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A nutrigenomic analysis of intestinal response to partial soybean meal replacement in diets for juvenile Atlantic halibut, *Hippoglossus hippoglossus*, L.

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ABSTRACT

Aquaculture feeds for carnivorous finfish species have been dependent upon the use of fish meal as the major source of dietary protein; however, the increasing demands upon the finite quantity of this high-quality protein source requires that feeds become increasingly comprised of alternative plant and/or animal protein. Soybean meal has been has been used to partially replace fish meal in the diets of several fish but it is known to cause enteritis in Atlantic salmon, *Salmo salar*. We have compared two groups of juvenile (207.2 ± 6.6 g) Atlantic halibut, *Hippoglossus hippoglossus*, L., fed diets containing fish meal (FM; control) or 30% soybean meal (SBM; experimental) as a protein source for 3 weeks. No detectable difference in feed intake or palatability was evident with the SBM diet relative to the FM diet. Histological examination of the distal intestine was performed to examine leukocyte infiltration of the lamina propria and other changes in morphology commonly observed with soybean-induced enteritis of salmonids. No significant difference was found between fish fed the FM and SBM diets. Global gene expression profiling performed using a high-density oligonucleotide microarray containing 9260 unique features, printed in quadruplicate, from Atlantic halibut revealed subtle underlying changes in the expression of several immune genes and genes involved in muscle formation, lipid transport, xenobiotic detoxification, digestion and intermediary metabolism. These results indicate that SBM can be used successfully as a replacement for animal protein in diet for juvenile Atlantic halibut, although long-term effects on the immune system may ensue.

1. Introduction

Atlantic halibut (*Hippoglossus hippoglossus* L.) is a highly valued food fish and it shows good potential for coldwater aquaculture (Berg, 1997; Mangor-Jenson et al., 1998). Although some progress has been made on diet development for halibut, information on the nutritional requirements of most coldwater marine fish including halibut is limited. Proteins and their constituent amino acids are essential components of marine fish diets. The dietary protein requirements of coldwater marine and salmonid finfish for maximum growth generally range from 40–55% assuming a sufficient and appropriate supply of available energy. Flatfish, such as plaice (Cowey et al., 1972) and turbot (Danielssen and Hjertnes, 1993) require 50% dietary protein and several studies have determined the optimal dietary protein levels for halibut to be similar, although somewhat higher amounts (~60%) are required by younger fish (Aksnes et al., 1996; Grisdale-Helland and Helland, 1998; Helland and Grisdale-Helland, 1998; Hamre et al., 2003). In general, smaller fish require more dietary protein and are more sensitive to dietary carbohydrates (Hamre et al., 2003; Hatlen et al., 2005).

Fish meal (FM) is a major source of protein in fish feeds. However, the increasing demands of the world's aquaculture production upon the finite quantity of this high-quality protein source necessitates that fish feeds become increasingly comprised of alternative economical and highly digestible protein sources of plant and/or animal origin that support similar fish performance and concurrently have little or no adverse effects upon the environment. Numerous studies have investigated the potential of alternate plant proteins, particularly soybean meal (SBM) and canola meal or their concentrates in rainbow trout and other salmonid fish diets (see Higgs et al., 1995; Storebakken et al., 2000 for reviews). Research conducted in the past two decades has

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established the maximum amounts of some plant protein sources that carnivorous fish can tolerate and the negative effects of their antinutritional factors or toxicants (depending upon the source some or most of the following may be present i.e., protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, alkaloids, tannins, cyanogens, glucosinolates, etc.) that adversely affect digestion, absorption and physiological utilization of protein and amino acids. Some differences in the anatomy of the digestive tract of flatfish, cod and salmonids exist; however, the effects of dietary protein sources of plant and animal origin and physiological differences among these fish on nutrient absorption mechanisms from the digestive tract are not known. The site of lipid and protein absorption in the digestive tract of turbot appears to be the hindgut and rectum where lipolytic activity is relatively high (Koven et al., 1997). However, in halibut fat absorption occurred to a greater extent in the anterior part of the intestine and may be linked to a few pyloric caeca located in the anterior part of the digestive tract (Martins et al., 2009).

Soybean protein has been used successfully for some species such as Atlantic cod, *Gadus gadus* (Hansen et al., 2006; Refstie et al., 2006b), Indian carp, *Cirrhinus mrigala* (Jose et al., 2006), three genera of catfishes (Usmani et al., 2003; Evans et al., 2005), yellowtail, *Seriola quinqueradiata* (Shimeno et al., 1997), Japanese flounder, *Paralichthys olivaceus* (Kikuchi, 1999), and Egyptian sole, *Solea aegyptiaca* (Bonaldo et al., 2006). In some species, morphological changes have been noted in fish fed high levels of soy protein. These include the liver of Asian sea bass, *Lates calcarifer* (Boonyaratpalin et al., 1998) and mangrove red snapper, *Lutjanus argentimaculatus* (Catacutan and Pagador, 2004) and distal intestine of sea bass, *Dicentrarchus labrax* (Penn et al., 2007) and carp (Uran et al., 2008).

