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Influence of dietary phospholipid on early development and performance of Atlantic salmon (*Salmo salar*)

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ABSTRACT

The present study aimed to confirm the requirement for dietary phospholipid in Atlantic salmon and better define the level and period of requirement. Thus, the effects of dietary supplementation with phospholipid supplied by krill or soy lecithin were investigated in Atlantic salmon, *Salmo salar*. First feeding fry were fed diets containing 55% protein and 17% lipid supplemented with krill oil or soybean lecithin in a regression design at five levels, 1.5 (unsupplemented), 2.6, 3.2, 3.6 and 4.2% total phospholipid and fish were sampled at 1 g (1400 °day post-fertilisation, dpf), 2.5 g (1990 °dpf), 5 g (2350 °dpf), 10–20 g (2850 °dpf) and smolt (3800 °dpf). Survival was high overall with a positive correlation ($r^2 = 0.59–0.72$) between survival and dietary phospholipid supplementation. Growth was improved by phospholipid with highest growth achieved in fish fed krill phospholipid at 2.6% and in fish fed soy lecithin at 3.6%. The pattern of growth differed between fish up to 2.5 g and that from 2.5 g onwards with SGR (0–2.5 g) being significantly higher in fish fed 2.6% krill phospholipid and 3.6% soy phospholipid compared to the basal diet, whereas there was no difference in SGR (2.5 g-smolt) between the treatments. Intestinal steatosis was observed in 2.5 g fish fed the unsupplemented diet (20% prevalence) and lower levels of soy (10% prevalence), whereas it was absent from 2.5 g fish fed krill oil and higher levels of soy lecithin (≥3.2%), and fish at all later stages. Prevalence of vertebral deformities was low but was reduced by increasing dietary phospholipid with krill oil generally being more effective. The results were consistent with salmon having a dietary requirement for dietary phospholipid in early life stages.

Statement of relevance: Determining the effects of dietary phospholipid level and source on growth, gut and skeletal health will help improve and optimise future diet formulations for early life stages (fry) of salmonids including, Atlantic salmon.

1. Introduction

Since the first report of a dietary phospholipid requirement in an aquatic organism, specifically shrimp (Teshima et al., 1982), it has become well established that inclusion of intact phospholipids in the diet can improve culture performance of various freshwater and marine fish species (Coutteau et al., 1997). The primary criteria used to define the beneficial effects of dietary phospholipid have been growth and survival, but effects on development and stress resistance have also been reported in some species (Cahu et al., 2003b; Coutteau et al., 1997). Phospholipid requirement appears to be restricted to early life stages and so the specific evidence supporting a requirement for dietary intact phospholipid in fish has been improved growth in both larvae and early juveniles, increased survival rates and decreased incidence of malformation in larvae, and perhaps increased stress resistance (Tocher et al., 2008). However, no requirement has been established in adult fish of any species, although this has been largely unstudied (Tocher et al., 2008). In Atlantic salmon (*Salmo salar*), a requirement for dietary phospholipid based on growth performance was demonstrated in Atlantic salmon of 0.18 g, 1.0 g and 1.7 g initial weight, whereas in fish of 7.5 g initial weight, no requirement was observed (Poston, 1990). Dietary supplementation with lecithin also improved survival of 0.18 g fish but not of larger fish (Poston, 1990).

Most commonly, the levels of phospholipid requirement can vary depending upon species and developmental stage (larvae or juveniles) from around 2% up to much higher levels of 12–14% of diet (Cahu et al., 2003a; Rinchard et al., 2007). In Atlantic salmon, growth rate was higher in fry of initial weight 0.18 g, 1 g or 1.7 g fed soy lecithin at 4% of diet compared to fish fed the basal, unsupplemented feed (Poston, 1990). The phospholipid content of the food-grade lecithin used was unclear but may have been around 60%, which means that

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the growth enhancement was obtained by supplementing the diet with 2.4% phospholipid. In a later trial, graded levels of feed-grade lecithin (an approximately 1:1 mix of soy and corn lecithin) were tested in 0.18 g first-feeding salmon fry and a supplementation level of 6% provided the highest growth rate (Poston, 1991). Again the precise phospholipid content of the lecithin mixture used was not clear but appeared generally similar to food-grade lecithin used previously although it was also reported as being around 90% as "nutritionally effective" as crude soy lecithin (Poston, 1991). Therefore, supplementation with 6% feed grade lecithin may represent somewhere between 3.2 and 3.6% dietary phospholipid.

Based on a few studies where semi-purified phospholipid preparations have been used, the rank order for efficacy generally appears to be phosphatidylcholine (PC) > phosphatidylinositol (PI) > phosphatidylethanolamine (PE) > phosphatidylserine (PS) (Kanazawa, 1983, 1993; Kanazawa et al., 1985), with PC being associated with improved growth performance whereas PI has been associated with increased survival and reduced deformities (Geurden et al., 1998a). The efficacy of other phospholipid classes or sphingolipids is not known (Tocher et al., 2008). In the early studies on Atlantic salmon, the phospholipid class compositions of the lecithin preparations used were not assayed or fully described although it was reported that soybean lecithin was predominantly PC, PE and PI (Poston, 1991) and that the food-grade lecithin used contained around 16% PC (Poston, 1990). Extrapolating from this figure would suggest that the growth promoting effect of lecithin supplementation was associated with a dietary PC level of around 0.6 to 1.0%.

