty acid composition (%)		
□ *saturated*[a]	22.4	14.2
□ *monounsaturated*[b]	45.8	53.3
18:2n−6	7.0	16.6
20:2n−6	0.2	0.1
20:3n−6	0.0	0.1
20:4n−6	0.4	0.1
□ *n−6 PUFA*[c]	7.9	17.1
18:3n−3	1.6	6.0
20:3n−3	0.15	0.05
20:4n−3	0.5	0.2
20:5n−3	7.0	2.9
22:5n−3	0.7	0.3
22:6n−3	9.6	4.6
□ *n−3 PUFA*	22.6	15.1
□ *PUFA*[d]	31.7	32.5
Total n−3 LC-PUFA	17.3	7.8

Fish and FO, fish oil and respective feed; LC-PUFA, long-chain polyunsaturated fatty acid (sum of 20:4n−3, 20:5n−3 22:5n−3 and 22:6n−3); n.d. not detected.
[a] Contains 15:0, 22:0 and 24:0.
[b] Contains 16:1n−9, 20:1n−11, 20:1n−7, 22:1n−9 and 24:1n−9.
[c] Contains 22:4n−6 and 22:5n−6.
[d] Contains C16 PUFA.

2. Materials and methods

2.1. Ethics statement

All procedures and protocols were performed in accordance with Local (Institute of Marine Research), Norwegian national, and European (EU) Regulations for the use of animals in scientific experimentation (Directive 2010/63/EU).

2.2. Dietary trial and sampling

Eggs were obtained from a land-locked Atlantic salmon population (Lake Vanern/Gullspång Swedish stock) and incubated and hatched in April 2013 at the facilities of the Institute of Marine Research (IMR) (Matre, Norway). Two isonitrogenous and isoenergetic feeds, formulated to satisfy the nutritional requirements of salmonid fish [15], were manufactured by the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima Fôrteknologisenteret, Fana, Norway) by vacuum coating identical dry basal extruded pellets with either fish oil (FO) or rapeseed oil (RO) and were named according to the oils used (Table 1). The dietary fatty acid profiles reflected the oil source with the FO diet containing high levels of LC-PUFA (17.3% vs. 7.8% in RO), whereas the levels of 18:3n−3 were higher in the RO diet than in the FO diet (6.0% vs. 1.0%) (Table 1). A classic factorial 2 trial was performed comparing land-locked (termed W) and farmed (termed F, Aquagen strain) parr fed the two diets to produce four experimental treatments (WFO, WRO, FFO and FRO).

Twelve 400 l tanks (1 × 1 m) were stocked with 50 parr (initial weight ~25 g) with 6 tanks of each population and the two experimental feeds were fed to triplicate tanks for 8 weeks until at least a doubling of weight (~60 g). At the end of the feeding trial, twelve fish per treatment (4 fish per replicate tank) were sampled and flesh (white muscle) collected in liquid nitrogen. In addition, liver of 12 fish was removed and divided into two portions. One portion (~100 mg) was stabilised in RNALater® for subsequent gene expression analyses, whereas the remaining portion was used to determine the hepatocyte fatty acyl desaturation and elongation activities. All fish were weighed at the end of the experimental period and specific growth rate (SGR) calculated as follows: SGR = 100 ∗ (lnWf − lnWo) / t, where Wo = initial weight (g) and Wf = final weight (g) at time t (days).

2.3. Determination of hepatocyte fatty acyl desaturation/elongation activities

Each pool (per tank) of liver was chopped, incubated with 1% collagenase, and dissociated cells sieved through 100 μm nylon gauze and isolated as described in detail previously [16]. 100 μl of each cell preparation was taken for protein determination [17] following incubation with 1 M NaOH/0.25% (w/v) SDS for 1 h at 60 °C. 5 ml of each cell preparation was dispensed into 25 cm^2 tissue culture flasks and incubated at 18 °C for 1 h with 0.25 μCi (final fatty acid concentration, 2 μM) of [1-^{14}C]18:3n−3 added as a complex with fatty acid free-bovine serum albumin [18]. After incubation, cells were harvested, washed and cell total lipid extracted as described in detail previously [16]. Fatty acid methyl esters were prepared as described below, separated by argentation chromatography on silver nitrate-impregnated TLC plates, subjected to autoradiography, and radioactivity in individual fatty acids determined by liquid scintillation counting, all as described in detail previously [16].