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The effect of dietary lipid composition on the intestinal uptake and tissue distribution of benzo[*a*]pyrene and phenanthrene in Atlantic salmon (*Salmo salar*)

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ABSTRACT

Uptake of polycyclic aromatic hydrocarbons (PAHs) across the intestine is suggested to occur in association with dietary lipids. Partial replacement of fish ingredients by vegetable ingredients in aquafeeds has led to increased levels of PAHs in marine farmed fish. We therefore investigated, intestinal uptake, tissue distribution and PAH metabolism after a single dose of 14C-benzo[*a*]pyrene (BaP) or 14C-phenanthrene (PHE) given to Atlantic salmon (*Salmo salar*) acclimatized to a fish oil or vegetable oil based diet. Both BaP and PHE were absorbed along the intestine. Fish oil based feed increased BaP concentration in the pyloric caeca and that of PHE in the proximal intestine. In contrast, vegetable oil increased BaP concentrations in the distal intestine. Extraction of whole body autoradiograms removed PHE-associated radiolabeling almost completely from the intestinal mucosa, but not BaP-associated radiolabeling, indicating the presence of BaP metabolites bound to cellular macromolecules. This observation correlates with the increased *cyp1a* expression in the proximal intestine, distal intestine and liver in the BaP exposed group. Furthermore, BaP-induced *cyp1a* expression was higher in the distal intestine of salmon fed fish oil compared to the vegetable oil fed group. PHE had no significant effect on *cyp1a* expression in any of these tissues.

We conclude that dietary lipid composition affects intestinal PAH uptake. Fish oil based feed increased intestinal PAH concentrations probably due to an enhanced solubility in micelles composed of fish oil fatty acids. Increased BaP accumulation in the distal intestine of vegetable oil fed fish seems to be associated with a reduced Cyp1a-mediated BaP metabolism.

1. Introduction

Aquaculture is a fast-growing global food-producing sector, with the production of approximately 1.9 million metric tons of Atlantic salmon (*Salmo salar*) in 2014 (FAO, 2014). Traditionally, marine fish oils and fishmeal have been used as the main feed ingredients in high energy commercial feeds for carnivorous farmed fish species such as Atlantic salmon. However, the rapidly growing aquaculture cannot continue to rely on fisheries for the supply of fish oil and fishmeal (Bostock et al., 2010; FAO, 2014; Richard et al., 2006). Therefore, there is a need to develop sustainable alternative aquafeed ingredients, such as oil and meal from vegetable sources. Partial or complete substitution of fish oils by vegetable oils does not seem to negatively affect growth, survival and/or feed nutrient utilization in several aquaculture species (Bell et al., 2001; Caballero et al., 2002; Izquierdo et al., 2003; Liland et al., 2013; Richard et al., 2006; Tocher et al., 2006; Torstensen et al., 2000, 2004). In 2013, less than 30% of the Atlantic salmon diet was composed of marine ingredients while approximately 56% was composed of vegetable ingredients (Ytrestøyl et al., 2015). The use of vegetable oils will increase dietary levels of saturated, monoene and $n-6$ fatty acids. Contrary, levels of $n-3$ long-chain C_{20} and C_{22} polyunsaturated fatty acids (PUFAs) will decrease (Bell et al., 2001; Torstensen et al., 2008).

Thermal processing of oil producing seeds and grains has been shown to elevate polycyclic aromatic hydrocarbons (PAH) contents in vegetable based fish feeds (Berntssen et al., 2010a; EFSA, 2007). As a consequence, the use of vegetable feed ingredients in aquafeeds also increases the concentration of contaminants that have not been associated before with Atlantic salmon farming, such as PAHs

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(Berntssen et al., 2005, 2015). PAHs are ubiquitous lipid-soluble non-persistent organic pollutants that are biotransformed by oxygenation by the cytochrome P4501A (Cyp1a) family (Billiard et al., 2002; Hellou et al., 2002). Atlantic salmon fed 'alternative' diets based on partial replacement of fish oil and fishmeal with vegetable oil and vegetable meal, were found to have elevated tissue levels of several PAH congeners, including benzo[*a*]pyrene (BaP) and phenanthrene (PHE) (Berntssen et al., 2010a). BaP and PHE (Fig. 1) differ in their physico-chemical properties as well as their potential toxic actions. BaP consists of five fused benzene rings, is highly lipophilic (octanol/water partition coefficient, $K_{ow} = 6.31$) and is an agonist to the aryl hydrocarbon receptor that induces Cyp1a-mediated metabolism (Lampen et al., 2004). PHE has three fused benzene rings, is less lipophilic than BaP ($K_{ow} = 4.5$) and is not an aryl hydrocarbon receptor agonist (Billiard et al., 2002), with, consequently, a slower metabolism than BaP (Cavret et al., 2004).

