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Dioxin and dioxin-like polychlorinated biphenyls (PCBs) in Scottish farmed salmon: effects of replacement of dietary marine fish oil with vegetable oils.

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Abstract

Duplicate groups of Atlantic salmon were fed one of four practical-type diets from first feeding to harvest after 115 weeks. The four diets were low fish oil (16% w/w, LFO), high fish oil (35% w/w, HFO), low vegetable oil (17%, linseed oil/rapeseed oil, 1:1 w/w; LVO) and high vegetable oil (35%, linseed oil/rapeseed oil, 1:1 w/w; HVO). Following sample collection of fish around 2 kg weight all groups were switched to the HFO diet for a further 24 weeks. The dioxin concentration in diets was in order, HFO > LFO > LVO > HVO with values ranging from 0.16-1.4 ng TEQ/kg. The dioxin-like PCB (DL-PCB) concentrations were in the same order with values ranging from 0.62-3.68 ng TEQ/kg. Concentrations of dioxins and DL-PCBs in flesh samples were correlated with feed concentrations although values in flesh were always lower than in feed. Flesh dioxin concentrations ranged from 0.10-0.53 ng TEQ/kg and DL-PCBs from 0.58-1.48 ng TEQ/kg. After 24 weeks feeding a fish oil-containing finishing diet (HFO) the flesh dioxin concentrations ranged from 0.20-0.54 ng TEQ/kg and the DL-PCBs from 0.66-1.07 ng TEQ/kg. Feeding the HVO diet resulted in significant reductions in flesh concentrations of 20:5n-3 (EPA) and 22:6n-3 (DHA) to around 25% of the values in fish fed the HFO diet. However, feeding the HFO finishing diet to all groups for 24 weeks resulted in restoration of flesh EPA and DHA concentrations to 80% of the values in fish fed the HFO diet throughout. This study suggests that salmon cultured on diets based on fish meal and oil (HFO) attain flesh dioxin concentrations that are <14% of the current European Commission limit. However, by replacing marine fish oils with vegetable oils for most of the production cycle dioxin and DL-PCB concentrations can be substantially reduced.

Keywords: salmon, polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), polychlorinated biphenyls (PCBs), fish oil, vegetable oils.

1. Introduction

Polychlorinated dibenzodioxins and polychlorinated dibenzofurans (dioxins) can be produced naturally, arising from, for example, forest fires and incomplete combustion of organic matter, as well as from industrial chemical processes. Dioxin-like polychlorinated biphenyls (DL-PCBs) arise from man made products such as electrical transformers, heat exchange fluids, hydraulic oils and plastic manufacture. Although production of these chemicals is now banned they have been deposited in the oceans, due to industrial activity over the last century, where they are distributed across the marine environmental biota (North Sea Task Force, 1993). Dioxins and DL-PCBs are highly lipophilic and have biological half-lives measured in decades which means they tend to accumulate in predators at the top of the food chain (Froescheis et al. 2000).

The use of fishmeals and oils in aquafeeds, to supply essential amino acids, fatty acids and dietary energy, has been scientifically and economically sound. However, the feed grade fisheries that supply fish meal and oil have reached sustainable limits (Sargent & Tacon, 1999) and, if aquaculture production is to expand, alternative raw materials must be investigated and introduced. Replacement of significant amounts of either fish oil or meal can be achieved without loss of growth performance or affects on fish health (Bell et al. 2002, 2003; Torstensen et al 2004; Watanabe et al. 1999; Kaushik et al. 2004). However, when significant inclusion of vegetable oils occurs there is a marked reduction in the n-3 highly unsaturated fatty acid (HUFA) content of the flesh (Bell et al. 2002, 2003; Torstensen et al 2004). The reduction in n-3 HUFA, principally eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid

(22:6n-3; DHA), could potentially be offset by reduced levels of dioxins and DL-PCBs in salmon flesh as vegetable oils generally contain lower levels of these pollutants than most marine fish oils (Ministry of Agriculture, Fisheries and Food, 1995; European Commission (EC), 2000a; Jacobs et al. 2002a & b). Atlantic salmon currently produced in Scotland, fed on diets containing only marine fish oil, are of high nutritional quality and are rich in EPA and DHA (Bell et al. 1998; Aursand et al. 2000). However, recent literature has suggested that farmed salmon from Northern Europe contains elevated concentrations of dioxins and DL-PCBs due to dietary input from fish oils and meals (Jacobs et al 2002a & b, Hites et al. 2004). The values quoted (Hites et al. 2004) of ~3 ng /kgWorld Health Organisation Toxic Equivalent values (WHO TEQ) for dioxins + DL-PCBs are well within the EU permitted values for salmon of 4.0 ng TEQ/kg, for dioxins alone, which were implemented in July 2002.

In the present study, Atlantic salmon were fed one of four diets from first feeding through to harvest. The diets were low fish oil (LVO), high fish oil (HFO), low vegetable oil (LVO) and high vegetable oil (HVO) where the vegetable oil was a mixture of rapeseed and linseed oil (1:1 w/w). The fish were sampled at ~2kg and thereafter all fish were switched to a HFO finishing diet for 24 weeks. The aim was to investigate the influence of feeding dietary VO on flesh dioxin and DL-PCB concentrations as well as the effects of feeding a FO finishing diet on flesh dioxin/DL-PCB and n-3 HUFA concentrations.

