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Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.)

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ABSTRACT

The effects of high levels of replacement of dietary fish meal (FM) by mixtures of plant protein (PP) sources on growth performance, lipid composition, protein and lipid digestibility and fatty acid profile were investigated in Atlantic salmon, *Salmo salar*. Experimental diets containing 35% protein and 28% lipid were formulated with a low level of FM that was replaced by increasing levels of PP resulting in four diets of 25/45 (% FM/% PP, F25), 18/50 (F18), 11/55 (F11) and 5/60 (F5). Dietary oil was supplied by a fish oil (FO) and rapeseed oil blend at a ratio of ~40/60 so this formulation was effectively a dual replacement of FO and FM. Diets were supplemented with crystalline amino acids, to compensate for the reduction in indispensible amino acids due to reduced FM content, and all diets were supplemented with lecithin. Salmon, initial weight 1.30 ± 0.1 kg, were fed one of the four experimental diets for 19 weeks. Feed consumption decreased as PP inclusion in diets increased, probably as a result of reduced palatability. Fish fed the F18, F11 and F5 diets had significantly lower final body weights than fish fed the F25 diet, with SGR decreased by 5%, 11% and 23%, respectively. The lower growth as FM inclusion in diets decreased was associated with decreased feed intake throughout the trial. In contrast, nutrient utilization was significantly affected in the first phase with increased FCR and decreased PER as FM inclusion decreased. However, there were no significant differences in these parameters in the second phase suggesting that there was metabolic adaptation to the diets. Changes in feed physical texture and/or chemical olfactory attractants possibly reduced the palatability of the diets. Essential fatty acid composition, in particular EPA, DHA and ARA in salmon flesh and liver were not negatively affected by dietary treatment and there was some evidence of increased retention and/or synthesis of LC-PUFA.

1. Introduction

Atlantic salmon *Salmo salar* are an important high value, carnivorous fish species generally farmed in intensive systems and fed high-energy extruded feeds containing high-quality protein. The protein content of feed for farmed salmon has traditionally been marine fish meals (FM) derived from industrial, reduction fisheries (Hardy, 1996; Sargent and Tacon, 1999; Pike, 2005). It is clear that FM (and fish oil, FO) supplies from these finite fisheries are strictly limited and, if aquaculture continues to expand worldwide, the requirements for FM and FO will soon exceed global supplies (FAO, 2006). The constraints that utilization of these marine products impose has resulted in increasing investigation of alternative protein and oil sources in aquafeeds to sustain aquaculture development.

Many studies have investigated replacement of FM in feeds with a variety of plant protein (PPs) at different levels of inclusion for a range of fish including Atlantic salmon (Storebakken et al., 1998a,b; Refstie et al., 2000, 2001; Carter and Hauler, 2000; Opstvedt et al., 2003; Mundheim et al., 2004; Dias et al., 2005). Wheat gluten can substitute up to 40% of FM in feeds for salmon and trout (Hardy, 1996), and partial substitution of FM with soybean meal at levels up to 30–40% showed no reduction in growth of various species (Smith et al., 1995; Nengas et al., 1996; Robaina et al., 1997; Opstvedt et al., 2003; Kaushik et al., 2004; Dias et al., 2005). Substitution of FM with soybean protein concentrate up to 80% or 100% in feeds for halibut (Berge et al., 1999) and rainbow trout *Oncorhynchus mykiss* (Kaushik et al., 1995) showed no adverse effects on growth performance or nutrient utilization. Addition of pea protein concentrate, corn gluten, sunflower meal, or dehulled peas at up to 30% of total protein showed no adverse effects on growth performance or carcass composition in salmonids and sea bream (Mente et al., 2003; Thiessen et al., 2003; Gill et al., 2006; Lozano et al., 2007). A blend of soybean meal and corn gluten meal could be used at up to 69% of total protein replacement without any negative effect on growth and feed intake in cod (Albrektsen et al., 2006). However, total replacement of FM with PP affected growth performance of rainbow trout (Gomes et al., 1995) and Atlantic

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salmon (Espe et al., 2006), although substitution of FM in feeds close to 100% was possible in salmon with no negative effect on growth if the amino acid profile was well balanced and if feed intake was comparable to a high FM feed (Espe et al., 2007).

