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^{1}H NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (*Salmo salar*)

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ABSTRACT

A ^{1}H NMR-based metabolomics approach was used to explore the impact of dietary sesamin on the liver and white muscle metabolic profile of Atlantic salmon (*Salmo salar*). Fish were fed diets containing different n-6/n-3 fatty acid ratios (V0.5 or V1) and sesamin contents [without (S0), low (SL) 1.16 g/kg feed, and high (SH) 5.8 g/kg feed] for 4 months. Liver and white muscle extracts of aqueous polar and chloroform lipid phases were collected. Multivariate data analyses (PCA and OPLS-DA) of liver chloroform phase showed that high levels of sesamin affected the metabolic profile impartially of the n-6/n-3 ratio. In the aqueous phase, the metabolome of liver and white muscle were affected in fish fed an n-6/n-3 ratio of 1.0 and 0.5, respectively. With high inclusion of sesamin, the levels of several metabolites (*e.g.* glucose, glycogen, leucine, valine, creatine, carnitine, lactate, nucleosides) were increased. These metabolites are mainly associated with energy metabolism, suggesting that high sesamin inclusion affects liver and white muscle metabolism in fish. This is consistent with lower body weights found in fish fed high sesamin content.

1. Introduction

The flesh of fatty fish, including Atlantic salmon (*Salmo salar*), is considered a healthy food due to *e.g.* its potential to reduce the risk of cardiovascular and inflammatory diseases and behaviour disorders (Mozaffarian & Rimm, 2006; Simopoulos, 2002). These health benefits have been attributed to the presence of omega-3 (n-3) long chain polyunsaturated fatty acids (LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), in fish muscle for human consumption (Jensen et al., 2012).

Aquaculture is the fastest growing food industry sector in the world, producing approximately 50% of all fish consumed globally. However, this percentage is expected to rise as a result of decreasing catches from wild fisheries that cannot currently provide enough seafood protein for the growing human population (Turchini, Torstensen, & Ng, 2009). However, the expansion of the aquaculture industry is associated with declining availability of fish oils (FO) as a component in fish feed production, although this is partially mitigated by the replacement of FO with vegetable oils (VO) such as soy, palm, rapeseed, and linseed oil. Freshwater fish species are able to convert the shorter 18-carbon essential fatty acids (FA) 18:3n-3 (linolenic acid, ALA) and 18:2n-6 (linoleic acid, LA) present in VO to LC-PUFA, including eicosapentaenoic acid (20:5n-3; EPA), docosahexaenoic acid (22:6n-3; DHA), and arachidonic acid (20:4n-6; AA). Therefore, essential FA requirements for freshwater fish can be met by the shorter C18 unsaturated PUFA. However, a natural drawback for consumers is not only the decreased amount of n-3 but also the modification of the n-6/n-3 ratio in fish tissues (Simopoulos, 2002). To counteract this negative effect, several studies have investigated the possibility of improving conversion of α-linolenic acid (ALA, 18:3n-3) to LC-PUFA by adding lipid modulators, such as bioactive compounds, to fish feeds (Alhazzaa, Bridle, Carter, & Nichols, 2012; Trattner, Kamal-Eldin et al., 2008a; Trattner, Ruyter et al., 2008b).

Bioactive compounds are defined as naturally occurring non-nutritive components present in small quantities in plant products and lipid-rich food (Shahidi, 2000). Minor bioactive lipid compounds such as sesamin found in sesame seeds are known to have antioxidant properties (Ikeda et al., 2003) and to regulate lipid metabolism in mammals (Ashakumary et al., 1999). Sesamin has been shown to increase the β-oxidation of FA in rodents via activation of the peroxisome proliferator activator receptor (PPAR) alpha

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(Ashakumary et al., 1999). In addition, sesamin modulates several genes related to lipid metabolism, such as Δ5 and Δ6 desaturases. In rats sesamin inhibits *in vivo* the Δ5-desaturase activity of n-6 FA, but not that of n-3 FA (Fujiyama-Fujiwara, Umeda-Sawada, Kuzuyama, & Igarashi, 1995). In fish, sesamin increases the DHA content of white muscle in the freshwater stage of salmonids (Trattner et al., 2008a). However, to the best of our knowledge, the effects of sesamin in diets rich in VO on modulation of the hepatic metabolome of a teleost fish species have not previously been investigated.

Metabolomics is defined as the comprehensive analysis of the whole metabolome under a given set of conditions (Goodacre, Vaidyanathan, Dunn, Harrigan, & Kell, 2004). Metabolomics can be used to gain insight into changes in fish metabolism after addition of sesamin. Previous studies have addressed the use of ^{1}H NMR technology to evaluate the compositional analysis of fish muscle (Bankefors et al., 2011; Gribbestad, Aursand, & Martinez, 2005), plasma (Kokushi, Uno, Harada, & Koyama, 2012) and liver (Bankefors et al., 2011). In a previous study Schiller Vestergren et al. (2012) found that VO-based diets affected the liver of Atlantic salmon in terms of lipid content and gene expression. Thus, analysis of liver samples by means of metabolomics-based approaches can provide additional information regarding FA composition (obtained from lipid extracts) and lipophobic small metabolites content (obtained from aqueous extracts), which reflect the metabolic state of the tissue. Liver plays a major role in metabolism and biosynthesis, and was therefore chosen as a major metabolic site. The analysis of white muscle may provide important information of this tissue in response to the diets, as well as for the final flesh quality.

