Use of Dietary Flaxseed Oil to Reduce PCB loadings in Farmed Sablefish

Erin N Friesen[1], Michael G Ikonomou[2], Dave A Higgs[3], Brent J Skura[4]

[1]Faculty of Land and Food Systems, Univeristy of British Columbia
[2]Institute of Ocean Science, Department of Fisheries and Ocean
[3]West Vancouver Laboratory, Department of Fisheries and Oceans
[4]Faculty of Land and Food Systems, University of British Columbia

Introduction

Public confidence in farmed fish products fell with the release of several studies that indicated that farmed fish contained higher levels of persistent organic pollutants (POPs) than their wild counterparts.[1,2] Marine fish oils have traditionally been used in aquaculture feeds. However these oils have been suggested to be the main source of POPs in cultured fish.[3] Vegetable oils and animal fats have long been studied as alternative lipid sources in fish feed as a method of reducing costs and alleviating the pressures on the limited marine fish oil stocks. Due to the fact that vegetable oils have been found to have significantly lower levels of POPs[4] and because the deposition of dioxins in fish flesh has been found to correlate with the levels found in fish feed,[5,6] the use of vegetable oils in fish feed has recently been examined as a method of reducing POPs in farmed products.[3,7]

The current study examined the use of cold pressed flaxseed oil as an alternative lipid source in the diets of post-juvenile sablefish at replacement levels of 0, 25, 50 and 75% of supplemental anchovy oil. The sablefish is a highly valued marine finfish due to its white flaky flesh and high oil content and is currently being researched as a new aquaculture species on the West coast of Canada. This is the first study to examine the use of graded levels of a vegetable oil on the accumulation of polychlorinated biphenyls farmed marine species, which in this case was sablefish.

Materials and Methods

Juvenile sablefish (~50grams each) were purchased from Cluxewe Enterprises, Cedar British Columbia and were transported to the Department of Fisheries and Oceans and University of British Columbia Center for Aquaculture and Environmental Research (CAER), West Vancouver, British Columbia. The fish were acclimated in 1100 L tanks supplied with ambient aerated saltwater (salinity 29-32‰; dissolved oxygen 7.0-10.0 mg/l; temperature 7.3-11.1˚C), and were fed a commercial fish feed until the start of the feeding trial. In November 2003, fish were distributed into twelve 1100 L saltwater tanks to obtain triplicate groups of 23 sablefish (initial mean weight of all fish154.4 ±31.4g) per diet treatment. All groups were fed by hand twice daily to satiation for 15 weeks and all unconsumed pellets were counted and their mean air dry weight was used to obtain accurate daily feed intakes.

Four dietary treatments were formulated to contain equivalent digestible protein (460g kg^{-1}), lipid (190g kg^{-1}) and energy (18.7 MJ kg^{-1}) on a dry weight basis (Table 1). The supplemental lipid level (134.7g kg^{-1}) either consisted of 100% South American anchovy oil (control treatment) or blends of anchovy oil with cold pressed flaxseed oil. The diets were made and steam pelleted at CAER, and the pellet diameter was increased from 4mm to 6mm as fish grew.

On the initial day of the experiment, 3 composite samples of 2 left fillets were taken at random for contaminant analysis and on day 105 of the growth trial, 3 composite samples of two left fillets were randomly collected from each tank to obtain a sample size of n=9 per dietary treatment. Each fish was filleted on a clean piece of hexane-rinsed aluminum foil. The fillet pairs were wrapped in hexane-rinsed foil with the skin still on and then were placed into contaminant-free plastic bags for storage at -20ºC until analysis. Diets and oils were also collected in hexane rinsed containers and frozen at -20ºC until analysis. Using contaminant-free methods at the Institute of Ocean Science (IOS) Sydney, British Columbia, the fillets were processed prior to homogenization to produce a sample representative of the edible portion of the fish (removal of skin, fins and kidney). Knives and homogenization equipment were washed

and solvent rinsed between samples to prevent cross contamination.