2. Materials and methods

2.1 Fish, culture conditions and diets

In March 2000, 24,000 Atlantic salmon fry were distributed randomly into 8 square fibreglass tanks (3m x 3m, depth 0.5m) at Kinlochmoidart smolt unit (Marine Harvest (Scotland) Ltd., Highland, Scotland) and weaned onto the experimental extruded diets. Each diet was fed to duplicate tanks of fry/parr at either 14% (L) or 25% total oil (H), resulting in four dietary treatments in total, LFO (low fish oil), HFO (high fish oil), LVO (low vegetable oil) and HVO (high vegetable oil). Fish were fed the experimental diets until sea water transfer in April 2001, at which point fish (average weight ~ 40g) were transferred into 5m x 5m net pens (600 fish/pen) at Loch Duich, Lochalsh, Scotland. The fish were fed the same diets in seawater as in freshwater although the dietary oil levels were increased to 17% in the low oil diets (LFO and LVO) and 30% (3mm pellet) with the latter increasing to 35% (6 and 9mm pellets) in HFO and HVO diets. The diets were formulated and manufactured by the major salmon feed producers; Ewos (freshwater phase; Ewos Technology Centre, Livingston, Scotland), BioMar (sea water ongrowing to ~1 kg; BioMar A/S, Brande, Denmark) and Skretting (seawater phase to 2kg and finishing diet; ARC Nutreco, Stavanger, Norway). All diets were formulated to satisfy the nutritional requirements of salmonid fish (National Research Council 1993). Diet formulations and proximate compositions of the 6 & 9 mm diets are shown in Table 1. Fish were ongrown until June 2002 at which time they had reached ~2 kg. Thereafter, all treatments were switched to the HFO feed for 24 weeks when they had reached ~4.5kg.

2.2 Sample collection

Samples of 6 mm and 9 mm feed, from each of the four dietary treatments, analysed for dioxin and DL-PCB were collected from the centre of a newly opened feed bag and placed in a glass jar. Equal quantities of each feed size were mixed before grinding. The 9 mm finishing diet was the same as the HFO diet and a sample of this feed was collected as described above. Dioxin and DL-PCB concentrations were measured in single analyses of each 6 and 9 mm composite or 9 mm finishing diet.

In June 2002, when fish had reached an average weight of ~2 kg, 12 fish per treatment were sampled for dioxin and DL-PCB analysis and a further 12 were sampled for flesh fatty acid analysis. The fish were killed by a blow to the head and a steak cut from the Norwegian Quality cut region. Each individual steak for dioxin analysis was immediately wrapped in aluminium foil and frozen on dry ice. The steaks were pooled to provide three samples of flesh from 4 individual fish for each dietary treatment. Before analysis, the steaks were blended in a food processor, after removal of skin, bones and the dorsal fat body. Similar samples of pooled flesh were processed in the same way and the homogenised flesh frozen prior to lipid extraction and fatty acid analysis.

2.3 Analysis of dioxins and DL-PCBs

All analytical procedures for dioxin and DL-PCB analysis were sub-contracted to the United Kingdom Accreditation Service (UKAS) accredited laboratories of Scientific Analytical Laboratories Ltd. (SAL), Medlock House, New Elm Road, Manchester M3 4JH. All analyses were conducted in accordance with SAL Standard Operating Procedures.

2.3.1 Sample extraction and clean up procedures

For feed samples a 50 g aliquot was ground and placed in a pre-extracted Soxhlet thimble, spiked with ^{13}C-labelled internal standards and extracted with 300 ml toluene for 16h in an automated soxhlet extraction apparatus. For flesh samples a 50 g aliquot was spiked with internal standards as for feed samples, and digested in concentrated HCl overnight. The solution was then back extracted with 200 ml of hexane/dichloromethane (1:1 v/v) for 16h. A method blank was processed in the same manner. The extracts were then purified by acid washing followed by elution through a column combining sulphuric acid impregnated silica, potassium hydroxide impregnated silica and anhydrous sodium sulphate. The eluate from this column was further purified on a column containing activated Florasil and then concentrated to near dryness prior to analysis on a gas chromatograph/mass spectrometer (GC/MS) apparatus. Immediately prior to GC/MS analysis the sample, dissolved in nonane was spiked with recovery standard, $^{13}C_6$-1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TCDD).

2.3.2 GC/MS methodology

The dioxin and DL-PCB analyses were carried out using a HP 5890A GC coupled to a VG Autospec Ultima mass spectrometer. Samples were injected using an HP7673B autosampler and data was collected and processed using the VG OPUS package. The GC was equipped with a DB-5ms capillary column (60m x 0.25mm i.d, 0.25μm film thickness) and helium was used as carrier gas with a head pressure of 30 p.s.i. The oven temperature program was 140ºC for 4 minutes then to 220ºC at 15ºC/min then to 240ºC at 1.5ºC/min, hold for 2 min then to 310ºC at 4ºC/min and held for 10 min. Injection was in splitless mode at 300ºC. The MS source was set at 30eV, the trap current was 700 μA and the source temperature was 250ºC. The samples were

analysed for the 17 PCDDs/PCDFs which have toxic equivalency to 2,3,7,8-TCDD and the 12 co-planar PCBs 77, 81,105, 114, 118, 123, 126, 156, 157, 167, 169 & 189. EC/WHO toxic equivalent factors (TEF) were used to convert dioxin/PCB values in ng/kg to WHO toxic equivalent values (TEQ).

