USING DECONTAMINATED FISH OIL OR A VEGETABLE / FISH OIL BLEND TO REDUCE ORGANIC CONTAMINANT CONCENTRATIONS IN DIETS AND FLESH OF FARMED ATLANTIC SALMON (*Salmo salar*)

Bell, J.Gordon., Sprague, Matthew., Bendiksen, Eldar.Å[*]., Dick, James.R., Strachan, Fiona., Pratoomyot, Jarunan., Berntssen, Marc.H.G[+]., and Tocher, Douglas.R

Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK.
[*]BioMar AS, Nordre gt. 11, N-7484 Trondheim, Norway. [+]National Institute of Nutrition & Seafood Research, N-5817, Bergen, Norway.

Introduction

The nutritional and health benefits of consuming seafood are well documented and are based on the virtually unique supply of n-3 highly unsaturated fatty acids (HUFA), as well as essential vitamins and minerals, in fish and shellfish[1]. Global food grade fisheries have reached a plateau at around 90m tonnes/annum while in 2004 aquaculture contributed over 28m tonnes to the human food basket[2]. Growth of aquaculture is predicted to continue over forthcoming decades, at a rate between 1.9 and 3.3%/annum, as the demand for fish in general and the consumption of aquaculture products increases to fill the gap in demand that cannot be met by capture fisheries. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), account for more than 80% of total European aquaculture production with Norway being the major producer in Europe with Chile producing similar production volumes. Salmon is an oil-rich species and contains high concentrations of health beneficial n-3 HUFA[3]. However, as with all oily carnivorous fish, the oil rich tissues can accumulate lipophilic organic pollutants, including dioxins/furans, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), derived largely from their feed[4]. There has been considerable recent focus on the transfer of organic pollutants from fish feed to farmed fish and possible consequences for human health[5], and subsequently on approaches to reduce levels in feed and farmed Atlantic salmon[6]. As fish oil is the main contributor of contaminants to fish feeds we investigated the effects of replacing northern fish oil, with high levels of contaminants, with either decontaminated fish oil or a blend of fish oil and a 1:1 (w/w) blend of soya and rapeseed oils. The results of these 3 treatments on dioxin, dioxin-like (DL) PCB and PBDE concentrations in fish feed and flesh are described below.

Materials and methods

Three diets (9 mm) with the same basal composition, but coated with 3 different oils, were prepared at the BioMar Tech Centre, Brande, Denmark. The diets were formulated to satisfy the nutritional requirements of salmonid fish, and contained 33% protein and 34% lipid. The 3 diets were a) 100% northern fish oil (cNFO) as control, b) 100% decontaminated northern fish oil (deNFO), and c) a blend of the vegetable oils (VO) with southern hemisphere fish oil (40% southern fish oil/30% rapeseed oil/30% soybean oil; SFO:RO:SO). The deNFO was the same product and batch as the cNFO following the decontamination process. Atlantic salmon (*Salmo salar*) of initial mean weight 0.78 ± 0.01 kg were fed for 11 weeks with one of the 3 diets in $5m^3$ net pens with 120 fish/pen at Fjord Research Station, Dønna, Norway. Each diet was fed to triplicate groups of salmon. Samples of diet and flesh [Norwegian Quality Cut (NQC)] were collected and wrapped in foil and immediately frozen at -20 °C. Each sample of NQC comprised 3 pooled samples per cage, the samples selected being close to the average fish weight in each cage, to provide three samples per treatment group. Samples of ground diet and freeze-dried flesh were extracted using *iso*-hexane in an automated solvent extractor (ASE™, Dionex, Camberley, UK) and the lipid extracts fractionated and purified using a Powerprep™ sample processing module (Fluid Management Inc., Waltham, MA, USA) to produce dioxin, non-ortho and mono-ortho PCB fractions. PBDEs were extracted by ASE and after sample clean-up and concentration were analysed by GC/MS (Thermo Fisher Scientific Trace DSQ, Hemel Hempstead, UK). Seven PBDE congeners were measured namely 28, 47, 99, 100, 153, 154 and 183. For the dioxins and PCBs the 17 dioxins and furans plus the 12 DL-PCBs assigned WHO TEF values were measured. After final sample clean up and concentration, dioxin/furan and PCB fractions from the Powerprep™ were analysed on a Thermo Fisher Scientific Polaris Q, GC/MS/MS. Fatty acid methyl esters, prepared from diets and flesh, were separated and quantified by gas-liquid chromatography (Carlo Erba

Vega 8160, Milan, Italy) using a 30m x 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-column injection.