Table 1 Main ingredient composition of experimental diets (g kg $^{-1}$ dry weight basis)

	Diets			
Ingredients	1	2	3	4
LT Anchovy Meal - South American	389.68	389.68	389.68	389.68
Blood Flour	39.13	39.13	39.13	39.13
Squid Meal	57	57	57	57
Krill Meal	77.69	77.69	77.69	77.69
Wheat Gluten Meal	56.79	56.79	56.79	56.79
Pregelatinized Wheat Starch	100	100	100	100
Lipid Supplement				
Anchovy Oil - South American	134.37	100.78	67.19	33.59
Flaxseed Oil	-	33.59	67.19	100.78
Vitamin and Mineral supplement	58.57	58.57	58.57	58.57
Soybean Lecithin	10	10	10	10
A-Cellulose	59.81	59.81	59.81	59.81
Permapell	10	10	10	10
DL-methionine	1.99	1.99	1.99	1.99
Chromic oxide	5	5	5	5

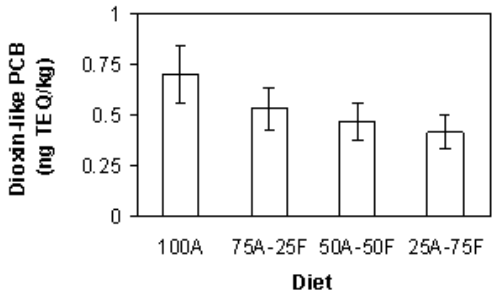
The methods used for PCB determination have been published previously[8] with a few modifications[9]. In short, 7 g-wet weight (ww) of fillet, 10g-ww of diets or 2g of oil were spiked with ^{13}C-labelled surrogate internal standards (Cambridge Isotope Laboratories). The samples were dried with sodium sulfate and extracted with 350 ml of 1:1 dichloromethane (DCM):hexane in extraction columns. Sample volumes were reduced to a few ml by rotary evaporation before being processed through 3 cleanup steps. Gel permeation techniques using 1:1 DCM:hexane and 70g of Biobeads S-X3 (Bio-Rad Laboratories) were used to remove larger lipid molecules. Acidic/basic silica columns eliminated polar lipids from the samples, and alumina columns were used to further remove non-target compounds by fractionation. The cleaned-up samples were fractioned using carbon fiber high-performance liquid chromatography. Extracted samples were spiked with surrogate recovery standards and were analyzed for PCBs by High Resolution Gas Chromatography / High Resolution Mass Spectrometry using 3 fused silica capillary columns. The two most abundant isotope ions of known relative abundance were monitored for each molecular ion. To ensure strict QA/QC procedures, each batch of 9 samples was processed with a procedural method blank, a replicate, a certified reference sample, and a glasswear proof. In this paper PCB concentrations are reported as toxic equivalents which were calculated using WHO toxic equivalent factors for the 12 congeners with dioxin-like properties.[10]

In addition to PCB analysis, the partial replacement of anchovy oil with flaxseed oil was evaluated on the basis of fish performance (weight gain, specific growth rate, feed efficiency etc.) fish health (haematological and immunological responses), carcass and muscle proximate composition, muscle fatty acid composition, flesh quality (color, aroma, texture, and taste), dietary protein and energy digestibility as well as analysis for full congener dioxins, furans, 20 organochlorine pesticides, polyaromatic hydrocarbons, toxaphene, and polybrominateddiphenyl ethers. However, the analyses of some of these parameters are ongoing and will not be discussed in this paper.

Results

PCB concentrations in the diets decreased from 0.70 to .418 ng TEQ/kg as the percentage of supplemental dietary flaxseed oil was increased from 0 to 75 (Figure 1). When sablefish were fed one of the four dietary treatments, the PCB concentration in the flesh was also found to decrease from 0.547 to .405 ng TEQ/kg as supplemental dietary anchovy was reduced to a level of 25% (Figure 2). When interpreting the results the following it should be mentioned that all have been processed through the CP-Sil 19CB and CP-Sil 5/18 CB columns. However some samples still require analysis on the DB-5 column. Hence only the data available for those samples with complete PCB congener profiles were used to calculate ratios for each congener relative to total PCB concentrations. These ratios were then used to estimate PCB concentrations for those samples with incomplete analysis. This method of estimation was verified by applying the ratio to samples with complete analysis and was found to predict congener concentration

with 80% accuracy.



