Aquaculture 498 (2019) 132–142

Contents lists available at ScienceDirect

Aquaculture

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

MicroRNAs related to cholesterol metabolism affected by vegetable diet in rainbow trout (*Oncorhynchus mykiss*) from control and selected lines

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ARTICLE INFO

Keywords:
MicroRNAs
Cholesterol metabolism
Plant-based diet
Rainbow trout

ABSTRACT

For the sustainable development of aquaculture, vegetable ingredients have been used to replace the traditional ingredients fishmeal and fish oil in aquafeeds. On the other hand, selective breeding has also been implemented at research level to obtain strains that are adapted to feeding on the plant-based diet. To better understand the underlying mechanisms prompting the adaptation to plant-based diets in fish, we investigated the hepatic expression of several microRNAs (miRNAs) that are involved in the post-transcriptional regulation of cholesterol and lipid metabolism at 8 h and 16 h after the last meal in two lines of rainbow trout: one selected for better adaptation to plant-based diets and the corresponding control line. Both groups were fed either a fishmeal and fish oil based diet or a 100% plant-based diet. Results showed that the expression of miR-33a in liver was greatly elevated in trout fed the plant-based diet, while the expression of miR-122 and miR-128 was much higher in the selected line at 8 h after the last meal regardless of the diet. Furthermore, our results indicated that some genes involved in immune processes (caspase 6 apoptosis-related cysteine peptidase like 2, casp6l2) and cAMP signal transduction (phosphodiesterase 4B cAMP-specific a, pde4ba) were also potentially regulated by miRNAs. They were newly identified as putative direct targets of miRNAs and affected in trout fed the plant-based diet. Though further investigations are still needed to establish a valid relationship between miRNAs and their target genes, our study found miR-33a, miR-122 and miR-128 as potential candidates for further study and provided new perspectives to understand the role of miRNAs in the selective breeding for adaptation to the plant-based diets.

1. Introduction

Increasing demand for world fisheries production has resulted in a great expansion of aquaculture over the last decades, accounting for about half of the world global seafood production (FAO, 2016). As capture fisheries are limited and showed no increase since the mid-1980, the increasing demand for fish in the future will be inevitably supplied by aquaculture. However, the production of fishmeal and fish oil coming from wild fishery stocks and constituting important traditional ingredients in aquafeed is also going to keep stable or even decrease (Jackson, 2012). Therefore, the utilization of plant ingredients to replace fishmeal and fish oil has been considered as the best solution to support the sustainable development of aquaculture in recent years (Tacon et al., 2010) and evident progress has been made regarding the substitution of fishmeal and fish oil by plant raw materials in salmonids and marine fish (Benedito-Palos et al., 2008; Torstensen et al., 2008). However, studies have indicated that total replacement of fishmeal and fish oil by the plant-based diets still reduced growth performances and flesh quality (Geay et al., 2011; Panserat et al., 2009). In order to further improve the use of plant food in carnivorous fish like salmonids, research efforts have been more recently focused on the selective breeding. Indeed, the selective breeding could be the alternative solution to enhance fish adaptation to the plant-based diets and has been well confirmed at a research level. For example, it has been demonstrated that survival rate and mean body weight were improved in the first generation of rainbow trout selected for the ability to adapt to a totally plant-based diet (Le Boucher et al., 2012). Moreover, in Atlantic salmon, the deposition and/or retention of dietary n-3 long-chain polyunsaturated fatty acids (LC-PUFA) in flesh, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is a highly heritable trait (Leaver et al., 2011) and Nofima has also carried out profitable work in this respect (Horn et al., 2017), prompting further interest in

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https://doi.org/10.1016/j.aquaculture.2018.08.058
Received 22 February 2018; Received in revised form 21 August 2018; Accepted 22 August 2018
Available online 23 August 2018
0044-8486/

exploring genotype-nutrient interactions.

The replacement of fishmeal and fish oil by plant ingredients caused great variations in nutrient supply, including the reduction of cholesterol and n-3 LC-PUFA. Cholesterol is derived from the animal organisms, so it is absent in plant ingredients (Tocher et al., 2008). In animals, cholesterol represents an important component of the membrane to modulate the fluidity and permeability and it is also the precursor of many biologically active compounds, including bile acids, steroid hormones and vitamin D (Goedeke and Fernández-Hernando, 2012).

To maintain cholesterol homeostasis, the expression of genes related to cholesterol metabolism is tightly controlled at both transcriptional and post-transcriptional level. The sterol regulatory element-binding proteins (SREBPs) and the liver X receptors (LXRs) are known to be well involved in the transcriptional regulation of cholesterol metabolism by targeting genes involved in the cholesterol synthesis (*hmgcr*, 3-hydroxy-3-methylglutaryl-CoA, reductase) and efflux (*abca1*, ATP-binding-cassette transporter-A1, *abcg5* and *abcg8*, ATP-binding-cassette transporter G5 and G8 and *cyp7a1*, cytochrome P450 cholesterol 7a-hydroxylase), respectively (Sakakura et al., 2001; Zhao and Dahlman-Wright, 2010). At the post-transcriptional level, a class of small non-protein coding RNAs of 19–24 nucleotides, designated as microRNA (miRNA), has been found to regulate gene expression either by mRNA decay or translational repression (Iwakawa and Tomari, 2015). Among the wide numbers of miRNAs, miR-1 (Zhong et al., 2013), miR-33a (Gerin et al., 2010; Najafi-Shoushtari et al., 2010), miR-122 (Esau et al., 2006), miR-128 (Adlakha et al., 2013) and miR-223 (Vickers et al., 2014; Wang et al., 2013) were reported to be involved in the regulation of cholesterol and lipid metabolism in mammals.