In most of the above studies FO still constituted the major lipid source in the feeds. However, FO supply is more pressured and, thus, imminently more limiting than FM and, currently, vegetable oils (VOs) are considered the most sustainable alternatives for FO replacement in aquafeeds due to the steadily increasing production, high availability and stable prices (Fountoulaki et al., 2009). Several studies have shown that the use of VO to replace FO in aquafeeds at levels of >50% replacement for all species, or indeed complete replacement in the case of salmon, is now feasible in practical feeds without affecting growth of fish, but does significantly impact on tissue fatty acid composition and metabolism (Bransden et al., 2003; Torstensen et al., 2004; Izquierdo et al., 2005; Pratoomyot et al., 2008; Petropoulos et al., 2009). Therefore, replacing FM and FO with alternative non-marine ingredients can affect not only production parameters such as growth, but also nutritional quality including fillet fatty acid composition.

In the present study, the effects of dual substitution of FM and FO were investigated in adult Atlantic salmon of initial weight of 1.3 kg that were grown to market size (>3 kg) over a period of 19 weeks on diets with 60% of dietary FO replaced by rapeseed oil, and increasing proportions of FM substituted by PPs (a mixture of sunflower meal, corn gluten meal, soybean meal, and wheat gluten). The level of FO substitution represented the upper level of FO replacement currently used in commercial ongrowing diets. The control diet contained 25% FM and 45% PP, which also represented the current minimum commercial level of FM inclusion. Three other diets had FM inclusion reduced to 18, 11 and 5%, with PP inclusion increased to 50, 55 and 60% of the diet. Effects on growth performance, feed utilization efficiency, protein and fat digestibility, sustainability index, and lipid and fatty acid compositions of flesh and liver were investigated.

2. Materials and methods

2.1. Diets and animals

Four diets were formulated to satisfy the nutritional requirements of salmonid fish (National Research Council, 1993), and manufactured at Biomar TecCentre, Brande, Denmark. All diets contained 35% crude protein and 28% crude lipid and were formulated to fixed digestible protein and digestible energy contents of 308 g kg^{-1} and 20.5 MJ kg^{-1}, respectively. The control diet was formulated to represent the maximum level of PP inclusion currently in commercial use and contained 45% PP (a blend of sunflower and corn gluten meals, and soybean protein concentrate) and 25% FM (Diet F25) (Table 1). The remaining three diets followed a regression with PP inclusion increased to 50%, 55% and 60% and FM inclusion reduced to 18%, 11% and 5% of total diet, diets F18, F11 and F5, respectively. All diets were coated with a 60:40 blend of rapeseed oil and FO. All diets were supplemented with crystalline amino acids, lecithin and carophyll pink as sources of amino acids, phospholipid and pigments (Table 1). The proximate composition, lipid class composition and fatty acid composition of the diets are shown in Tables 1–3, respectively.

One thousand eight hundred Atlantic salmon (*Salmo salar* L.) of initial mean weight 1.3 ± 0.1 kg were randomly distributed among 12 cages of 125 m^3 (5 × 5 × 5 m) with 150 fish/cage at the Marine Harvest Fish Trials Unit, Ardnish, Scotland, and fed with one of the four diets in triplicate cages. The experiment was conducted over 19 weeks from October 2007 to February 2008 under natural photoperiod. Fish were fed to apparent satiation by a combination of manual feeding and automatic feed hoppers (Arvo-tec, Sterner Arvo-tec UK, Inverness, Scotland). Daily feed intake was determined in each cage from the difference between the feed ration (1 or 2 meals depending on temperature and day-length) per day and the mass of uneaten pellets registered 15–45 min after each meal in a waste feed lift-up system. Mortalities, feed consumption and waste feed were recorded daily.

Table 1
Feed formulation (g kg^{-1}) and analyzed compositions (%) of the experimental diets.