The aim of the present work was to explore changes in the metabolic profile in the liver and white muscle of Atlantic salmon fed different levels of sesamin in VO-based diets with varying n-6/n-3 FA ratio.

2. Materials and methods

2.1. Feeding trial

Atlantic salmon reared at the Skretting ARC fish station in Stavanger, Norway, with an initial body weight of 104.6 ± 9.9 g, were distributed in groups of 35 fish into six experimental tanks (500-L, provided with flow-through seawater at 12 °C). During the trial, fish were fed with the experimental diets twice per day for 4 months (November–March). The experiments were carried out in accordance with EU legislation (i.e. Directive 2010/63/EU), and Norwegian Animal Welfare Regulations.

The experimental diets were formulated as previously described (Schiller Vestergren et al., 2012). In brief, there were six different diets reflecting an n-6/n-3 FA ratio of either 0.5 (V0.5) or 1.0 (V1). Different ratios were chosen, based on the study of Trattner et al. (2008a), showing the effect of sesamin on lipid metabolism at the higher n-6/n-3 ratio. The different fatty acid ratios were combined with three different sesamin levels: a control without sesamin (S0), low sesamin = 1.16 g/kg feed (SL), and high sesamin = 5.8 g/kg feed (SH). Linseed oil (LO), rapeseed oil (RO) and palm oil (PO), were used in different amounts: V0.5 diets included 135 g/kg LO, 53.5 g/kg RO and 50 g/kg PO, V1 diets contained 40 g/kg LO, 138.5 g/kg RO and 60 g/kg PO. A schematic presentation of diet codes and sesamin levels in the experimental diets is given in Fig. 1.

At the end of the 4-month feeding trial, the fish were anaesthetised (ethylene glycol monophenyl ether, 5 mL/L) and euthanized with a blow to the head. Liver and white muscle samples were taken from 6 fish per diet, frozen in liquid nitrogen and stored at −80 °C for metabolomics analyses.

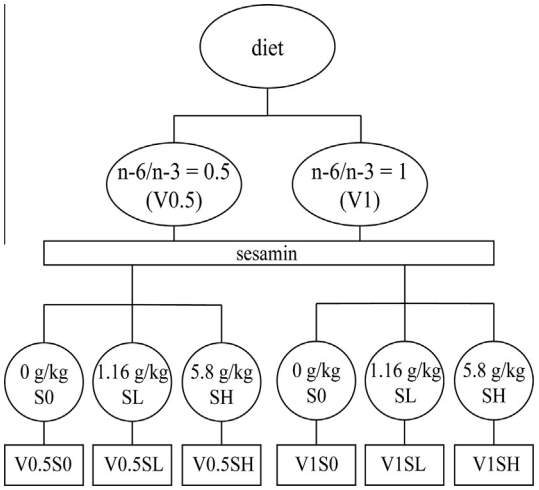

Fig. 1. Schematic representation of diet codes and sesamin levels in the experimental diets.

2.2. ^{1}H NMR-based metabolomics assays for liver

Fish liver samples were extracted using a method described previously (Moazzami, Andersson, & Kamal-Eldin, 2011), after slight modification. In brief, frozen liver samples (100 mg) were homogenised for 1 min in 3 mL ice-cold methanol–chloroform (2:1, v/v). Samples were then vortexed for 1 min after addition of 1 mL ice-cold water and 1 mL ice-cold chloroform. Phase separation was achieved by centrifugation at 1.800g for 35 min at 4 °C.

Hydrophilic extract. The aqueous supernatant (polar phase) was collected, mixed with 600 μL sodium phosphate buffer (0.25 M, pH = 7.0), and dried using an evacuated centrifuge (Savant, SVC 100H, Techtum Instrument AB, Umeå, Sweden). The dried extracts were reconstituted by adding 595 μL D_2O and 5 μL sodium-3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate in D_2O (TSP-d_4, 1 mmol/L, Cambridge Isotope Laboratories, Andover, MA, USA) as internal standard to assure quantitative analysis. The samples were analysed in 5 mm outer diameter NMR tubes using a Bruker DRX 400 spectrometer (Karlsruhe, Germany). The NMR experiment was performed at 25 °C with 400 scans and 32,768 data points over a spectral width of 8012.82 Hz. The acquisition time was 2 s and the relaxation delay was 3 s. The one-dimensional ^{1}H NMR spectra with water pre-saturation were recorded using Noesypr1D (Bruker Spectrospin Ltd., BioSpin, Karlsruhe, Germany) pulse sequence. Two-dimensional NMR spectroscopy for the identification of signals, including correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY), were performed using standard pulse sequences from the Bruker library (Moazzami et al., 2011).

Lipophilic extracts. The chloroform phase was collected and dried under nitrogen. The dried extracts were dissolved in 580 μM $CDCl_3$ (99.96 atom% D) and 20 μM CD_3OD (99.8 atom% D) as internal standard. ^{1}H NMR spectra were obtained on a DRX 400 Bruker instrument (Karlsruhe, Germany) using zg30 pulse sequence (Bruker Spectrospin Ltd.) at 25 °C with 65,536 data points and 180 scans over a spectral width of 8223.68 Hz. The acquisition time was 4 s and the relaxation delay was 3 s.

2.3. ^{1}H NMR-based metabolomics assays for white muscle

The sample preparation for white muscle was the same as for liver, with slight modification. In brief, 100 mg white muscle was