Full-fat soybean meal in diets for Atlantic halibut: growth, metabolism and intestinal histology

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Abstract

Isonitrogenous and isocaloric diets containing 0, 18 or 36% toasted full-fat soybean meal (FFSM) were fed to Atlantic halibut. The diets were fed to five tanks of fish each for 34 days (period 1). Four tanks from each treatment were then retained in the growth experiment for a further 32 days (period 2), while the groups of fish from one tank from each of the 0 and 36% FFSM groups were split and transferred to two metabolism tanks each. The initial weight of the fish in the growth trial was 169 ± 1 g (mean ± SEM, $n = 12$; weight range 89–253 g) and the final weight was 317 ± 5 g. There was no significant effect of dietary treatment on specific growth rate (range, 0.8–1.1% day^{-1}), feed consumption (0.5–0.7% body weight day^{-1}), feed efficiency (1.3–1.6 g wet gain g dry feed^{-1}), protein retention (48–55%) or energy retention (49–57%). The fat, protein and energy concentrations in the fish increased during the trial and were not affected by the diet. The hepatosomatic index in fish fed with 36% FFSM diet was significantly lower (1.7%) than in the other groups (2.2%) ($P < 0.05$). No differences in intestinal morphology were observed between dietary treatments and no pathological reactions were identified in any of the samples. In the metabolism trial, there were no significant differences in oxygen consumption or ammonia excretion between fish fed with 0 and 36% FFSM diets. In conclusion, up to 36% FFSM may be added to diets for Atlantic halibut without negative effects on growth, feed efficiency or intestinal morphology.

KEY WORDS: Atlantic halibut (*Hippoglossus hippoglossus*), feed utilization, full-fat soybean meal, growth, intestinal enteritis, protein sources

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Introduction

Uncertainty about the supply of fish meal has prompted investigations of alternative protein sources, but information about the use of such protein sources for production of Atlantic halibut (*Hippoglossus hippoglossus*) is scarce. During the last three decades, soybean products have been tested as protein sources in fish diets, but results have varied depending mainly on the fraction of the beans used and the processing and inclusion level of the soybean product. The inclusion of 28% soybean protein concentrate (SPC) in a fish meal based diet (replacement of 44% of the nitrogen) had no effect on halibut growth or protein digestibility, but feed utilization was poorer (Berge *et al*. 1999). The replacement of fish meal protein with 14–56% SPC protein also had no effect on the growth of Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), whereas a linear decrease in growth occurred when 14–56% full-fat soybean meal (FFSM) protein was included in high protein (43%) diets (Olli & Krogdahl 1994; Olli *et al*. 1994). In contrast, the inclusion of 30% FFSM in a 24% protein diet for fingerling tilapia (*Oreochromis niloticus* X *O. aureus*) (Shiau *et al*. 1990) and in a 40% protein diet for rainbow trout (Oliva-Teles *et al*. 1994) resulted in good growth compared with fish fed with fish meal control diets. Differences between studies may have resulted from the soybean products used, but may also be partly related to the quality of the fish meal used.

The aim of this trial was to examine the effect of replacing low temperature produced (LT) fish meal with FFSM in a high protein, medium fat diet on the growth and metabolism of Atlantic halibut.

Materials and methods

Diets

Three isonitrogenous and isocaloric diets containing 0, 18 or 36% toasted FFSM were prepared through replacement of

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fish meal, wheat and fish oil (Table 1). The extruded diets (6 mm pellets) were produced by NorAqua Innovation AS (Dirdal, Norway). The diets were analysed for dry matter (DM) (105 °C, until constant weight), fat after HCl hydrolysis (Soxtec HT6, Tecator, Höganäs, Sweden), crude protein (nitrogen × 6.25; Kjeltec Auto System, Tecator, Höganäs, Sweden) and ash (550 °C, overnight). Energy was measured using an adiabatic bomb calorimeter (Parr 1271, Parr Instrument Company, Moline, IL, USA).