2.4 Lipid extraction and fatty acid analysis

Total lipid contents of salmon flesh were determined gravimetrically after extraction by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Fatty acid methyl esters (FAME) were prepared from total lipids by acid-catalyzed transesterification in 2 ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (1982) and FAME extracted and purified as described previously (Ghioni et al. 1996). FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as carrier gas and temperature programming was from 50ºC to 150ºC at 40ºC/min and then to 230ºC at 2ºC/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman 1980). Data were collected and processed using the Chromcard for Windows (version 1.19) computer package (Thermoquest Italia S.p.A., Milan, Italy.

2.5 Statistical analysis

Significance of difference ($P < 0.05$) between dietary treatments was determined by one-way ANOVA. Differences between means were determined by Tukey's test. Data identified as non-homogeneous, using Bartlett's test, were subjected to log or

arcsin transformation before applying the ANOVA. ANOVA was performed using a GraphPad Prism (version 3.0) statistical package (GraphPad Software, San Deigo, CA, USA).

3. Results

Dioxin concentrations in the four experimental diets were in order HFO > LFO > LVO > HVO with the concentrations ranging from 1.40 to 0.16 ng TEQ/kg (Fig.1A). The finishing feed had a slightly higher concentration, than the combined 6 mm & 9 mm HFO feeds, of 1.7 ng TEQ/kg (Fig. 1A). DL-PCB concentrations in the four experimental diets were in the same rank order as for dioxins with concentrations ranging from 3.68 to 0.62 ng TEQ/kg although the concentration of DL-PCB in the finishing feed was considerably lower than that in the HFO experimental feeds at 1.31 ng TEQ/kg (Fig. 1B).

Dioxin concentrations in salmon flesh broadly followed the concentrations in the feeds with the highest value in fish fed the HFO diet (0.53 ng TEQ/kg) and the lowest in fish fed the LFO diet (0.10 ng TEQ/kg) although there were no significant differences between the dioxin concentrations in fish fed the LFO, LVO and HVO diets (Fig. 2A). DL-PCB concentrations in salmon flesh also followed the pattern in diets with the highest values in fish fed the HFO diet (1.48 ng TEQ/kg) with the lowest values in fish fed the LFO diet (0.58 ng TEQ/kg) although, as for dioxins, there were no significant differences between the DL-PCB concentrations in fish fed the LFO, LVO and HVO diets (Fig. 2B).

After the initial sampling of fish fed the four experimental diets the remaining fish were switched to a FO-containing finishing diet (the HFO 9 mm diet) for a further 24

weeks. Following the 24 week finishing diet period, fish were sampled again for flesh dioxin and DL-PCB analysis. Dioxin concentrations, after 24 weeks on the finishing diet, were still significantly higher in fish fed the HFO diet throughout (0.54 ng TEQ/kg) compared to fish previously fed the other three diets. The lowest values were in fish previously fed the HVO diet (0.20 ng TEQ/kg) and they were significantly lower than fish previously fed both the LFO and HFO diets (Fig. 3A). Apart from fish previously fed the HFO diet fish from all other groups had significantly higher flesh dioxin concentrations after the finishing diet period compared to the concentrations after the experimental diet phase 24 weeks earlier (Fig. 3A). DL-PCB concentrations, after the 24 weeks on the finishing diet, were significantly higher in fish fed the HFO diet throughout (1.07 ng TEQ/kg) compared to fish previously fed the LVO and HVO diets (Fig. 3A). Fish previously fed the LFO and HVO diets had significantly higher flesh DL-PCB concentrations after the finishing diet period compared to the concentrations after the experimental diet phase 24 weeks earlier (Fig. 3B).

Flesh EPA concentrations were significantly different for each of the treatment groups with the highest values in fish fed HFO (7.0%) and the lowest in fish fed HVO (1.4%) (Fig. 4A). After feeding the FO finishing diet for 24 weeks flesh EPA concentrations in fish previously fed HVO were still significantly lower than fish previously fed HFO or LVO but had been restored to 80% of the value in the HFO fish (Fig. 4A). Flesh DHA concentrations were significantly different for each of the treatment groups with highest values in fish fed HFO (18.5%) and the lowest in fish fed HVO (4.9%) (Fig. 4B). After feeding the FO finishing diet for 24 weeks flesh

DHA concentrations in fish previously fed HVO were still significantly lower than fish previously fed HFO or LFO but had been restored to 80% of the value in the HFO fish (Fig. 4B).

4. Discussion

The dioxin and DL-PCB concentrations in the experimental diets followed a predictable pattern based on the FO content of the diets with the highest values in the HFO diet and lowest values in the HVO diet. The 9 mm finishing diet had a higher dioxin concentration, but a lower DL-PCB concentration, compared to HFO diet which was a composite of the 6 and 9 mm feeds. The FO in the finishing diet was capelin oil while that used in the 6 mm diet was herring oil. The concentration of dioxins and DL-PCB in capelin is generally lower than that in herring although the values can vary seasonally and geographically for both species (Rappe et al. 1989; Lundebye-Haldorsen and Lie, 1999; EC 2000a). All diets used in this study were considerably lower than the European Union (EU) permitted value for dioxins in fish feeds of 2.25 ng TEQ/kg (EC 2000a). The highest dioxin concentration in the feed used was 1.7 ng TEQ/kg for the 9 mm feed containing capelin oil followed by the 6 mm/9 mm composite at 1.4 ng TEQ/kg where the 6 mm feed contained herring oil. The lowest dioxin concentration was in the HVO feed being 0.16 ng TEQ/kg or 9% of the value in the finishing feed and 7% of the EU recommended limit (EC 2000a). In the recent publication by Hites et al. (2004) dioxin concentrations in Scottish feed samples ranged from 3-7 ng TEQ/kg with lowest values ~0.5 TEQ/kg being found in feeds from British Columbia, Canada. However, the dioxin values quoted by Hites et al (2004) were based on dioxins plus DL-PCBs. In the feeds from the present study