In salmonids, inflammation of the distal intestine (van den Ingh et al., 1991; Rumsey et al., 1994; Baeverfjord and Krogdahl, 1996) and ulcer-like lesions in the stomach (Refstie et al., 2006a) caused by antinutritional factors in the plant protein has presented problems such as reduced intestinal absorptive ability and increased disease susceptibility (Krogdahl et al., 2000; Bakke-McKellep et al., 2007a). Proliferation of distal intestine enterocytes of SBM-fed Atlantic salmon, *Salmo salar* was observed using antibodies against PCNA (Sanden et al., 2005) and changes in trypsin activity and gene expression have also been observed in both Atlantic salmon (Krogdahl et al., 2003; Lilleeng et al., 2007b) and rainbow trout, *Oncorhynchus mykiss* (Romarheim et al., 2006). In addition to morphological studies, analyses of immunological (Bakke-McKellep et al., 2000, 2007a), metabolic and hormonal factors (Bakke-McKellep et al., 2007b) have been performed to investigate the salmonid response to soy protein. Proteomics has also been used to analyse global changes in protein expression in response to the introduction of soy protein diet (Martin et al., 2003) or other plant protein diet (Vilhelmsson et al., 2004).

Studies with Atlantic halibut have shown that up to 36% full-fat SBM can be added to the diet without adversely affecting growth, feed efficiency or intestinal histology (Grisdale-Helland et al., 2002). Inclusion of 28% soy protein concentrate also did not affect growth or protein digestibility but feed utilization was lower (Berge et al., 1999). Although the morphology of the Atlantic halibut digestive system has been well-characterized (Murray et al., 1993, 1994, 1996) as has the ontogeny of digestive enzyme production (Gawlicka et al., 2000; Murray et al., 2006), the effect of feeding non-fish based diets on gut histology has only received limited attention (Grisdale-Helland et al., 2002) and there are no reports of changes in intestinal gene expression in this species in response to the inclusion of plant protein in the diet. In this study we use a combination of morphological observations and nutrigenomics with a custom-made Atlantic halibut oligonucleotide microarray to assess changes in intestinal gene expression in juvenile Atlantic halibut over the first 3 weeks after introduction of a diet containing SBM.

2. Materials and methods

2.1. Fish rearing

Atlantic halibut juveniles (average weight 207.2 ± 6.6 g) were cultured at Scotian Halibut Ltd. (Clarks Harbour, Nova Scotia, Canada) on October 18, 2006. Each of six tanks was stocked with 42 fish and the fish raised under constant incandescent light (approximately 1000 lx at the surface) in 0.26 m^3 tanks with flow-through oxygenated salt water (30 ppt) maintained at 11 ± 0.2 °C using a heat exchanger. The halibut were hand fed to satiation on a commercial FM diet (North East Nutrition Inc., Truro, NS, CAN) three times daily from the time of metamorphosis until the initiation of the trial. On October 24, 2006, the SBM diet was introduced to fish in three randomly assigned tanks whereas FM diet was introduced to fish in the three remaining tanks. Sampling of fish tissues occurred after 1, 10 and 21 days. The mean weight of the fish in each tank was calculated at the beginning and the end of the trial and medians for each tank used to determine weight gain. Feed consumption weights (amount of food administered), oxygen saturation and temperature were measured daily. All animals were maintained and sampled according to the guidelines set by the Canadian Council of Animal Care (Olfert et al., 1993).

2.2. Diet composition

We have used two experimental diets and, since information on the amino acid requirements of halibut is unavailable, formulation of these diets was based on the amino acid requirements of salmonids (NRC, 1993). The FM and SBM diets were isonitrogenous and isocaloric, containing 50% total protein and 22% total lipid (Table 1). In addition, the fibre and carbohydrate compositions were designed to be similar. The two diets differed in the partial replacement of FM with SBM at an inclusion level of 30%. Solvent-extracted dehulled SBM was used. Dry ingredients of the diets were finely ground (<800 µm) using a Perten Laboratory Mill (Model 3100, Perten Instruments, Huddinge, Sweden) before being combined with the liquid ingredients

Table 1
Formulation of the experimental diets (as-fed basis).

	Fish meal diet (g/kg)	Soybean meal diet (g/kg)
Ingredient		
Fish meal[a]	640.0	472.0
Soybean meal (dehulled)[b]	0.0	300.0
Wheat middlings[c]	150.0	0.0
Whey[d]	30.0	30.0
Fish oil[e]	152.0	170.0
Vitamin mixture[f]	18.0	18.0
Mineral mixture[g]	10.0	10.0
Calculated analysis		
Crude protein	503.5	499.9
Lipid	220.8	220.5
Fibre	16	13
Carbohydrates	183	199
Gross energy (MJ/kg)	22.0	22.1

[a] Scotia Garden Seafood Incorporated, Yarmouth, NS.
[b] Bunge Canada, Oakville, ON.
[c] Walker's Livestock Feeds, Dartmouth, NS.
[d] Farmer's Co-operative Dairy Ltd., Truro, NS.
[e] Stabilized with 0.06% ethoxyquin. Commeau Seafood, Saulnierville, NS.
[f] Vitamin added to supply the following (per kg diet): vitamin A, 8000 IU; vitamin D3, 4500 IU; vitamin E, 300 IU; vitamin K_3, 40 mg; thiamine HCl, 50 mg; riboflavin, 70 mg; d-Ca pantothenate, 200 mg; biotin, 1.5 mg; folic acid, 20 mg; vitamin B_{12}, 0.15 mg; niacin, 300 mg; pyridoxine HCl, 20 mg; ascorbic acid, 300 mg; inositol, 400 mg; choline chloride, 3000 mg; butylated hydroxy toluene, 15 mg; butylated hydroxy anisole, 15 mg.
[g] Mineral added to supply the following (per kg diet): calcium phosphate (mono), 6000 mg; manganous sulphate (32.5% Mn), 40 mg; ferrous sulphate (20.1% Fe), 30 mg; copper sulphate (25.4% Cu), 5 mg; zinc sulphate (22.7% Zn), 75 mg; sodium selenite (45.6% Se), 1 mg; cobalt chloride (24.8% Co), 2.5 mg; sodium fluoride (42.5% F), 4 mg.