The ability to grow on the plant-based diet has been proved to be genetically variable in fish (Le Boucher et al., 2011; Quinton et al., 2007). Although the growth potential has been effectively enhanced by selective breeding, the molecular mechanisms underlying this selection still remain unknown. In the present study, we used two lines of rainbow trout, the selected line which had better adaptation to the plant-based diet and the non-selected control line in order to investigate the potential role of miRNA, such as miR-1, miR-33a, miR-122, miR-128 and miR-223, in the adaptation to the plant-based diet. Therefore, the hepatic expression of these miRNAs and the genes related to cholesterol metabolism were analyzed. Furthermore, as miR-33a, miR-128 and miR-223 were found to be affected by the plant-based diet or selective breeding, we expanded our study to some genes that were identified in silico as potential direct targets of these miRNAs based on the complementarity between the seed sequence of the miRNAs and the 3′ untranslated region (UTR) region of the rainbow trout transcripts.

2. Materials and methods

2.1. Ethics statement

Experimentation was conducted in the INRA experimental facilities (Peima facilities, Sizun, France and UMR Numéa, St-Pée-sur-Nivelle, France) authorized for animal experimentation by the French veterinary service which is the competent authority (B 29–277-02 and A 64–495-1). The experiments were in strict accordance with EU legal frameworks related to the protection of animals used for scientific research (Directive 2010/63/EU) and according to the National Guidelines for Animal Care of the French Ministry of Research (decree n°2013–118, February 1st, 2013). The scientists in charge of the experimentation received training and personal authorization (N° B64 10,003 and A29 102). In agreement with ethical committees “Comité d'Ethique Aquitaine Poissons Oiseaux” (C2EA-73) and “Comité d'Ethique Finistérien en Expérimentation Animale” (C2EA-74), the experiment reported here does not need approval by a specific ethical committee since it implies only classical rearing practices with all diets used in the experiment formulated to cover the nutritional requirements of rainbow trout (National Research Council, 2011). Fish were monitored daily during the experiment. If any clinical symptoms (i.e. morphological abnormality, restlessness or uncoordinated movements) were observed, fish were sedated by immersion in 2% benzocaine solution and then euthanized by immersion in a 6% benzocaine solution (anesthetic overdose) during 3 min.

2.2. Fish, diet and experimental design

The experiment was conducted on two groups of rainbow trout: one line (S) which has been selected for better ability to grow and survive with a totally plant-based diet for three generations (Callet et al., 2017; Le Boucher et al., 2012) and a control line (C) maintained with high effective size and without artificial selection. 3600 eggs from S line and 3600 eggs from C line were obtained by within line mating (18 dams and 31 sires for each line). At 19 days post fertilization (dpf), the eyed eggs were randomly distributed into 12 tanks (0.25 m^3) with 600 eyed eggs in each tank. The fish were reared in the 12 flow-through tanks (three tanks per treatment) in INRA experimental facilities (PEIMA, Sizun, France) at 11 °C and under artificial photoperiod condition (from 8 am to 8 pm). From the first-feeding stage, S and C fish were fed ad libitum with either a plant-based diet totally devoid of marine products (V diet) or a diet containing fishmeal and fish oil (M diet) during six months. Diets were manufactured in our experimental facilities of Donzacq (France) using a twin-screw extruder (Clextral, France). The M diet contained fishmeal and fish oil as main protein and lipid source, respectively. The V diet was formulated with a blend of vegetable oils (palm, rapeseed and linseed oils) and a blend of plant protein sources (corn and wheat gluten, soybean meal, soy protein concentrate, light white lupin, dehulled pea and extruded whole wheat). Diets were formulated to fulfill the requirements of rainbow trout according to NRC recommendations (National Research Council, 2011). Synthetic L-lysine, L-arginine, dicalcium-phosphate and soy-lecithin were added to the V diet to correct the deficiency in essential amino acids, phosphorous and phospholipid supply. Diet formulations and compositions are described in Table 1.

2.3. Sampling procedure

At the end of the feeding trial, fish were anesthetized with benzocaine (30 mg/L) and killed by a sharp blow to the head. Six fish were sampled from each tank at 8 h and 16 h after the last meal, respectively. Blood was removed from the caudal vein into EDTA syringes and centrifuged (1000 *g*, 10–15 min); then recovered plasma was immediately frozen in liquid nitrogen and kept at −80 °C for the measurement of cholesterol and triglycerides. Liver was dissected and weighed for hepato-somatic index determination and then immediately frozen in liquid nitrogen and kept at −80 °C. About 100 mg was cut from the whole liver for RNA extraction and then subsequent gene and miRNA expression analyses.

2.4. Diet and whole body composition analysis

Proximate analysis of the experimental diets was determined according to Association of Official Analytical Chemists (AOAC, 2000) as follows: dry matter was analyzed by drying the samples to constant weight at 105 °C for 24 h. Crude protein was determined using the Kjeldahl method after acid digestion and estimated by multiplying nitrogen by 6.25. Crude lipid was quantified by petroleum diethyl ether extraction using Soxhlet method. Gross energy content was determined in an adiabatic bomb calorimeter (IKA). Ash was examined by combustion in a muffle furnace at 550 °C for 16 h. Dietary sterols were assayed on a total lipid extract by means of the Liebermann-Burchard method (Stadtman, 1957).