the values for dioxins plus DL-PCBs ranged from 5.1 (HFO) to 0.78 ng TEQ/kg (HVO).

In general, the flesh dioxin and DL-PCB concentration varied in accordance with the concentration in the diet although flesh values were always considerably lower than those in the diet fed. However, fish fed the LFO diet had the lowest mean flesh dioxin concentration, but the second highest diet dioxin concentration, although the value was not significantly different from fish fed LVO and HVO. The lower dioxin concentration in LFO flesh may have been due to these fish having a significantly lower mean weight at harvest (1.44 ± 0.37kg) compared to fish fed the LVO (1.71 ± 0.36), HVO (2.02 ± 0.57) or HFO (1.88 ± 0.57) diets (Tocher et al. 2004). Salmon generally deposit more flesh lipid as a function of growth rate, feed intake and dietary lipid concentration (Lie, 2001).

In the period from 1995-1999 the EU undertook a comprehensive analysis of dioxin and PCB levels in foods for human consumption across the EU and EFTA members states (EC 2000b). In farmed salmon they found dioxin values in the range 0.43-1.04 ng TEQ/kg wet weight and in farmed trout the range was 0.24-0.81 ng TEQ/kg. The values in the present study, for fish fed HFO, fall within the lower end of this range (0.53) while values for fish grown on VO diets were below this range both at 2kg harvest weight (~0.15) and in HVO fish fed the HFO finishing diet for 24 weeks (0.2 ng TEQ/kg). Values quoted for fatty fish in the EC (2000b) report, including anchovy, herring, mackerel, sardine, tuna and wild salmon, ranged from 0.01-7.04 ng TEQ/kg, with lowest concentrations in tuna and highest concentrations in Baltic

salmon. When the tuna values, along with those for fish caught in the Baltic, were removed the range was 0.29-2.10 ng TEQ/kg (EC 2000b).

Some studies describing dioxin and DL-PCBs in foodstuffs present data as pg TEQ/g lipid. When presented in this way the dioxin concentrations in fish from this study had values ranging from 1.87 pg TEQ/g lipid, in fish fed the HVO diet, to 7.69 in fish fed the HFO diet. In two studies by Jacobs et al. (2000, 2002b), the range for samples of Scottish and Norwegian farmed salmon was 4.7-11.5 pg TEQ/g lipid. Our own value for Scottish salmon grown on FO for the whole production cycle is intermediate within this range while fish grown on the HVO diet have a considerably reduced flesh dioxin concentration compared to any of the farmed fish sampled by Jacobs et al. (2000, 2002b). Interestingly, the highest value for flesh dioxin found by Jacobs et al. (2000, 2002b) was 18.21 pg TEQ/g lipid from a wild Scottish salmon caught in the River Deveron.

In the present study, the DL-PCB concentration (12 congeners) in salmon flesh was in the range 0.58-1.48 ng TEQ/kg wet weight. These values are lower than those quoted in a recent report by the RIVO Fisheries Institute of Holland (2000) which found values up to 4 ng TEQ/kg in farmed salmon from Norway and Scotland. When the DL-PCB values from the present study are quoted as pg TEQ/g lipid the range is from 8.7 pg TEQ/g lipid, in fish fed the HVO diet, to 22.84 in fish fed the LFO diet. By comparison, the range of Jacobs et al. (2000, 2002a) was 9.05-24.82 (7 congeners) in farmed Scottish and Norwegian salmon, ~20 pg TEQ/g lipid found by the RIVO Fisheries Institute of Holland (2000), 15.4-43.7 in Scottish salmon (Jacobs et al. 2002b). A study of one Atlantic and three Pacific farmed salmon from Canada found

slightly lower concentrations of DL-PCBs (12 congeners) ranging from 3.81-10.54 pg TEQ/g lipid (Easton et al. 2002). Values for wild Pacific salmon were considerably lower in the range 0.26-1.11 pg TEQ/g lipid (Easton et al. 2002) although those for wild Atlantic salmon were similar to farmed salmon being in the range 17.02-23.99 pg TEQ/g lipid (Jacobs et al. 2000, 2002 a & b).