Growth trial

Individually tagged (Passive Integrated Transponder) Atlantic halibut were used in the trial. At the start of a 6-day adaptation period, 28 fishes were placed in each of 15 1-m^2 tanks (water depth 50 cm), held under continuous light and fed with a commercial diet. The tanks were filled with seawater at 12 ± 0.1 °C (flow rate 10 L min^{-1}). Oxygen concentration in the effluent water was measured weekly and never fell below 8 mg L^{-1}. After the adaptation period and a 2-day fast, the fish were individually weighed and one fish from each of 12 tanks was taken for the initial sample (the extra fish in the other three tanks were removed from the experiment). The three diets were fed to five tanks of fish each during 34 days. Following a 1-day fast, the fish in each tank were weighed in bulk. Four tanks from each treatment continued in the growth trial for a further 32 days, while two tanks of fish (fed with 0 or 36% FFSM diets) were used in a metabolism trial (see below). The fifth tank fed with 18% FFSM diet was removed from the trial. At the end of the growth trial, the fish in each of the 12 tanks were individually weighed. In order to allow histological samples of the intestine to be taken, the fish were not fasted before weighing. Only the fish from the 12 tanks that were used during the 2-month period were included in the analysis of the growth trial. At the start of the growth trial, the fish in the 12 tanks ranged in weight from 89 to 253 g and the mean (±SEM, $n = 12$) fish weight in each tank was 169 ± 1 g.

Table 1 Formulation and chemical composition of the diets

	Soybean inclusion level (%)		
	0	18	36
Formulation (g kg^{-1})			
Fish meal (Norse-LT 94)[1]	590	508	425
Full-fat soybean meal[2]	0	180	360
Wheat grits[3]	199.7	139.7	80.7
Fish oil	183	156	129
Arbocel[4]	22	11	0
Vitamins and minerals[5]	4.3	4.3	4.3
Betain[6]	1	1	1
Chemical analysis (g or MJ kg^{-1})			
Dry matter	940	942	919
Composition in dry matter			
Protein	484	494	492
Fat	273	268	278
Ash	74	77	74
Carbohydrate[7]	169	161	156
Energy	24.72	24.57	24.84

[1] Norsildmel, Bergen, Norway.
[2] Soyax-Aqua LF®, Schouten Industries B.V., Glessen, the Netherlands. Chemical composition, g kg^{-1}: dry matter, 919; of dry matter: protein, 429; fat, 213; ash, 56; gross energy, 23.42 MJ kg^{-1}. Declared maximum trypsin inhibitor activity 1 mg g^{-1}.
[3] Chemical composition, g kg^{-1}: dry matter, 892; of dry matter: protein, 161; fat, 43; ash, 23; gross energy, 18.38 MJ kg^{-1}.
[4] Arbocel, J. Rettenmaler & Söhne GmbH & Co., Ellwangen-Holzmühle, Germany. Declared approximate composition (%): cellulose, 80; lignin, 20; ash, 1.5.
[5] NorAqua Innovation's standard formulations for vitamins and minerals for salmonids were used. These are formulated according to or higher than the requirements published in National Research Council (1993).
[6] Betafin®, Finnsugar Bioproducts, Helsinki, Finland.
[7] Carbohydrates calculated as 1000 − (protein + fat + ash).

During the growth trial, the fish were fed from automatic feeders for 12.5 min every 2 h. Waste feed was collected from the outlet water by a wire mesh collector, separated from faeces, weighed and frozen. Based on DM analysis of waste feed and percentage recovery of waste feed DM, the daily feed intake was calculated (Helland *et al.* 1996). The feeding level was adjusted based on the previous 4 days intake in each tank and allowing for 15–20% waste.

