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Aquaculture 244 (2005) 283–291

Aquaculture

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Macronutrient composition of formulated diets for Atlantic halibut (*Hippoglossus hippoglossus*, L.) juveniles, II: protein/lipid levels at low carbohydrate

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Received 8 September 2004; received in revised form 10 December 2004; accepted 10 December 2004

Abstract

The aim of the present study was to show that Atlantic halibut on the weaning stage, fed low carbohydrate levels, tolerates a wide variation in lipid as long as protein requirement is fulfilled. The fish (0.4 g) were fed diets with lipid levels increasing from 50 to 300 g kg^{-1} concomitant with protein decreasing from 860 to 620 g kg^{-1} with 50 g kg^{-1} intervals. No carbohydrate was added and the diets contained less than 12 g kg^{-1} digestible starch. The experiment lasted for 95 days. An increase in growth rate with increasing dietary lipid was registered for the whole population, but for the largest half of the population, growth was not affected by the dietary treatments. No specific pathology was observed in liver or intestine on histological examination, but a consistent increase in hepatocellular vacuolization was observed with increasing dietary lipid levels. Increasing dietary lipid led to decrease in survival and the small fish were affected most severely by mortality. We suspect that high levels of soy lecithin supplemented as 25% of the lipid in the present experiment may have caused the dietary effect on mortality. Based on this assumption and combined with previous studies, our results indicate that weaning diets for Atlantic halibut should contain no more than 50 g kg^{-1} carbohydrate, a minimum of 580 g kg^{-1} protein and 50–300 g kg^{-1} lipid.

Keywords: Atlantic halibut; Dietary macronutrients; Protein; Lipid

1. Introduction

Weaning marine fish from live food to a formulated diet represents a critical phase in juvenile production, and the diet should be of optimal quality, where the composition of macronutrients is an important issue. For Atlantic halibut there are some reports on macro-

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doi:10.1016/j.aquaculture.2004.12.007

nutrient requirements in the ongrowing phase (Hjertnes et al., 1991; Aksnes et al., 1996; Nortvedt, 1997; Grisdale-Helland and Helland, 1998; Helland and Grisdale-Helland, 1998). Because of rapid growth and ontogenesis in young fish, nutrient requirements may be different from those of larger fish especially with respect to dietary protein demand (Einen and Roem, 1997).

A study with optimisation of macronutrients in weaning diets for Atlantic halibut has been performed by our group (Hamre et al., 2003), where we used a three-component mixture design. Protein, lipid and carbohydrate were varied between 530–830, 50–300 and 0–15 g kg^{-1}, respectively, with 50 g kg^{-1} intervals. We found that the diets should contain no more than 50 g kg^{-1} carbohydrate. At 0 and 5 g kg^{-1} carbohydrate, there was no difference in growth between fish fed 50–250 g kg^{-1} lipid, whereas fish fed 50 g kg^{-1} carbohydrate, 300 g kg^{-1} lipid and 580 g kg^{-1} protein showed a dramatic reduction in growth. Since we used 21 diets, the experiment was performed with one tank per diet, and the latter result should be confirmed using replicate treatments.

The results on lipid tolerance are supported by Aksnes et al. (1996) who fed diets with lipid varying from 126 to 325 g kg^{-1} and protein varying from 549 to 719 g kg^{-1}, at low carbohydrate, to Atlantic halibut from 5 g growing to approximately 560 g. There was no effect of dietary composition on growth, mortality or feed conversion. On the other hand, Hjertnes et al. (1991) found that an increase of lipid from 240 to 290 g kg^{-1}, concomitant with a decrease in protein from 580 to 530 g kg^{-1} (low carbohydrate) for halibut growing from 35 to approximately 300 g, led to a reduction in growth. The authors propose a protein requirement of 580 g kg^{-1}, which is in line with the findings of Hamre and Næss (unpublished). The results of Hamre et al. (2003) indicate a slightly higher protein requirement for the weaning stage. The aim of the present experiment was to confirm the results of Hamre et al. (2003), indicating that Atlantic halibut juveniles fed low levels of carbohydrate can tolerate a wide range of lipid supplementation, as long as protein is sufficient. Further, we conducted histological studies of liver and intestine to assess possible pathological changes due to high lipid levels.

2. Materials and methods

2.1. Fish

Atlantic halibut (*Hippoglossus hippoglossus*) eggs were stripped from one female and fertilized with sperm from two males and thereafter kept in egg incubators for 10 days. Before hatching the eggs were administered to yolk sac incubators (silos, Harboe et al., 1994) for 43 days. The larvae were then first fed according to Harboe et al. (1998) on *Artemia* enriched for 24 h on Selco (INVE, Belgium). After 53 days the fry were stocked in the experimental tanks.

2.2. Rearing conditions

Each of the 6 diets was fed to fish in triplicate tanks by automatic feeders. In addition, the fish were handfed twice per day to ensure satiation. The tanks were cleaned twice a day by siphoning. Initially, 20 fish were stocked per tank and the experiment lasted for 95 days. The tanks were circular, 40 cm in diameter and 20 cm in height. They were connected to recirculation units, consisting of a biofilter, a skimmer and a heat pump for temperature control. Temperature and oxygen were measured daily with an instrument, Handy Delta, from OxyGuard. Ammonia was measured once a week using a Shimatzu UV-160 spectrophotometer and pH was measured using a Radiometer PHM210 standard pH-meter. Determination of unionised ammonia was done according to Fivelstad (1988). Temperature was 12.0 ± 0.2 °C in all tanks during the experiment. Oxygen concentration varied between 92% and 87% of saturation, measured in the outlet water. The concentration of unionised ammonia was on average 0.0007 mgl^{-1}.

2.3. Diets

Protein sources of the diets (Table 1) were fillet of cod and squid mantle (local suppliers) at a fixed ratio in all diets. Lipids were added as a highly purified fish oil (EPAX 3000 TG, Pronova Biocare A/S, Sandefjord, Norway) and soy lecithin (Norsk Medisinaldepot, Bergen, Norway). Since phospholipids are essential for absorption of triacylglycerol in young fish (Geurden et al., 1998), the ratio between soy lecithin and fish oil was held constant in the