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Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils

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ABSTRACT

The present study compared the effects of diets formulated with reduced fishmeal (FM) content and either 100% fish oil (FO) or 100% of a vegetable oil (VO) blend in post-smolts of three family groups of Atlantic salmon. Two groups were selected as being either "Lean" or "Fat" based on estimated breeding values (EBV) for flesh adiposity of their parents derived from a breeding programme, while the third group (CAL) was a mix of non-pedigreed commercial families unrelated to the two groups above. The VO blend comprised rapeseed, palm and a new product, Camelina oil in a ratio of 5/3/2, and diets were fed to duplicate pens of each salmon group. After an ongrowing period of 55 weeks, to reach a mean weight of 3 kg, the fish from all treatments were switched to a decontaminated FO for a further 24 weeks to follow restoration of long-chain n-3 polyunsaturated fatty acids (LC-PUFA) in the fish previously fed VO. Final weights were significantly affected by family group and there was also an interaction between diet and group with Fat and Lean FO fish being larger than the same fish fed VO. Specific growth rate (SGR) was highest in CAL fish (1.01), feed conversion ratio (FCR) was highest in the Lean fish but there were no significant effects on thermal growth coefficient (TGC). Condition Factor (CF) was lowest in CAL fish while the hepato-somatic index (HSI) was highest in Lean fish and viscero-somatic index (VSI) highest in Fat fish. Flesh and viscera lipid content was affected by both family group and diet with a significant interaction between the two. Flesh lipid in fish fed FO was in the order Fat>CAL>Lean although this order was Fat = Lean>CAL when fed VO. Flesh fatty acid compositions were affected mainly by diet although some minor fatty acids were also influenced by group. Fish fed VO had n-3 LC-PUFA reduced by ~65% compared to fish fed FO but this could be restored by a 16week FO finishing diet phase. The differences observed in lipid and fatty acid deposition suggested that genetics affected lipid deposition and metabolism and that breeding programmes could select for fish that retained more n-3 LC-PUFA in their flesh, particularly when fed diets low in these fatty acids.

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1. Introduction

Worldwide demand for seafood continues to expand while traditional fisheries are at best stable or in decline, and recent evidence suggests that aquaculture production now provides half of all seafood (FAO, 2009). In recent times global production of Atlantic salmon increased from ~0.9 million metric tonnes (mMT) in 2000 to 1.5 mMT in 2006 and as such is the largest production of a marine fish species globally (FAO, 2008). As with most carnivorous fish, salmon has

traditionally been cultured using diets rich in fishmeal (FM) and fish oil (FO), which has placed excessive demand on these raw materials, resulting in increased prices and concerns about ethical sustainability. Production of FO globally has ranged from 1.0 m to 1.5 m MT between 1980 and 2006 and there is no likelihood of this increasing (Tacon and Nates, 2007; Tacon and Metian, 2008). Recent estimates of FO use by aquaculture suggested 87% of global production is currently utilised with salmonid culture being the major user at 66.4% of total FO production (Tacon et al., 2006). Tacon et al. (2006) suggested that aquaculture would use 88% of FO production up to 2012 while an earlier study suggested that 98% could be consumed by 2010 (Pike and Barlow, 2003). All of these predictions could be eclipsed if a severe El Nino event occurs in the next few years, as has been widely predicted.

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In summary, the need to establish alternatives to FM and FO has never been more urgent if aquaculture production is to increase and seafood supply and nutritional quality is to be maintained.

Several studies conducted recently have shown that terrestrial vegetable oils (VOs), either singly or as blends, can replace some or all of the added FO in salmonid feeds largely without any compromise in growth or fish health and condition (Bell et al., 2001, 2004; Torstensen et al., 2000, 2004a, 2005; Richard et al., 2006; Karalazos et al., 2007). However, replacement of FO, rich in the n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), eicosapentaenoic (20.5n-3; EPA) and docosahexaenoic acids (22:6n-3: DHA), with VOs devoid of n-3 LC-PUFA but rich in α -linolenic acid (18:3n-3) and especially linoleic acid (18:2n-6), can significantly reduce the flesh n-3 LC-PUFA concentration by more than 50% if total FO replacement is implemented (Bell et al., 2004; Torstensen et al., 2005; Richard et al., 2006). While salmon grown on diets rich in VO have reduced n-3 LC-PUFA, such products are still rich in n-3 LC-PUFA compared to most other fish species (Bell and Tocher, 2009). However, given the well established efficacy of n-3 LC-PUFA in preventing and attenuating a range of inflammatory disorders, including cardiovascular disease, immune dysfunction and neurological conditions, it is vitally important to maintain flesh n-3 LC-PUFA in salmon at levels close to those found in salmon fed largely on FO (Young and Conquer, 2005; Ruxton et al., 2005; Bell, 2008). Finishing diets rich in marine FO have been used to restore n-3 LC-PUFA levels in market size salmon that had previously been fed high levels of VO. Feeding FO finishing diets to fish previously fed VOs, for periods of 16-24 weeks, have successfully restored flesh levels of n-3 LC-PUFA to 80–100% of their concentrations in FO-fed fish (Bell et al., 2004; Torstensen et al., 2005).