Results and discussion

Dietary dioxin, PCB and PBDE concentrations

The two-stage fish oil decontamination process performed by FF Skagen, Denmark involves an initial adsorption using activated carbon that should remove ~90% of the dioxins followed by a thin-film deodourisation step that removes up to 95% of PCBs including pesticides and other contaminants, as well as removing free fatty acids and peroxides. Similarly, Berntssen et al [6] showed that active carbon removes PCDD/F from fish oils but to a lesser degree DLPCB, of which the mono-ortho PCB are removed with least effectively. PBDEs are not removed at all by active carbon. The use of short path distillation techniques gave, depending on the process conditions, a maximum 98-99% reduction in PCDD/F and DLPCB, as well as PBDE. The concentrations of DL-PCBs in feeds (pg/g wet weight) are shown in Figure 1. The reduction of mono-ortho PCBs due to fish oil decontamination averaged 98%, compared to the cNFO diet, with individual congener reductions in the range 71-99%. The reduction of non-ortho PCBs due to fish oil decontamination also averaged 98% with individual congener reductions in the range 93-100% (Figure 1). The reduction in mono-ortho DL-PCBs between the cNFO diet and the SFO:RO:SO diet averaged 98% with individual congener reductions reduced by 98-99%. Similarly for non-ortho PCBs the average reduction in the SFO:RO:SO diet compared to the cNFO diet was 97% with individual congener concentrations being reduced by 93-99%. A reduction of PCDD/DF of 97% was seen for both the deNFO and SFO:RO:SO diets also. When quoted as sum ng TEQ/kg diet, for the 17 dioxins and 12 DL-PCBs with assigned WHO TEF values, the concentrations were cNFO, 17.36; deNFO, 0.44 and SFO:RO:SO, 0.53. The current EU limit values for combined dioxins and DL-PCBs are 7 ng TEQ/kg feed. The value for the cNFO diet significantly exceeds this limit value while the deNFO and SFO:RO:SO would represent 6.3 and 7.6% of the EU limit[7], respectively.

The concentrations of the 7 PBDE congeners in feeds (ng/g wet weight) are shown in Figure 2. The reduction of the sum PBDEs was 68% for the deNFO compared to the cNFO and 95% due to replacement of cNFO with SFO:RO:SO. The individual PBDE congeners were reduced by 35-94% in the deNFO diet and by 93-100% in the SFO:RO:SO diet compared to the cNFO diet.

Salmon flesh dioxin, PCB and PBDE concentrations

There were no differences in the growth of salmon in the 3 study groups and all fish grew well attaining final weights of ~2.2kg after the 11 week trial period. In the initial flesh of fish entering the trial, the sum of the 12 DL-PCBs were 1034 ng/kg wet weight. After feeding the cNFO diet for 11 weeks the flesh concentration of the 12 DL-PCBs increased to 9269 ng/kg wet weight while the flesh of fish fed the deNFO and SFO:RO:SO had reduced sum DL-PCB concentrations of 629 and 517 ng/kg wet weight, respectively. If these results are expressed as ng TEQ/kg flesh, for the 29 congeners described above, the concentrations for the initial product were 0.73 ng TEQ/kg. After 11 weeks feeding the experimental diets the value for fish fed the cNFO diet was increased to 6.82 ng TEQ/kg flesh while the values for fish fed the deNFO and SFO:RO:SO diets were reduced to 0.27 and 0.24, respectively. The current EU limit values for combined dioxins and DL-PCBs are 8 ng TEQ/kg flesh[8]. Thus, the dioxin + DL-PCB concentration for the fish fed the cNFO diet is just below the current EU limit value of 8 ng TEQ/kg while the flesh concentrations in fish fed the deNFO and SFO:RO:SO diets would represent 3.4 and 3.0% of the EU limit value, respectively.