The dioxin concentration in salmon flesh samples quoted by Hites et al. (2004), which quoted dioxins to include dioxins plus DL-PCBs, was ~3 ng TEQ/kg for Scottish salmon. In the present study, the values for flesh dioxins plus DL-PCBs ranged from 2.00 ng TEQ/kg (HFO) to 0.73 ng TEQ/kg (HVO) with lowest in LFO flesh (0.68 ng TEQ/kg). The values in the latter two groups were found in wild Pacific salmon (Hites et al 2004). The combined values for dioxins and DL-PCBs are relevant to current EU advice for human consumption which recommends a maximum combined intake of 14 pg TEQ/kg body weight/week (SCF 2000). For a 75 kg adult this equates to ~1000 pg TEQ/week. Based on the values from the present study this would allow around two 200g portions of Scottish salmon per week when grown on FO diets or around six 200g portions if salmon grown on the HVO diet were consumed. However, it is important to consider that the majority of dioxins consumed in Europe comes from other foodstuffs, particularly dairy products, and not principally from seafood. Taking this into account, the present recommendation of the UK Food Standards Agency that we consume one portion of oily fish per week then the values for dioxin plus DL-PCBs in Scottish salmon would still leave consumers well within the EU recommended consumption limit.

Feeding diets containing 100% of added oil as LO and RO (1:1 w/w) from first feeding to harvest at 2 kg resulted in flesh EPA and DHA concentrations being reduced to around 25% of the concentrations found in fish fed the HFO diet over the same period. However, after feeding the HFO finishing diet for 24 weeks flesh EPA and DHA concentrations in fish previously fed HVO were still significantly lower than fish previously fed HFO but were restored to 80% of the values in the fish fed HFO throughout. This result supports a number of recent studies in salmon post-smolts fed diets containing either RO or LO where restoration of flesh concentrations of EPA and DHA was achieved by feeding a FO finishing diet for periods between 16 and 24 weeks (Bell et al. 2003, 2004 Torstensen et al. 2004). These data have been further supported by modelling studies demonstrating the application of a dilution model to predict tissue fatty acid changes following alteration of dietary oils in Atlantic salmon and other species (Jobling, 2004, Robin et al. 2003).

5. Conclusion

This study has shown that Atlantic salmon can be cultured, over the whole production cycle, using diets in which 100% of added dietary fish oil (FO) can be replaced by a blend of rapeseed (RO) and linseed oils (LO) without detriment to growth performance. While flesh concentrations of EPA and DHA were significantly reduced in fish fed vegetable oils (VO) these values could be restored, to 80% of the values in fish fed FO, by feeding a FO-containing finishing diet for 24 weeks. Feeding the 100% VO diet reduced flesh dioxin and dioxin-like PCB concentrations by 75% and 64%, respectively, compared to fish fed FO. After feeding the FO finishing diet for 24 weeks the flesh dioxin and dioxin-like PCB concentrations were

still 60% and 47% lower than in salmon fed FO throughout. While Scottish salmon cultured using diets based on fish meal and oil have flesh dioxin concentrations that are < 14% of current EU limits these values can be substantially reduced by the use of vegetable oils in feed formulations.

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Figure legends

Fig. 1. Concentrations of dioxin (A) and dioxin-like PCBs (B) in diets (ng TEQ/kg) used during the trial.

Fig. 2. Concentrations of dioxin (A) and dioxin-like PCBs (B) in flesh (ng TEQ/kg) samples collected after 115 weeks. Columns assigned a different superscript letter are significantly different ($P < 0.05$).

Fig. 3. Concentrations of dioxin (A) and dioxin-like PCBs (B) in flesh (ng TEQ/kg) samples collected after 115 weeks and after feeding the HFO finishing diet for 24 weeks. Columns assigned a different letter, within each feeding period, are significantly different ($P < 0.05$). Columns assigned an asterisk in the post-finishing diet group are significantly different from the same dietary group in the pre-finishing diet sample.

Fig. 4. Concentrations (g/100g total fatty acids) of flesh eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic acids (22:6n-3; DHA) before (0 weeks) and after (24 weeks) feeding the HFO finishing diet. Columns assigned a different letter, within each time point, are significantly different ($P < 0.05$).

Table 1

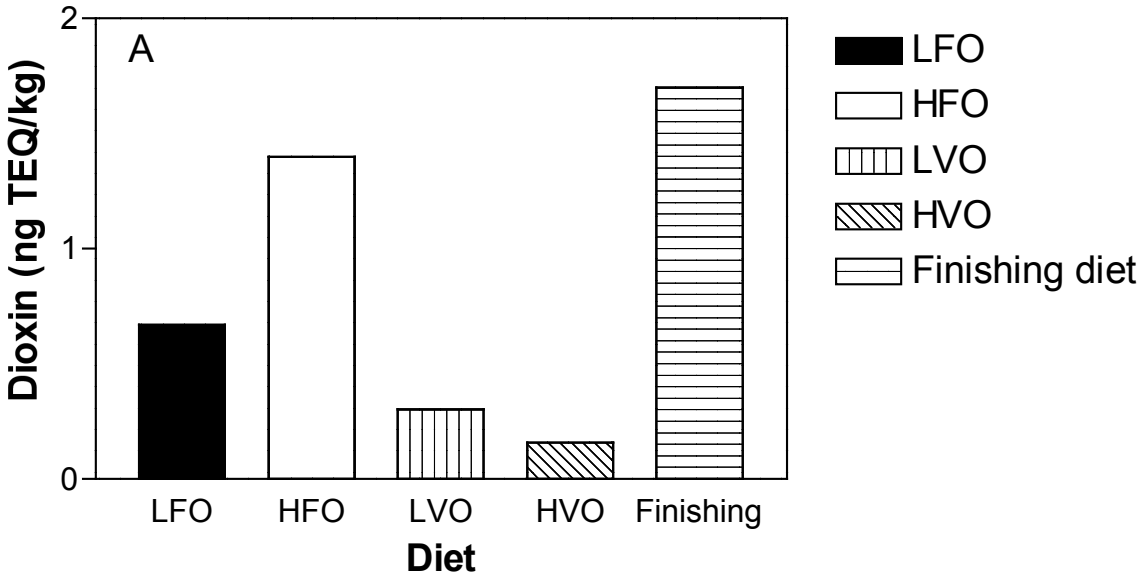
Diet formulations and proximate compositions (g/100g).