As described above, there is an extensive literature on the replacement of FO with both single and blended VOs in a range of fish species and there is a similar body of literature on the replacement of FM with plant proteins. In salmonids, partial or total replacement of FM with plant proteins has been demonstrated by a number of authors, although in some cases growth reduction was observed, which may have been due to the presence of anti-nutritional factors that impacted on feed intake and gut function (Kaushik et al., 1995; de Francesco et al., 2004; Espe et al., 2006). Interestingly, lipid retention was increased in rainbow trout but decreased in Atlantic salmon when fed high levels of plant protein (Kaushik et al., 2004; Espe et al., 2006). While the current project focused more on the complete replacement of FO with VO on different salmon family groups, the levels of FM and plant protein used were lower and higher, respectively, than in commercial salmon diets used in 2006. Hence, a secondary aim was to develop and test feed formulations that significantly reduced dependence on marine resources and result in more sustainable aquafeed products.

The proximate composition of fish flesh, and especially the lipid content, has an impact on the sensory quality of the flesh that also impacts on consumer preferences that may vary between different geographic regions and cultures. It is therefore important for the aquaculture industry to produce fish that use the dietary nutrients to promote muscle growth over adipose growth as well as meeting consumer perceptions in fulfilling optimal quality parameters. Thus, the ability to develop breeding programs in farmed fish that exploit desirable phenotypic and genotypic traits that influence lipid deposition or adiposity has resulted in recent research activity (Neira et al., 2004; Quillet et al., 2005; Tobin et al., 2006). There is also evidence that lipostatic regulation can be influenced by dietary lipid and protein content in fat and lean strains of fish and chickens (Bourneuf et al., 2006; Tobin et al., 2006; Kause et al., 2009). Evidence suggests that retention of n-3 LC-PUFA is subject to genetic control in poultry (Mennicken et al., 2005).

The primary aim of this study was to grow Atlantic salmon smolts, for the whole marine production phase, on diets with reduced FM and that contained either 100% of added oil as FO or a blend of VOs. Each of

these two diets was fed to either "Lean" or "Fat" salmon families as well as a commercial strain used in previous studies on FO replacement (Bell et al., 2004; Torstensen et al., 2005). Flesh fatty acid compositions were determined at final sampling for the ongrowing phase. Thereafter, all fish were placed on a FO diet, containing a decontaminated FO for the finishing diet phase, and flesh fatty acid concentrations measured after 8, 16 and 24 weeks on the FO finishing diet.

2. Materials and methods

2.1. Fish populations

Three genetically characterised and contrasting groups of farmed Atlantic salmon post-smolts were provided for the feed trials. Two groups comprised full-sib families selected from the Landcatch Natural Selection Ltd breeding program (Argyll, Scotland) based on estimated breeding values (EBVs) of their parents for high or low flesh adiposity. The parental EBVs were derived from Torry Fatmeter (Distell Industries, West Lothian, UK) lipid assessments (recorded from microwave reflectance of fat as a percent of body weight) obtained on live and harvested sibs of those parents. These assessments have a heritability (proportion of observed variation due to family differences) ranging from 0.17 to 0.39 in this dataset. Four unrelated full-sib families were then created from the selected parents at the November 2004 stripping season, two families from the extreme lower end of the EBV distribution for Torry fat meter assessed lipid content ("Lean") and two families from the extreme upper end of the distribution ("Fat"). The average EBV for the lipid content as determined by Torry fat meter of the two Fat families was 2.00 percentage units higher than that of the two selected Lean families, representing a standardised selection differential of 2.33 standard deviations (sds). The corresponding standardised selection differential of the two Fat families for weight EBV at harvest was 0.62 sds; .i.e, the Lean families being genetically slightly heavier than the Fat families. A third group was of non-pedigreed mixed families derived from the Caledonian 20 strain (CAL; Marine Harvest Scotland Ltd.), which had been used in previous replacement trials (Bell et al., 2004; Torstensen et al., 2005).

Two thousand smolts of each of the three groups were stocked into $12\times 5~\text{m}^3$ net pens at the Ardnish Fish Trials Unit (Marine Harvest Scotland, Lochailort, PH38 4LZ, Highland; 500 fish/pen) and the initial mean weights were 52, 88 and 85 g for the CAL, Fat and Lean fish, respectively. Thus, there were four pens for each of the three groups with each of the two diets being feed to duplicate pens for each group. The temperature over the experimental period (May 2006 to December 2007) ranged from 5.5 to 17.0 °C with a mean temperature of 11.5 \pm 2.9 °C.

2.2. Experimental diets

The fish were fed one of two experimental diets, prepared by Skretting ARC (Stavanger, Norway) in three different pellet sizes (3, 6 and 9 mm) for a period of 55 weeks until the fish reached a weight of ~3 kg. The diets were formulated to fully satisfy the nutritional requirements of salmonid fish (NRC, 1993). Duplicate pens of each of the three genetic groups were fed a similar basal diet containing either 100% Northern FO or a VO blend comprising rapeseed, palm and Camelina oils in a ratio of 5:3:2. The oil of the plant Camelina sativa is a new addition to aquaculture feeds although it has been used since Neolithic times as a lamp oil and also in cosmetics. It is low in saturated fatty acids and contains 18:3n-3 and 18:2n-6 PUFA in a ratio of ~2:1. It is unusual in being rich in the monoene fatty acid, 20:1n-9, representing around 15% of the fatty acids and is more usually found in marine FO where it is a preferred source of dietary energy. Its seeds contain more than 40% oil and it can be grown in arid conditions not tolerated by other oilseeds and requires minimal use of

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