The concentrations of the sum of 7 PBDE congeners in the initial salmon flesh were 0.26 ng/g wet weight which increased to 0.94 ng/g after feeding the cNFO diet for 11 weeks. In fish fed the deNFO diet the value was similar to the initial flesh value at 0.25 ng/g but decreased to 0.09 ng/g in fish fed the SFO:RO:SO diet.

Dioxins and furans can arise from natural processes such as forest fires and incomplete combustion of organic matter, as well as from industrial processes. The DL-PCBs are synthetic products used in electrical transformers, heat exchange fluids, hydraulic oils and plastic manufacturing. Although production of PCBs has been banned since 1976, they have been deposited in the oceanic benthos and they are widely distributed across the marine

biota. However, levels of both dioxins and PCBs in the environment have been declining since the 1950s, although, due to their persistent nature, they will remain in the biota for a considerable period. The EU has recently revised dioxin limits and assigned new limits for the 12 dioxin-like (DL) PCBs, such that combined values of 8 ng TEQ/kg for fish products and 7 ng TEQ/kg for fish feeds are now in place[7,8]. These new limits mean that some fish oils, particularly those from Northern latitudes that may be affected by previous industrial activity, are no longer useable in fish diets.
The polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in a wide variety of household furniture and electrical equipment since their introduction in the 1980s. However, due to their lipophilic and persistent characteristics the PBDEs are currently increasing in the environment, including fish products.

Recently, the authors of the present study have investigated the use of VOs in salmon diets as a way of reducing contaminant loading in farmed salmon. In the present study, the cNFO was chosen to be high in contaminants in comparison to the deNFO and SFO:RO:SO diets and does not reflect fish oils currently used by the salmon industry. By comparison to the DL-PCB value seen in fish fed cNFO of 7.82 ng TEQ/kg flesh, the value measured in fish fed FO by Bell et al.[9] was 1.48 ng TEQ/kg while salmon fed 100% VO had a value of 0.58 ng TEQ/kg. In a similar study by Berntssen et al[6,10] fish fed FO had DL-PCB concentrations of 1.81 ng TEQ/kg flesh while those fed 100% VO had 0.12 ng TEQ/kg. The values quoted for Scottish farmed salmon, fed FO-based diets by Hites et al.[5] were ~3 ng TEQ/kg but these also included dioxins and furans so are not directly comparable with the values for DL-PCBs alone. Direct comparison between studies is, however, difficult since factors such as time of exposure, feed conversion, and growth rate strongly determine the fillet concentrations in addition to feed concentrations[11].

Flesh n-3 HUFA levels were unaffected by the dietary oil decontamination process as the deNFO fish had similar n-3 HUFA levels to those fed the cNFO diet. However, the n-3 HUFA were reduced by up to 40% in fish fed the SFO:RO:SO, compared to fish fed the cNFO diet.

In conclusion, these values confirm that replacing marine fish oils with VO in aquafeed formulations can significantly reduce dioxin and DL-PCB concentrations in farmed salmon flesh. While the reduction in flesh concentrations of dioxins and PCBs arising from the use of VO in aquafeeds is to be welcomed, the reduction in n-3 HUFA that accompanies the use of VO is potentially detrimental. However, in the present study the use of deNFO significantly reduced the concentrations of contaminants in fish flesh while leading to no losses of health beneficial n-3 HUFA. Clearly, the use of diets high in VO for the majority of the salmon production cycle, where most of the dietary fatty acids are used to provide energy for growth, can significantly reduce contaminant concentrations in fish flesh. Use of deNFO as a pre-market finishing diet could effectively restore n-3 HUFA levels in the flesh while resulting in no or minimal increases in contaminant concentrations. Using this methodology farmed Atlantic salmon can be regarded as a very safe product that is rich in n-3 fatty acids and at the same time containing minimal levels of organic contaminants.

