Toxicology Reports 3 (2016) 211–224

Contents lists available at ScienceDirect

Toxicology Reports

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

Omega-3 and alpha-tocopherol provide more protection against contaminants in novel feeds for Atlantic salmon (*Salmo salar* L.) than omega-6 and gamma tocopherol

Liv Søfteland [a,*], Marc H.G. Berntssen [a], Jennifer A. Kirwan [b], Trond R. Størseth [c], Mark R. Viant [b], Bente E. Torstensen [a], Rune Waagbø [a], Pål A. Olsvik [a]

[a] National Institute of Nutrition and Seafood Research, Norway
[b] School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK
[c] SINTEF Fisheries and Aquaculture, Norway

ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form
23 December 2015
Accepted 11 January 2016
Available online 14 January 2016

Chemical compounds studied in this article:
Chlorpyrifos
Endosulfan
Phenanthrene
Benzo(a) pyrene
Arachidonic acid
Eicosapentaenoic acid
α-tocopherol
γ-tocopherol

Keywords:
Interactions
Nutrients
PAH
Pesticides
Lipidomics
Metabolomics
RT-qPCR transcriptomics

ABSTRACT

Extended use of plant ingredients in Atlantic salmon farming has increased the need for knowledge on the effects of new nutrients and contaminants in plant based feeds on fish health and nutrient-contaminant interactions. Primary Atlantic salmon hepatocytes were exposed to a mixture of PAHs and pesticides alone or in combination with the nutrients ARA, EPA, α-tocopherol, and γ-tocopherol according to a factorial design. Cells were screened for effects using xCELLigence cytotoxicity screening, NMR spectroscopy metabolomics, mass spectrometry lipidomics and RT-qPCR transcriptomics. The cytotoxicity results suggest that adverse effects of the contaminants can be counteracted by the nutrients. The lipidomics suggested effects on cell membrane stability and vitamin D metabolism after contaminant and fatty acid exposure. Co-exposure of the contaminants with EPA or α-tocopherol contributed to an antagonistic effect in exposed cells, with reduced effects on the VTG and FABP4 transcripts. ARA and γ-tocopherol strengthened the contaminant-induced response, ARA by contributing to an additive and synergistic induction of CYP1A, CYP3A and CPT2, and γ-tocopherol by synergistically increasing ACOX1. Individually EPA and α-tocopherol seemed more beneficial than ARA and γ-tocopherol in preventing the adverse effects induced by the contaminant mixture, though a combination of all nutrients showed the greatest ameliorating effect.

© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Increased use of plant feed ingredients has introduced a new cocktail of plant-oil derived contaminants, such as polycyclic aromatic hydrocarbons (PAHs) and pesticides, formerly not related with farming of salmonids [5,23]. Plant oils intended for animal feed production can be contaminated with PAH like phenanthrene and benzo(a) pyrene [81,12] due to atmospheric deposition of particles on crops before harvesting or later during the thermal processing of the oil seeds [20,64]. Residue levels of pesticides like endosulfan and chlorpyrifos have been reported in products from plants such as soya or maize [33,49] which are commonly used as ingredients in salmon feeds [4]. Both PAH and pesticides have been shown in several *in vitro* and *in vivo* experiments to cause lipid and endocrine disturbances and to induce cytochrome P450 enzymes in teleost fish [43,53,68,79,100,102]. PAHs are genotoxic [16,86,35] and exposure has been suggested to cause vitamin D signalling disruption [79] as well as an effect on cell membrane integrity [65,70]. The pesticides endosulfan and chlorpyrifos have been shown to induce lipid accumulation in Atlantic salmon liver cells both *in vitro* and *in vivo* [43,23,79].

