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Aquaculture 252 (2006) 453–461

Aquaculture

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Myosin heavy chain mRNA expression correlates higher with muscle protein accretion than growth in Atlantic salmon, *Salmo salar*

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Received 1 April 2005; received in revised form 8 July 2005; accepted 11 July 2005

Abstract

In order to link growth and protein accretion in Atlantic salmon the mRNA expression of muscle myosin heavy chain (*MyHC*) was analysed. *MyHC* gene is expressed throughout muscle development and is consistent with the hypertropic growth in fish. Total RNA was isolated from white muscle tissues ($N=32$) from salmon fed a fish meal based diet with three levels of solubilised protein incorporated as fish protein hydrolysate (FPH) control (0 g kg^{-1}), medium (180 g kg^{-1}) and high (300 g kg^{-1}) FPH inclusion. The salmon were PIT-tagged and fed the experimental diets for a period of 68 days. A high level of dietary FPH resulted in significant up-regulation of *MyHC* by a factor of 2.4 compared to fish fed the medium FPH inclusion. The fish with highest *MyHC* expression also contained significant higher levels of muscle protein. *MyHC* expression correlated higher with protein accretion than individual specific growth rate (SGR) in salmon. These observations indicate that *MyHC* mRNA expression can be a useful marker for understanding of growth and protein accretion in Atlantic salmon.

Keywords: Myosin heavy chain (*MyHC*); White muscle; Solubilised protein; FPH; Atlantic salmon

1. Introduction

In fish nutrition studies performed in tanks or cages with large numbers of individuals it is difficult to control individual feed intake and give individual estimates on protein accretion. Growth is thought to have a close relationship with protein accretion (Carter et al., 2001), and there is a need for increased knowledge on the physiological regulation of muscular growth. One possible candidate linking growth and protein accretion is the *myosin heavy chain* (*MyHC*) gene which is present throughout muscle development in fish (Gauvry and Fauconneau, 1996). During growth, when white muscle fibers in fish increase in size (hypertrophic growth), the growth in body girth relies also on new muscle fiber recruitment (hyperplasia), but is largely driven

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doi:10.1016/j.aquaculture.2005.07.003

by fiber hypertrophy (Johnston et al., 2000). In rainbow trout muscle fiber hypertrophy is favoured in periods of rapid growth whereas fiber hyperplasia dominates in periods of slow growth (Kiessling et al., 1991). During the seawater phase, one isoform of *MyHC* is expressed in fast myotomal muscle of Atlantic salmon, (Martinez et al., 1993; Pierre-Yves Rescan, pers. comm.) and studies in post-smolt rainbow trout have shown only one isoform of fast *MyHC* (Weaver et al., 2001), but allelic variant may exist (Gauvry and Fauconneau, 1996).

Previously the RNA/DNA ratio has been used to estimate protein synthetic capacity in muscle tissues of Atlantic cod (Lied et al., 1982), rainbow trout (Peragon et al., 2001) and Atlantic salmon (Espe et al., 1993a; Arndt et al., 1996; Sveier et al., 2000). Experiments analysing the RNA/DNA ratio of myosin heavy chain in white muscle of Atlantic salmon have also been conducted (von der Decken et al., 1992). But these methods are quite laborious and do not necessarily provide sufficient information to evaluate the protein synthesis occurring in vivo. Overturf and Hardy (2001) observed differences in the relative levels of muscle *MyHC* mRNA expression between groups of rainbow trout held on a restricted feed regime using real-time RT-PCR. It was proposed that this expression data could be used to measure differences in muscle protein synthesis for fish associated with various nutrient intake levels. Real-time RT-PCR is a recommended method to compare expression levels of mRNA in different sample populations (Orlando et al., 1998; Bustin, 2000; Overturf and Hardy, 2001).

In the growing Atlantic salmon industry there is an increased demand for dietary marine protein. Fish protein hydrolysate (FPH) has potential as an economical way of converting fish by-products into acceptable protein ingredients for the fish feed industry. We previously reported that Atlantic salmon fed dietary solubilised protein (FPH) showed increased specific growth rate (SGR) when were fed medium levels of solubilised protein followed by significantly reduced growth when were fed high levels of solubilised protein (Espe et al., 1999; Hevrøy et al., 2005). Further, the salmon fed medium level of FPH showed a tendency towards higher feed intake compared to salmon fed both lower and higher inclusion levels of FPH (Hevrøy et al., 2005).

The aim of the present study was to elucidate if expression of *MyHC* mRNA is related to protein accretion and growth rate in the fast growing Atlantic salmon. The fish was fed three levels of FPH to obtain differences in protein accretion and growth.

2. Materials and methods

2.1. Experimental procedures

Individually PIT-tagged post-smolt (1+) Atlantic salmon, *Salmo salar* ($N=493$) of the NLA strain with an average initial body weight of 175 ± 28 g were fed each of three diets in duplicate tanks ($2 \times 2 \times 0.8$ m) for a period of 68 days. The experiment conducted at Norwegian Institute of Fisheries and Aquaculture Research, Austevoll (N 60°05′, E 05°16′), Norway, was performed indoors with stable water flow (80 l min^{-1}), temperature (11.5 ± 0.4 °C) and salinity (33.6 ± 0.6 b l^{-1}) condition. Level of oxygen from the water outlet was not lower than 7.0 mg l^{-1} (saturation >80%). The fish were fed by automatic feeders that were adjusted every day to secure 10% excess feeding, by the use of feed collection systems (Hølland Teknologi AS, Sandnes, Norway). The feed was offered in two main periods (0500 to 0800 and 1400 and 1500) in intervals of 20 s feeding and 200 s off feeding during the periods.

The fish were fed diets in which fish meal nitrogen was exchanged with FPH-nitrogen at three graded levels, fish meal diet (control) without any added FPH, a medium diet with 180 g kg^{-1} FPH and a diet with high level of FPH (300 g kg^{-1}). The different dietary groups were named as control, medium and high, respectively (Table 1). FPH was produced from whole herring using Alcalase® (Novozymes A/S, Baegsvard, Denmark) by Biomega A/S, Skaganeset, Norway, as described by Liaset et al. (2003). The feed pellets (5 mm) were produced by the use of a double screw test-extruder at the Norwegian Institute of Fisheries and Aquaculture Research, Titlestad, Norway. As expected the diets to which FPH was added possessed a higher level of soluble protein (Table 1, Hevrøy et al., 2005). All diets were isonitrogenous and isoenergetic, containing the same amount of