References:

1. Simopoulos, A.P. *Am. J. Nutr.* 1999; 70: 560S.
2. FAO, 2007. Fishery Statistical Collections. Fishery Information Data and Statistics Unit (FIDI) – FIGIS Data Collection. FAO, Rome.
3. Bell, J.G., McEvoy, J., Webster, J.L., McGhee, F., Millar, R.M. and Sargent, J.R. *J. Agric. Food Chem.* 1998; 46: 119.
4. Bell, J.G., McGhee, F., Dick, J.R. and Tocher, D.R. *Aquaculture* 2005; 243: 305.
5. Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A. and Schwager, S.J. *Science* 2004; 303: 226.
6. Berntssen, M.H.G., Oterhals, Å, Lie, Ø. Lundebye, A.-K. *Organohalogen Compounds* 2006; 68: 624.
7. European Commission Directive 2006b/13/EC amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs.
8. European Commission Regulation 199/2006a amending Regulation (EC) 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs.

9. Bell, J.G., McGhee, F., Dick, J.R. and Tocher, D.R. *Aquaculture* 2005; 243: 305.
10. Berntssen, M.H.G., Lundebye, A-K. and Torstensen, B.E. *Aquacult. Nutr*. 2005; 11: 219.
11. Berntssen, M.H.G. Giskegjerde, T.A.Rosenlund,G.,Torstensen,B.E., and Lundebye, A-K. *Environ. Chem Toxicol*. 2006; 13:23.

Figure 1: Concentrations of DL-PCB congeners in the control fish oil (cNFO), decontaminated fish oil (deNFO) and 40% southern fish oil/30% rapeseed/30% soya oil diet (SFO:RO:SO).

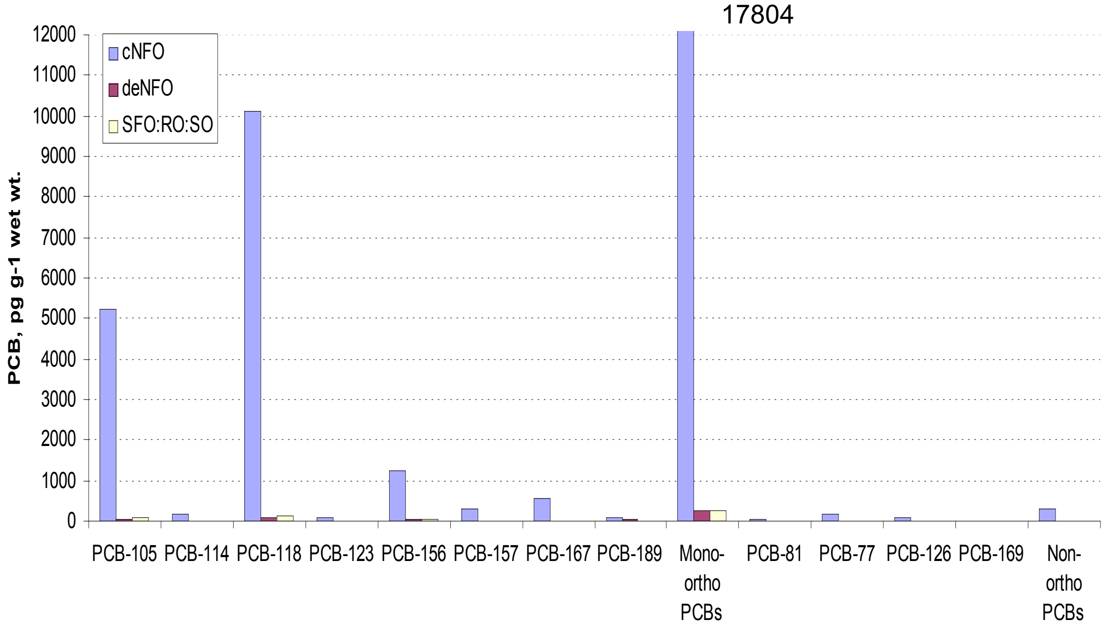

Figure 2: Concentrations of PBDE congeners in the control fish oil (cNFO), decontaminated fish oil (deNFO) and 40% southern fish oil/30% rapeseed/30% soya oil diet (SFO:RO:SO).