Effects of Dietary Supplementation of Rapeseed Oil on Metabolism of [1-14C]18:1n-9, [1-14C]20:3n-6, and [1-14C]20:4n-3 in Atlantic Salmon Hepatocytes

C. Moya-Falcón, M.S. Thomassen, J.V. Jakobsen, and B. Ruyter

Abstract:

Atlantic salmon were fed fish meal-based diets supplemented with either 100% fish oil (FO) or 100% rapeseed oil (RO) from an initial weight of 85 g to a final average weight of 280 g. The effects of these diets on the capacity of Atlantic salmon hepatocytes to elongate, desaturate, and esterify [1-14C]18:1n-9 and the immediate substrates for the Δ9 desaturase, [1-14C]20:3n-6 and [1-14C]20:4n-3, were investigated. Radiolabeled 18:1n-9 was mainly esterified into cellular TAG, whereas the more polyunsaturated FA, [1-14C]20:3n-6 and [1-14C]20:4n-3, were primarily esterified into cellular PL. More of the elongation product, [1-14C]20:1n-9, was produced from 18:1n-9 and more of the desaturation and elongation products, 22:5n-6 and 22:6n-3, were produced from [1-14C]20:3n-6 and [1-14C]20:4n-3, respectively, in RO hepatocytes than in FO hepatocytes. Further, we studied whether increased addition of [1-14C]18:1n-9 to the hepatocyte culture media would affect the capacity of hepatocytes to oxidize 18:1n-9 to acid-soluble products and CO2. An increase in eicosanoid concentration of 18:1n-9 from 7 to 100 µM resulted in a nearly twofold increase in the amount of 18:1n-9 that was oxidized. The conversion of 20:4n-3 and 20:3n-6 to the longer-chain 22:5n-6 and 22:6n-3 was enhanced by RO feeding in Atlantic salmon hepatocytes. The increased capacity of RO hepatocytes to produce 22:6n-3 was, however, not enough to achieve the levels found in FO hepatocytes. Our data further showed that there were no differences in the hepatocyte FA oxidation capacity and the lipid deposition of carcass and liver between the two groups.


The use of vegetable oils as a substitute for fish oil (FO) in high-energy diets of farmed fish has increased in recent years owing to the limited availability of FO (1–10). A major reason for adding dietary marine oils to salmon feed is to provide adequate amounts of PUFA of the n-3 series in the fish muscle so as to secure the nutritional quality of the fish for human consumption. Many studies in the last decade have shown that salmonids can use vegetable oils in seawater without negative effects on fish growth (1,2,5,7) provided the diets contain enough n-3 PUFA to satisfy EPA requirements (3,4,6,8,11). Rapeseed oil (RO) is a good supplement for FO in salmon feeds as it has moderate levels of 18:3n-3, is relatively rich in 18:2n-6, and is very rich in 18:1n-9 (7). Culture of salmonids on diets containing high levels of vegetable oils does, however, considerably alter the FA composition of the fish muscle, giving high levels of C18 FA and much lower levels of the PUFA 20:5n-3 and 22:6n-3, which are known to have several positive health effects in humans (2,7).

The level of these healthful PUFA in fish muscle may be of major importance for the human acceptance of the salmon product. For this reason, there is currently considerable interest in creating diets with FA compositions that can enhance the ability of the fish to produce 20:5n-3 and 22:6n-3 from 18:3n-3, by stimulation of the Δ5 and Δ6 desaturases and the elongases involved in the conversion (10). Atlantic salmon can elongate and desaturate C18 FA of the n-3 and n-6 series to C22 FA may inhibit the conversion of 18:3n-3 and 18:2n-6 to 22:6n-3 and 22:5n-6, respectively, which has been shown in studies of Atlantic salmon hepatocytes. The activity of the Δ5 desaturase in Atlantic salmon is also found to be higher in hepatocytes from fish fed a diet containing a 1:1 blend of linseed oil and RO than it is in fish fed a diet containing FO (12). This is also the case for fish fed soybean oil diets (17). C18 FA were used as radiolabeled substrates for all these studies of desaturation and elongation capacities. It is known, however, that high levels of C18 FA may inhibit the conversion of 18:3n-3 to 22:6n-3 in Atlantic salmon, owing to the competition between C18 FA and C22 FA for the same Δ6 desaturase (18). In this trial, we incubated with the longer-chain radiolabeled specific Δ5 desaturase substrates, 20:4n-3 and 20:3n-6, to test whether RO feeding affected the production of 22:6n-3 and 22:5n-6 from C20 FA in Atlantic salmon hepatocytes.

To avoid increased lipid deposition, it is important that the FA of the dietary oil is easily oxidized by the fish. In vitro studies of mitochondrial β-oxidation in fish suggest that saturated and monounsaturated FA are preferred over PUFA as FA substrates (reviewed by Henderson, 19), but it is not yet known whether RO feeding affects the capacity of salmon liver to oxidize 18:1n-9, which is the dominant FA of RO. Another aim of this study was to investigate whether a dietary supplementation of RO to Atlantic salmon affects the capacity of hepatocytes to oxidize...
18:1n-9 and, further, whether it affects lipid deposition in the carcass and the liver.