Component	LFO	LVO	HFO	HVO	LFO	LVO	HFO	HVO
Pellet size	6 mm[1]				9 mm[2]			
LT Fishmeal	35.2		43.5		32.9		44.5	
Wheat	22.0		13.5		33.2		14.8	
Soybean meal	12.0		6.5		11.4		0	
Other plant meals[3]	11.8		7.2		10.2		8.5	
Micrantrients[4]	1.0		1.0		0.4		0.4	
Fish oil[5]	14.6		29.2		12.0		31.8	
Rapeseed oil	7.3		14.6		6.0		15.9	
Linseed oil	7.3		14.6		6.0		15.9	
Protein (%)	38.0	37.2	39.7	37.6	39.2	39.4	38.9	39.5
Oil (%)	16.9	16.9	36.9	36.5	16.5	16.8	33.6	34.2
Moisture (%)	7.9	8.7	1.8	5.8	5.6	5.9	5.7	5.7
Ash (%)	7.6	7.6	8.8	8.7	6.4	6.6	6.5	6.6

[1]Diets formulated and prepared by BioMar A/S, Brande, Denmark. [2]Diets formulated and prepared by Nutreco ARC, Stavanger, Norway. [3]Wheat flour, wheat and maize glutens. [4]Vitamin and mineral premixes prepared according to individual company

specifications. Finnstim and Carophyll pink® to provide 60/mg astaxanthin. [5]Herring oil for 6 mm pellet and capelin oil for 9 mm pellet.

Fig.1.