The mechanism underpinning the role of dietary phospholipids in early life stages of fish is not fully understood. The role appears to be not related to the delivery of other essential dietary components such as essential fatty acids, phosphate or the bases choline and inositol (Tocher et al., 2008). For instance supplemental choline did not replicate the effect of dietary soy lecithin in the early studies on Atlantic salmon (Poston, 1990, 1991). However, studies showed that phospholipid-deficient diets could lead to the accumulation of lipid vacuoles/droplets (steatosis) in enterocytes in fish larvae leading to the suggestion that dietary phospholipids may be required for the efficient export of dietary lipids from the intestine (Fontagné et al., 1998; Geurden et al., 1998b; Liu et al., 2002; Olsen et al., 1999; Salhi et al., 1999). Furthermore, as phospholipid is required for lipoprotein assembly, it was proposed that the stimulating effects of phospholipids on growth were due to early life stages of fish having limited ability for de novo phospholipid biosynthesis (Fontagné et al., 1998; Geurden et al., 1995, 1999). Thus, dietary phospholipids increase the efficiency of transport of dietary fatty acids and lipids from the gut to the rest of the body possibly through enhanced lipoprotein synthesis (Tocher et al., 2008).

Our overall aim is to determine the precise molecular mechanism(s) underpinning the requirement for dietary intact phospholipid in fish and so one objective of the present study was to provide experimental material for mechanistic studies. Our target species was Atlantic salmon due to the fact that, despite the huge commercial importance of this species, its dietary requirements are generally poorly studied and largely extrapolated from rainbow trout data (NRC, 2011). Dietary phospholipid requirements in Atlantic salmon were last investigated a quarter of a century ago in relatively short-term studies (12–16 weeks), using lecithin preparations with rather undefined phospholipid content and class composition. Therefore, the particular aims of the present study were to confirm the requirement for dietary phospholipid in Atlantic salmon, better define the level of requirement, and further establish the period of requirement. To this end, Atlantic salmon fry were fed diets containing either krill oil or soybean lecithin in a regression design at five levels from first feeding through to parr–smolt transformation. Analyses focused on growth, mortality, vertebral malformation and intestinal histology (steatosis).

2. Materials and methods

To be consistent with previous studies on phospholipid requirements of fish, the term "phospholipid" in the present paper will specifically refer to the glycerophospholipids, the major classes being PC, PE, PS and PI.

2.1. Experimental diets

Diets were formulated and manufactured by BioMar AS (BioMar Tech Centre, Brande, Denmark). Commercially available feed ingredients were used and diets were formulated to meet the dietary requirements of appropriately sized salmon, including vitamin and mineral requirements (NRC, 2011). Prior to use, the lipid class compositions of the phospholipid products were analysed (see below). The soybean lecithin (Cargill, Germany) was 49% polar lipids containing 10.4% PC, 9.2% PI, 7.5% PE and 1% PS, with the remaining polar classes being predominantly glycolipids. The neutral lipids (51%) were predominantly triacylglycerol (37%) with 7% each of sterols and free fatty acids. The krill oil (Aker Biomarine, Norway) was 40% polar lipids with 26% PC and 2% PE with trace amounts of PI and PS, with the remaining polar lipids being non-glycerophospholipid (e.g. sphingo- and glycolipids) and pigment. The neutral lipids (60%) contained 30% triacylglycerol with 15% each of sterols and free fatty acids.

A total of nine diets were formulated to deliver target phospholipid levels of basal (unsupplemented) and 4 levels each of krill oil or soybean lecithin. The basal diet mix contained 400 g kg^{-1} of fishmeal (Koster Marine Proteins Gmbh, Germany), 100 g kg^{-1} each of soy protein concentrate (Selecta SPC, Brazil), corn gluten (Cargill, Germany) and wheat gluten (Vest Korn, Norway), 30 g kg^{-1} pea protein (R2 Group, Denmark), 80 g kg^{-1} wheat flour (R2 Group), and 64 g kg^{-1} vitamin/mineral premix (BioMar Premix, Vilomix, Denmark). The added oil component of the diet accounted for 126 g kg^{-1}, which was supplied in the basal (unsupplemented) feed by a 3:2 mix of fish oil (Peruvian FO, ED&F Man, Germany) and rapeseed oil (Emmelev, Denmark). To reach targeted dietary phospholipid levels, krill oil and soybean lecithin were included in the formulations, replacing as much as possible fish oil and rapeseed oil, respectively, as shown in Table 1. The proximate compositions and the lipid class compositions of the nine feeds are shown in Table 2. This shows that the basal, unsupplemented feed still delivered 1.5% phospholipid, with 4 pairs of feeds delivering on average 2.6, 3.2, 3.6 and 4.2% total phospholipid (sum of PC, PE, PI and PS). The feed design aimed to balance dietary fatty acid compositions as much as possible but this was not fully successful at the highest inclusion of phospholipid (Table 3). The feeds were all produced by extrusion and were fed from first feeding through to smolt with appropriately increasing pellet sizes from 0.5 mm. The data presented in Tables 1–3 show mean values for the 0.5, 0.8 and 1.1 mm feeds that covered the fish from first feeding to 5 g.

2.2. Experimental fish

The feeding trial was carried out at the University of Stirling freshwater facilities (Niall Bromage Freshwater Research Facility, Stirling, UK) with all experimental procedures conducted in compliance with

Table 1
Inclusion levels (g/kg) of oil components in the nine experimental feeds.

	Target phospholipid level (percentage of diet)								
	Base	2.5		3.0		3.5		4.0	
		Krill	Soy	Krill	Soy	Krill	Soy	Krill	Soy
Krill oil	0	26	0	50	0	76	0	126	0
Soybean lecithin	0	0	24	0	49	0	73	0	122
Fish oil STD 18	75	50	75	25	75	0	53	0	4
Rapeseed oil	51	50	26	51	1	50	0	0	0