* Corresponding author.
E-mail address: lso@nifes.no (L. Søfteland).

http://dx.doi.org/10.1016/j.toxrep.2016.01.008
2214-7500/© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

In addition to an altered contaminant profile, the plant-based feed ingredients also change the nutrient profile of the fish. Marine oils contain high levels of the n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [36] while common plant oils can contain high levels of n-6 PUFA like linoleic acid (LA, 18:2n-6) [92]. Since the fatty acid composition of oily fish reflects the fatty acid composition of their feed [85], replacing fish oil with vegetable oil in fish feed typically reduces the concentration in Atlantic salmon fillet of the n-3 PUFAs EPA and DHA and increases concentrations of the n-6 PUFAs LA and arachidonic acid (ARA, 20:4n-6) [25,62,84,85]. The liver is a central organ for lipid metabolism [7] and the synthesis of cholesterol and bile [42]. Immediately after uptake in the liver, the PUFA can be converted to energy by β-oxidation [74], stored in adipocytes and in intracellular lipid droplets in different tissues [55] or incorporated into phospholipid membranes [92]. The n-3 and n-6 PUFAs can be converted to their respective group of eicosanoids or lipid mediators by the lipoxygenase (LOX), cyclooxygenase (COX) and cytochrome P450 (CYP) enzyme pathways [92]. The n-6 eicosanoids are a general group of pro-inflammatory eicosanoids [92]. By contrast, the n-3 PUFA can be converted to n-3 eicosanoids that have anti-inflammatory abilities [92]. Several studies have reported increase in liver lipid when fish oil was replaced with plant oils indicating that nutrients in fish oil such as EPA and DHA, n-6, saturated fatty acids, cholesterol and phytosterols play a role (Torstensen et al., 2011) [45,47,62]. High levels of the n-3 PUFAs DHA and EPA in fish feed can protect against induction of liver steatosis in Atlantic salmon [45,47]. α-tocopherol is an essential nutrient for fish [28] and is also the main form of vitamin E in fish fillet [76]. Dietary α-tocopherol is taken up more rapidly than γ-tocopherol [93], which is the main vitamin E congener in most plant seeds [34] and maize, rapeseed and soya oils [77]. γ-tocopherol seems to be a more effective trap for lipophilic electrophiles than α-tocopherol [34] and in contrast to α-tocopherol, γ-tocopherol possesses anti-inflammatory properties [34]. Similar to the n-3 PUFAs, high levels of tocopherol can inhibit induction of liver steatosis [91] and protect organisms against lipid oxidation [28].

The aim of this study was to examine how relevant nutrients can modulate the toxicological outcome of a contaminant mixture associated with plant feed ingredients using metabolomic, lipidomic and transcriptomic methods to search for novel biomarkers and possible interaction effects. The study utilised Atlantic salmon primary hepatocytes as a biological model system.

2. Materials and methods

2.1. Chemicals

Endosulfan (6,7,8,9,10-hexachloro-1,5,5a,6,9,9,a-hexahydro-6,9-metano-2,4,3-benza-dioxathiepin-3-oxide, α + β–2 + 1; PESTANAL®, analytical standard), chlorpyrifos (*O*,*O*-diethyl-*O*-3,5,6-trichlor-2-pyridyl phosphorothioate, PESTANAL®, analytical standard), phenanthrene (≥98% pure), benzo(a) pyrene (≥96% pure), arachidonic acid (ARA ≥99%), eicosapentaenoic acid (EPA, ≥99% pure), potassium hydroxide, α-tocopherol (αT, >95.5% pure) and γ-tocopherol (γT, ≥96% pure) were all purchased from Sigma–Aldrich (Oslo, Norway). Dimethyl stock solution was purchased from Scientific and Chemical Supplies Ltd. (Bilston, UK), chloroform (HPLC grade) was purchased from Fisher Scientific (Loughborough, UK) and ammonium acetate was purchased from Sigma–Aldrich Co., Ltd. (Dorset, UK). The fatty acid free-BSA (FAF–BSA) was purchased from PAA (Parching, Austria).