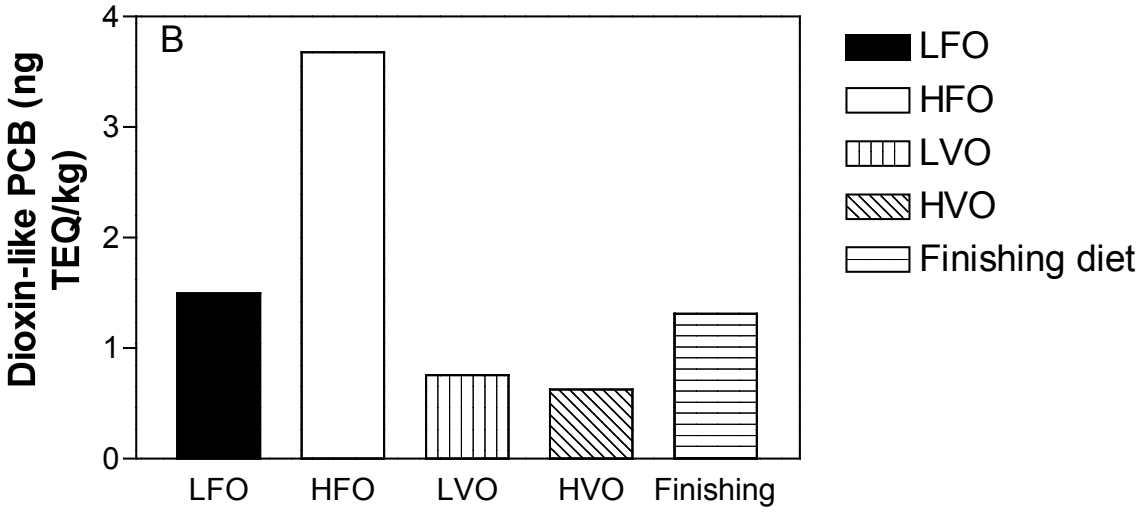


Fig. 2

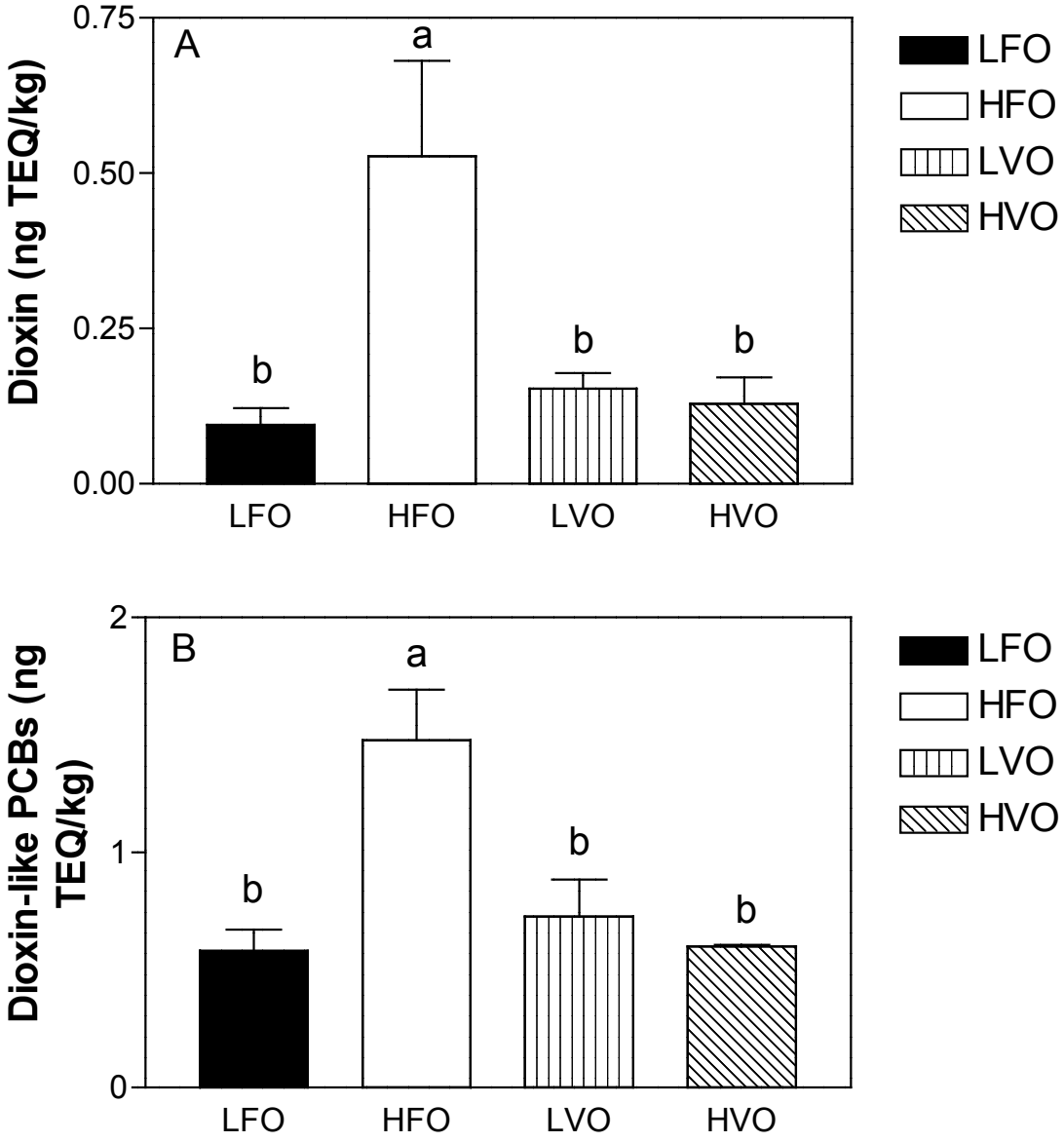

A
Dioxin (ng TEQ/kg)
0.75
0.50
0.25
0.00
a
b
b
b
LFO
HFO
LVO
HVO
B
Dioxin-like PCBs (ng TEQ/kg)
2
1
0
a
b
b
b
LFO
HFO
LVO
HVO