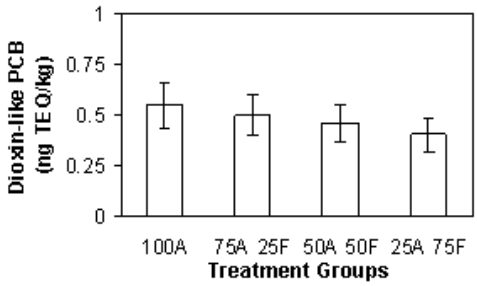


Figure 1 Concentration of dioxin-like PCBs (ng TEQ/kg) in diets composed of various percentages of supplemental anchovy oil (A) and flaxseed oil (F). Error bars represent 20% estimation due to incomplete analysis.

Figure 2 Accumulation of dioxin-like PCBs (ng TEQ/kg) in the flesh of sablefish fed dietary treatments with different percentages of supplemental anchovy oil (A) and flaxseed oil (F). Error bars represent 20% estimation due to incomplete analysis

Discussion

The level of PCBs in the flaxseed oil (data not shown) were significantly lower (~100 fold) than the anchovy oil. Consequently, the concentrations of dioxin-like PCBs in the feed and flesh of sablefish declined as the level of supplemental flaxseed oil increased. Hence, the higher inclusion levels of flaxseed oil resulted in less deposition of dioxin like PCBs in the edible muscle. When flaxseed oil comprised 75% of the supplemental lipid source, the dioxin-like PBC concentration in the feed was 40% lower then the control treatment. However, flesh concentrations were only reduced by 26%.

Experiments conducted on Atlantic salmon indicate that flesh POP concentrations reflect the levels in the dietary treatment.[3,6,7] In the current study, the fish fed the lowest level of anchovy oil had flesh dioxin-like TEQ concentrations similar to the dietary treatment (0.41 and 0.42 ng TEQ/kg respectively). In contrast, fish fed the diet coated with 100% anchovy oil had flesh PCB levels that were lower than the dietary treatment (0.55 versus 0.70 ng TEQ/kg in the feed).

A 15-week feeding trial was chosen for this study and results indicate that the time period was sufficient to cause reductions in flesh PCB concentrations when supplemental anchovy oil was partially replaced with flaxseed oil. The use of alternative ingredient sources is a cost-effective nutritional approach to reduce POP levels in farmed fish and will likely enhance consumer confidence in farmed fish. Further research should aim to reduce POPs to levels below those found in wild fish, while maintaining adequate flesh levels of the beneficial n-3 highly unsaturated fatty acids in market sized fish.

Acknowledgements

The authors would to express appreciation for the help given M. Rowshandeli, J. Oakes, C. Dubetz, and the staff at IOS and CAER.

References

1. Hites R.A., Foran J.A., Carpenter D.O., Hamilton M.C., Knuth B.A. and Schwager S.J. (2004) *Science* 303: 226-229

2. Easton M.D.L., Luszniak D. and Von derGeest E. (2002) *Chemosphere* 46: 1053-1074

3. Berntssen M.H.G., Lundebye A.-K. and Torstensen B.E. (2005) *AquacultNutr*. 11: 219-231

4. Jacobs M.N., Covaci A., Gheorghe A. and Schepens P. (2004) *J Agric Food Chem.* 52: 1780-1788

5. Karl H., Kuhlmann H. and Ruoff U. (2003) *Aquacult Res.* 34: 1009-1014

6. Lundebye A.K., Berntssen, M.H.G., Lie O., Ritchie G., Isosaari P., Kiviranta H. and Vartiainen T. (2004) *AquacultNutr.* 10: 199-207

7. Bell J.G., McGhee F., Dick J.R. and Tocher D.R. (2005) *Aquaculture* 243: 305-314

8. Ikonomou M.G., Fraser T.L., Crewe N.F., Fischer M.B., Rogers I.H., He T., Sather P.J. and Lamb R.F. (2001) A comprehensive multiresidue ultra-trace analytical method, based on HRGC/HRMS, for the determination of PCDDs, PCDFs, PCBs, PBDEs, PCDEs and organochlorine pesticides in six different environmental matrices. Can Tech. Rep. Fish. Aquat. Sci. 2389: 1-95

9. Ikonomou M.G. et al. (2005) Comprehensive Analysis of Polychlorinated Biphenyls (Manuscript in process).

10. Van den Berg M., Birnbaum L., Bosveld, A.T.C. et al. (1998) *Environ Health Perspect*. 106, 775-792