Feed ingredients	F25	F18	F11	F5
Fishmeals[a] (67/10)[b]	250	180	110	50
Sunflower expeller (37/10)[b]	115	77	40	–
Corn gluten (62/2)[b]	85	135	175	215
Soy concentrate (60/2)[b]	85	135	175	225
Wheat gluten (77/3)[b]	–	2	18	20
Rapeseed oil[c]	173	175	178	180
Fish oil [d]	116	117	118	120
Binders	160	160	160	160
Micronutrients [e]	11.95	17.59	23.59	28.99
L-lysine[f]	0.62	1.72	3.44	4.26
L-threonine[f]	–	–	0.43	0.67
DL-methionine[f]	0.57	1.03	1.56	2.01
Lecithin	5.0	5.0	5.0	5.0
Astaxanthin	0.40	0.40	0.40	0.40
Antioxidant[g]	4.25	4.25	4.25	4.25
Analyzed composition				
Crude protein (N × 6.25)	34.3 ± 0.4[2]	35.1 ± 0.3[1]	35.0 ± 0.1[1]	34.7 ± 0.3[1,2]
Crude lipid	29.8 ± 0.1[1]	29.5 ± 0.1[1]	27.9 ± 0.1[2]	27.3 ± 0.2[3]
Moisture	6.7 ± 0.1[2]	6.0 ± 0.0[4]	6.2 ± 0.0[3]	6.9 ± 0.0[1]
Ash	6.0 ± 0.1[1]	5.6 ± 0.0[2]	5.2 ± 0.1[3]	4.8 ± 0.0[4]
Crude fiber	3.5	3.3	2.6	3.0
NFE[h]	19.7 ± 0.3[2]	20.5 ± 0.4[2]	23.1 ± 0.4[2]	23.3 ± 0.5[1]

[a] Peruvian fishmeals produced from *Anchoveta*.
[b] Figures in parentheses are crude protein/crude lipid values, respectively.
[c] Non-GM double-low rapeseed oil.
[d] North-Atlantic standard fish oil.
[e] Vitamin and mineral premixes with limestone carrier added according to the commercial standards of BioMar AS.
[f] Purified (99%) crystalline amino acids.
[g] Blend of antioxidants and starch carrier added according to the commercial standards of BioMar AS.
[h] NFE (nitrogen-free extract) calculated by subtraction, 100 − (crude protein + crude fat + moisture + ash + crude fiber).

2.2. Sampling protocols

Fish were bulk weighed at the initiation, at the end of week 8 and at the termination of the trial, week 19. At the end of the trial, 2 fish per pen (6 fish per dietary treatment) were anaesthetized with metacaine sulphonate (MS222; 50 mg/L) and killed by a blow to the head. Flesh samples were taken from the Norwegian Quality Cut and were homogenized in a food processor after removal of skin and bones and stored at −20 °C prior to lipid analysis. Livers were also collected from the six fish and a 1–2 g sample placed into glass vials

Table 2
Lipid class composition (percentage of total lipid) and pigment content (g kg^{-1}) of the experimental diets.

Parameters	F25	F18	F11	F5
Lipid classes				
PC	2.8 ± 0.2	2.7 ± 0.2	2.1 ± 0.3	1.9 ± 0.6
PE	3.5 ± 0.6	3.6 ± 0.5	3.9 ± 0.6	3.2 ± 1.1
PI/PS	1.5 ± 0.3	2.0 ± 0.4	3.1 ± 0.6	2.8 ± 0.2
Sphingomyelin	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	nd
Lyso-PC	0.1 ± 0.0	0.1 ± 0.0	tr	nd
Polar lipid	8.2 ± 0.8	8.6 ± 0.7	9.3 ± 1.4	7.9 ± 1.0
Neutral lipid	91.8 ± 0.8	91.4 ± 0.7	90.7 ± 1.4	92.1 ± 1.0
Triacylglycerol	74.2 ± 1.8	72.7 ± 0.7	73.9 ± 1.4	75.6 ± 1.0
Sterol	8.5 ± 0.6	8.6 ± 0.5	6.9 ± 0.4	6.9 ± 0.3
Free fatty acid	9.1 ± 1.5	10.1 ± 0.7	9.9 ± 0.8	9.6 ± 0.8
Steryl ester	tr	tr	tr	tr

Results are means ± SD ($n = 4$). There were no significant differences between feeds for any parameter as determined by ANOVA. nd, not detected; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; TNL, total neutral lipids; TPL, total polar lipids, tr, trace.