2.1.1. Bovine Serum Albumin (BSA) coupling of fatty acids

Binding of fatty acids (FA) to fatty acid free-BSA (FAF–BSA) was performed as per Ghioni et al. [22]. To summarize, FA dissolved in 0.04 ml chloroform per mg FA was added to a glass sample tube and N_2 was used to evaporate the chloroform. Potassium hydroxide (KOH) was applied to the FA in a 1:3 ratio and the solution was shaken for 10 min using a vortex mixer. FAF–BSA was employed in a 2.5:1 relationship to the FA and the solution was mixed for 45 min before it was sterile-filtered and preserved at −80 °C in anoxic conditions.

2.2. Isolation of primary cultures of hepatocytes

Juvenile Atlantic salmon were obtained and kept at the animal holding facility at Ilab, University of Bergen (UiB), Bergen, Norway. The fish were fed once daily with a special feed produced without addition of synthetic antioxidants and with low levels of contaminants, supplied by EWOS, Norway (Harmony Nature Transfer 75). All glassware, instruments and solutions were autoclaved prior to liver perfusion. Hepatocytes were isolated from eight Atlantic salmon (278–381 g) with a two-step perfusion method previously described in Ref. [78]. The final cell pellet was resuspended in L-15 medium containing 10% fish serum (FS) from salmon (Nordic BioSite, Oslo, Norway), 1% glutamax (Invitrogen, Norway) and 1% penicillin–streptomycin–amphotericin (10,000 units/ml potassium penicillin, 10000 μg/ml streptomycin sulphate and 25 μg/ml amphotericin B) (Lonzo, Medprobe, Oslo, Norway). The Trypan Blue exclusion method, performed in accordance with the manufacturer's protocol (Lonzo, Medprobe, Oslo, Norway), was used to determine cell viability. The different cell suspensions used in this study had cell viability between 85–90%. The cell suspensions were plated on 5 $\mu g/cm^2$ laminin (Sigma–Aldrich, Oslo, Norway) coated culture plates (TPP, Trasadingen, Switzerland), and the hepatocytes were kept at 10 °C in a sterile incubator without additional O_2/CO_2 (Sanyo, CFC FREE, Etten Leur, The Netherlands). The following cell concentrations were used; 7.2×10^6 cells per well in 6-well plates (in 3 ml complete L-15 medium), 2.6×10^6 cells per well in 12-well plates (in 2 ml complete L-15 medium), 0.2×10^6 cells per well in xCELLigence 96-well plates (in 0.2 ml complete L-15 medium).

2.3. Chemical and nutrient exposure

The primary cells were cultured for 36–40 h prior to chemical exposure with one change of medium (containing 10% FS) after 18–20 h. The cells were exposed for 48 h to single nutrients to establish cytotoxic dose-response curves and to nutrients and a contaminant mixture according to a factorial experimental design for interaction evaluation. Cytotoxicity dose-response curves were established for α-tocopherol (1, 10, 100, 1000 and 10,000 μM) and EPA (100, 200, 4000 and 600 μM) and cells from three fish ($n = 3$) were used to make the dose-response curves. Based on individual cytotoxicity dose-response curves for the nutrients, non-cytotoxic concentrations of the α-tocopherol (100 μM) and the fatty acid EPA (200 μM) were used (data not shown). To be able to assess the effect of both marine and plant derived nutrients on the toxicity of a mixture of PAHs and pesticides, comparable concentrations were used for the plant derived nutrients γ-tocopherol (100 μM) and ARA (200 μM) in a factorial design. The contaminant mixture used here was selected based on a previous study by Søfteland et al. [79] and was composed of 100 μM of the PAHs benzo(a) pyrene and phenanthrene and 1 μM of the two pesticides chlorpyrifos and endosulfan. These concentrations were chosen after earlier individual assessment of the four contaminants aiming for levels just below the onset of cytotoxicity, and the contaminant mixture used in the present study was a potent combination [79]. A full factorial design was used with two levels (low and high concentrations), a zero

Download English Version:

https://daneshyari.com/en/article/2572124

Download Persian Version:

https://daneshyari.com/article/2572124

Daneshyari.com