Tissue concentration, distribution and bioavailability of PAHs depend on transport, uptake, metabolism and excretion. Intestinal transport and uptake of dietary PAHs has been suggested to occur in association with dietary lipids (Dulfer et al., 1998; Kelly et al., 2004; Vasiluk et al., 2008). Vetter et al. (1985) observed that lipids and BaP were co-transported from the intestinal lumen and co-incorporated in chylomicrons within 1 h after digestion. Because PAHs have a low solubility in the aqueous environment of the gut lumen, it has been proposed that dietary lipids influence the uptake of highly hydrophobic xenobiotics (Dulfer et al., 1998; Vasiluk et al., 2008; Vetter et al., 1985). In practice, aquafeeds are composed of vegetable and fish oil blends and an alteration in feed composition could affect the uptake of PAHs. In fish, lipids are absorbed throughout the entire intestine but predominantly in the proximal region and the pyloric caeca (Jutfelt et al., 2007; Krogdahl et al., 1999; Tocher, 2003). When PAHs enter the gastrointestinal tract, transport from the lumen to the apical brush border membrane of enterocytes can be facilitated by micelles (Doi et al., 2000; Vasiluk et al., 2008; Vetter et al., 1985). The solubility of hydrophobic xenobiotics is higher in micelles composed of unsaturated long-chain fatty acids compared to saturated short-chain fatty acids (Doi et al., 2000; Laher and Barrowman, 1983). Furthermore, lipid digestion rates increase with the degree of unsaturation, and decrease with increased chain length of the constituting fatty acids (Sigurgisladottir et al., 1992). The lower digestibility of vegetable $n-9$ and $n-6$ fatty acids compared to fish $n-3$ fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is mainly due to the lower degree of unsaturation in plant PUFAs (Torstensen et al., 2000).

For dietary exposures, the intestine is the first barrier for PAH uptake from the diet. The intestine plays an important role in PAH metabolism with near similar abilities as liver (on protein basis) in metabolizing BaP (McElroy and Kleinow, 1992). PAHs are metabolized to epoxides and hydroxylated derivatives during phase I metabolism. Excretion of phase I products is facilitated by conjugation to more water-soluble glucuronides and sulfates during phase II metabolism. Fatty acid levels and in particular PUFAs can induce BaP metabolism by increasing intestinal cytochrome P450 activity (Yang and Yoo, 1988). All in all, the increased inclusion of vegetable oils in aquafeeds increase PAH concentrations in farmed fish while the presence of vegetable and fish oils in the diet could alter the solubility of PAHs, their intestinal uptake, bioavailability, metabolism and, ultimately, the animal's exposure to PAHs.

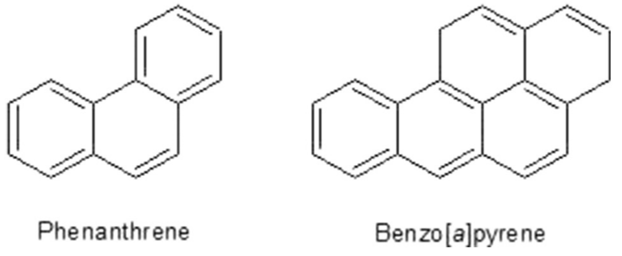

Fig. 1. Chemical structure of BaP ($C_{20}H_{12}$) and PHE ($C_{14}H_{10}$).

Differences in solubility of PAHs in micelles and different digestion and absorption rates of different fatty acids can affect the uptake of PAHs from the diet. The objective of the present study was to investigate the effects of dietary lipid composition on the uptake and distribution of BaP and PHE across the intestinal tract in Atlantic salmon fed either a "traditional" fish oil based diet or an alternative vegetable oil based diet.

2. Material and methods

2.1. Animals and diet formulation

The study was conducted at the Industrial and Aquatic Laboratory (I-Lab), Bergen, Norway. Locally bred seawater-acclimated Atlantic salmon (*S. salar*) weighing approximately 200 g were kept in indoor 1-m^3 fiber glass tanks (26 fish per tank) supplied with running seawater with a salinity of approximately 31‰. The study was performed at a temperature of 10.0 ± 0.2 °C (average ± SD).

Fish were fed for three weeks with either a diet based on fish oil or a diet based on vegetable (rapeseed) oil to acclimatize the intestinal tract to the diet. The fish were reared under 12:12 dark:light conditions and fed by automatic feeders twice a day for 2 h according to standardized in-house growth tables for salmonids modified after Austreng et al. (1987), with a feed intake of approximately 1.2% of body weight per day. The three weeks acclimatization period was chosen to ensure approximately 15 intestinal passages of the diet based on an assumed mean transit times of 28–29 h in 140–145 g rainbow trout at 10 °C fed two meals a day with a pelleted feed (Fauconneau et al., 1983).

Table 1 shows that the fish oil based diet was enriched in long-chain poly-unsaturated omega $-$ 3 fatty acids (LC PUFA-$n-3$), whereas the vegetable oil based diet had a low LC-PUFA-$n-3$ content. The diets were designed to meet the nutritional requirements of Atlantic salmon. The choice of alternative feed ingredients as well as the composition of vegetable protein and oils was based on earlier studies on the replacement of fish oil and -meal (Berntssen et al., 2010a; Torstensen et al., 2008). The two diets were selected for low background levels of

Table 1
Proximate feed (g/kg) and fatty acid compositions (area % of total fatty acids) in pellets (4 mm) of the fish oil and the vegetable oil based feeds. Q1

	Fish oil based feed	Vegetable oil based feed
Proximate composition		
Fish meal ingredients	150	150
Plant meal ingredients	569	569
Vitamin and mineral mixture	44	44
Fatty acid composition		
14:0	2.4	0.6
16:0	15.1	14.8
18:0	2.2	2.3
Sum saturates	20.4	18.3
16:1$n-7/9$	2.3	0.6
18:1$n-9/7$	35.6	42.8
20:1$n-9$	2.6	1.3
22:1$n-11/9$	2.6	0.8
Sum monoenes	43.7	45.9
18:2$n-6$	13.5	16.6
20:4$n-6$	0.2	0.0
Sum $n-6$	14.2	16.7
18:3$n-3$	10.1	13.2
18:4$n-3$	0.7	0.1
20:4$n-3$	0.2	0.0
20:5$n-3$	2.8	0.7
22:5$n-3$	0.4	0.1
22:6$n-3$	2.4	0.7
Sum $n-3$	16.7	14.8

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