Fig. 3

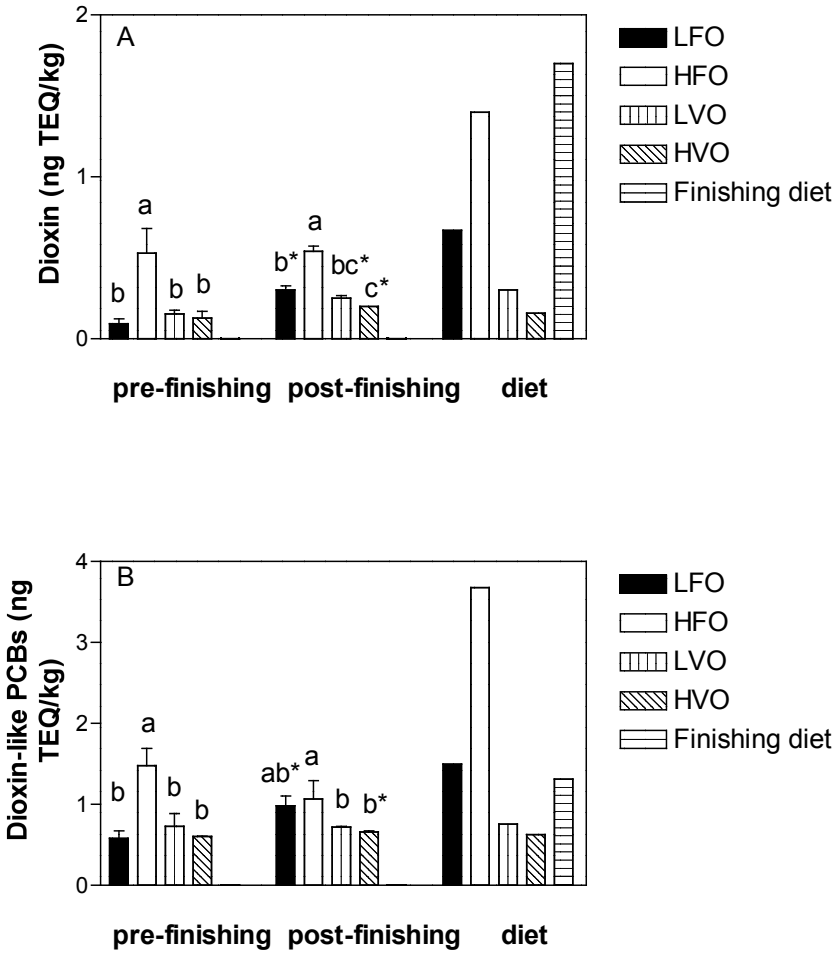
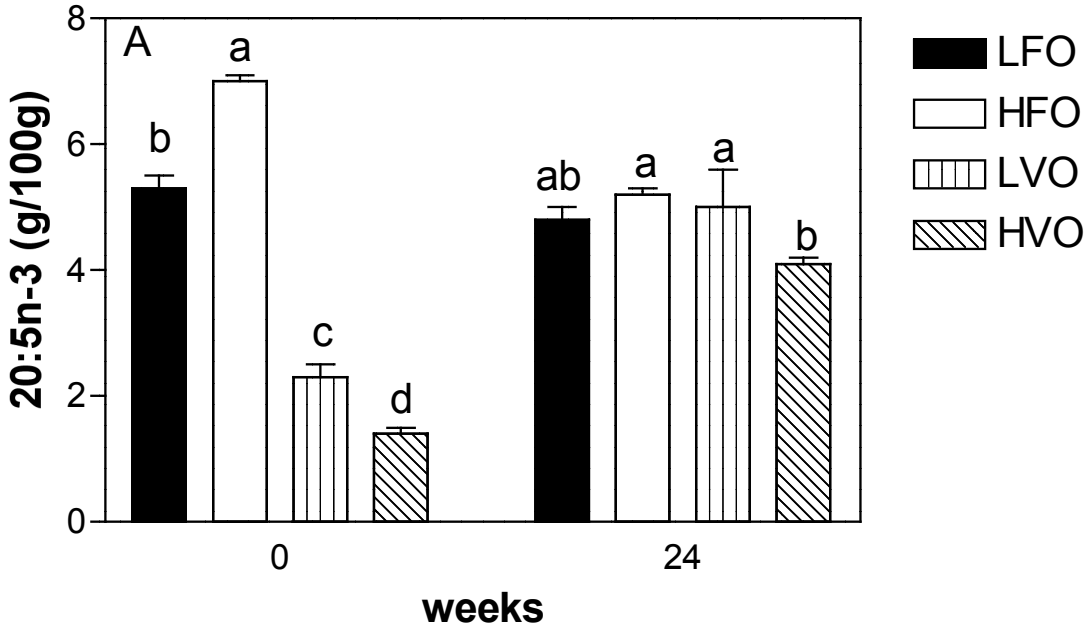
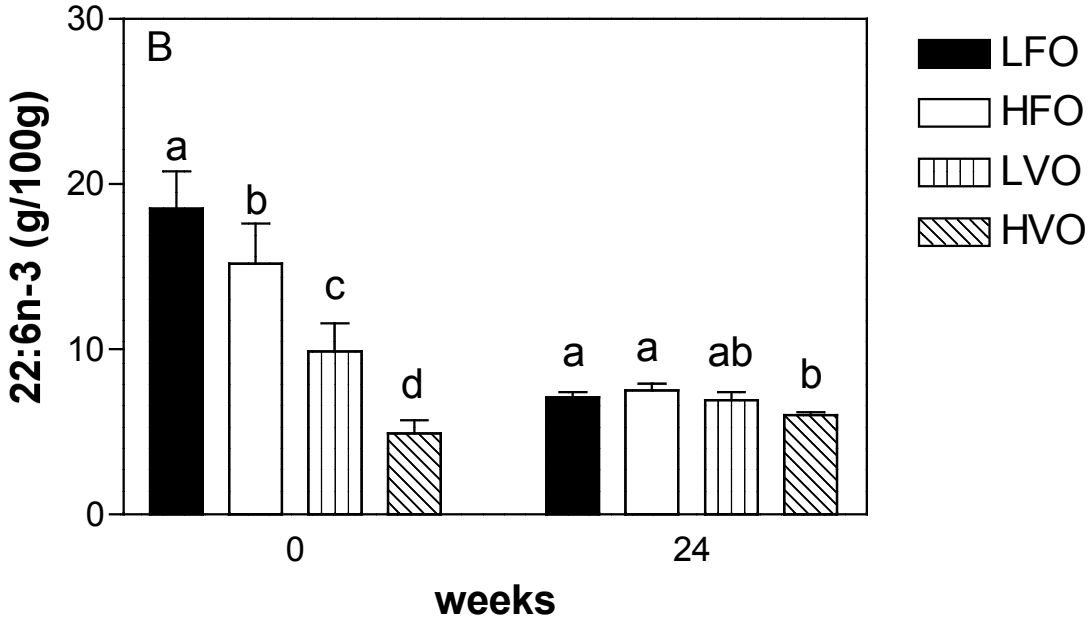

A
Dioxin (ng TEQ/kg)
2
1
0
b
a
b
b
b*
a
bc*
c*
pre-finishing
post-finishing
diet
LFO
HFO
LVO
HVO
Finishing diet
B
Dioxin-like PCBs (ng TEQ/kg)
4
3
2
1
0
b
a
b
b
ab*
a
b
b*
pre-finishing
post-finishing
diet
LFO
HFO
LVO
HVO
Finishing diet

Fig. 4.


A
20:5n-3 (g/100g)
8
6
4
2
0
a
b
c
d
ab
a
a
b
0
24
weeks
LFO
HFO
LVO
HVO


B
22:6n-3 (g/100g)
30
20
10
0
a
b
c
d
a
a
ab
b
0
24
weeks
LFO
HFO
LVO
HVO