MATERIALS AND METHODS

Chemicals. The radiolabeled FA [1-14C]18:1n-9, [1-14C]20:3n-6, and [1-14C]20:4n-3 were obtained from American Radiolabeled Chemicals (St. Louis, MO); and nonlabeled FA, BSA that was essentially free of FA, HEPES, 2',7'-dichlorofluorescein, and collagenase type 1 were obtained from Sigma Chemical Co. (St. Louis, MO). Metacaine (MS-222) was obtained from Norsk Medisinaldepot (Oslo, Norway). Acetic acid, chloroform, petroleum ether, and methanol were all obtained from Merck (Darmstadt, Germany). Benzene was obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Methanolic HCl and 2,2-dimethoxypropane were purchased from Supelco Inc. (Bellefonte, PA). Silica gel K6-coated glass plates were obtained from Whatman International Ltd. (Maidstone, England).

Fish, facilities, and experimental design. The trial was conducted at the AKVAFORSK Research Station, Sunndalsøra, Norway. Atlantic salmon (Salmo salar) with a mean initial weight of approximately 85 g were placed into six cylindrical-conical tanks (0.85 m diameter) with 40 fish per tank. The tanks were supplied with seawater at a constant temperature of 12°C. The fish were fed commercial diets before the start of the experiment. The growth trial consisted of one period of 8 wk. Two diets were randomly assigned to triplicate tanks. The feed was distributed by electrically driven disc-feeders (Akvaprodukter AS, Sunndalsøra, Norway). Acetic acid, chloroform, petroleum ether, and methanol were all obtained from Merck (Darmstadt, Germany). Benzene was obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Methanolic HCl and 2,2-dimethoxypropane were purchased from Supelco Inc. (Bellefonte, PA). Silica gel K6-coated glass plates were obtained from Whatman International Ltd. (Maidstone, England).

Diets. The experimental fishmeal-based diets were provided by EWOS Innovation SA (Dirdal, Norway) in the form of 3-mm pellets. The feed contained 0.1% yttrium oxide (Y2O3) as an inert marker for determining digestibility (21). The experimental diets, FO and RO, were produced from one common feed mix. The two diets were obtained by coating the common feed pellet with the different oils. The diets contained either 100% FO (capelin oil) or 100% RO. Table 1 shows the formulations and chemical compositions of the diets.

Initial and final sampling. The fish were fasted for 2 d before the initial sampling. The fish from each tank were anesthetized in MS-222, weighed, and measured at the beginning and at the end of the experiment. The fish were not fasted before the final sampling, because of the collection of feces. Five fish from each tank were stripped to collect fecal samples following the procedure described by Austreng (22). Fecal samples from each tank were pooled. The samples were stored at −20°C prior to analyses. A further five fish per tank were anesthetized, killed by a blow to the head, and used for determination of whole body chemical composition.

Chemical analysis. Fish sampled at the beginning and at the end of the experiment were analyzed for dry matter (DM), fat, protein, ash, and energy. DM was determined by drying the fish homogenate to constant weight at 105°C. All diets and fecal samples were analyzed for DM, fat, protein, ash, energy, and yttrium. The amount of protein was determined using a Kjeltec Autoanalyser-N*6.25, whereas the amount of ash was determined by heating to 550°C until constant weight was reached. The amount of fat was determined by ethyl-acetate extraction [NS 9402, 1994 (Atlantic Salmon—Fat Measurement, Norwegian Standards Association, Oslo)], and the amount of yttrium by using inductively coupled plasma-atomic emission spectros copy at Jordforsk, Ås, Norway, after wet-ashing the samples. Energy in the diets, feces, and whole fish homogenates was analyzed by adiabatic bomb calorimetry using a Parr 1271 bomb calorimeter.

Preparation of salmon hepatocytes. Hepatocytes were isolated from three fish from each tank. The fish were anesthetized in MS-222. The abdominal cavity was exposed and the vena porta cannulated. The liver was perfused, and parenchymal cells were isolated using a two-step collagenase perfusion procedure as described by Seglen et al. (23) and modified by Danneving and Berg (24). After collagenase perfusion, the parenchymal cells were easily isolated by gently shaking the liver in Leibowitz-15 medium (L-15). The suspension of parenchymal cells was filtered through a nylon filter with 100-µm mesh. Hepatocytes were isolated from the filtrate by centrifuging it three times, each time for 2 min at 50 × g. The hepatocytes were resuspended in L-15 culture medium containing 2% FBS, 2 mM L-glutamine, and 0.1 mg/mL gentamycin. Cell viability was assessed by staining with Trypan blue (0.4%). The protein content of the cell suspension was determined using the method described by Lowry et al. (25). Approximately 9 ×10⁶ cells (9–10 mg of protein) were plated onto 75 mL Nunc flasks and left to attach overnight at 12°C.

Incubations with radiolabeled FA. The hepatocytes were thoroughly washed with L-15 medium without additional serum and then incubated at 12°C before the incubation with