In addition to the initial sample of 12 fishes, 10 fishes per tank were sampled at the end of the growth trial. The fish were anaesthetized in MS-222 (metakain, 80 mg L^{-1}), individually weighed and then killed with a blow to the head. The intestines, liver and carcass were weighed individually and then all tissue samples were pooled by tank for analysis of whole fish. As the fish sampled at the end of the trial were not fasted, feed and faeces were pressed out of the stomach and intestine before weighing. The pooled samples were stored at –20 °C before being analysed for DM, crude protein, fat, ash and energy as described above (fat analysis without HCl hydrolysis).

Histological examinations were done on the intestines of five of the 10 fish sampled per tank. The fish were processed with minimal time delay, just after weighing. The abdominal cavity was opened and the gastrointestinal system was removed. After careful dissection, 1 cm pieces of intestinal tissue were taken from each of four locations: (1) middle part of the most distal of the four pyloric caecae; (2) one-third of the distance between the gastrointestinal and intestinal-rectal junctions; (3) two-thirds of the distance between the gastrointestinal and intestinal-rectal junctions; and (4) midway

between the intestinal–rectal and rectal–anal junctions. The samples were cut open in order to get better exposure of the intestinal mucosa to fixative, carefully rinsed in saline, and then fixed in 4% phosphate-buffered formalin. Further processing, light microscopy and evaluation were performed as described in Baeverfjord & Krogdahl (1996). The remaining parts of the intestines were included in the samples used for whole fish analysis. It is not expected that removal of the small sections of the intestines would greatly affect the composition of the whole fish samples.

Calculations

Feed consumption (% body weight (BW) day^{-1}) = 100 × (dry feed intake fish^{-1}) × [(initial BW + final BW)/2]$^{-1}$ × days fed^{-1}.

Specific growth rate (SGR) (% per day) = 100 × (ln final BW – ln initial BW) × days fed^{-1}.

Feed efficiency = wet BW gain × dry feed intake^{-1}.

Retention of protein or energy (%) = 100 × [(final BW × final body nutrient content) – (initial BW × initial body nutrient content)] × (nutrient content in diet × feed intake)$^{-1}$.

Hepatosomatic index = 100 × liver weight × BW^{-1}.

Dressing percentage = 100 × carcass weight × BW^{-1}. The carcass consisted of the whole fish minus the gastrointestinal system and the liver.

Metabolism trial

The fish in the two tanks removed from the growth trial were split into two groups of 13 or 14 fish each and placed in four 150-L self-cleaning tanks (diameter 0.75 m) with lids. This density gave approximately the same bottom area per fish as in the growth trial. At the start of the metabolism trial, the mean weights of the fish fed with control and 36% FFSM diets were 260 and 249 g, respectively. The duplicate tanks of fish were fed with two diets for 25 days and daily feed intake measured, as described for the growth trial. The tanks were supplied with the same source of water as in the main trial, and the intermittent-flow system was essentially the same as that described by Grisdale-Helland & Helland (1995). Metabolism measurements were done automatically on each tank of fish during 15-min closed periods every 2 h. Daily measurements were calculated by averaging the data from each day. Oxygen consumption was measured during the last 17 days of the feeding period, using a water-jacketed (tank water temperature) O_2 electrode connected to an oxygen meter (E101 electrode and OM400 oxygen meter, Cameron Instrument Company, Port Arkansas, USA). Because of equipment problems, ammonia production was only determined on seven of the last 9 days of the feeding period (TRAACS 800, Bran + Luebbe, Hamburg, Germany). The fish were weighed at the end of the trial after a 2-day fast. The weight of fish in each tank on the days metabolic measurements were taken was calculated as: initial BW + (feed intake to date × weight gain in period × feed intake in period^{-1}).

Statistical analyses

The data were checked for homogeneity of variance using Bartlett's test and percentage data were subjected to arcsin transformation (Sokal & Rohlf 1995). Because of dissimilar variances in the metabolism data, Welch's approximate *t*-test (Sokal & Rohlf 1995) was used to test for differences between the two dietary treatments. Final fish weight was included in the model for body composition data. The data with tank means were subjected to one-way analysis of variance (ANOVA) including the effect of diet. Individual growth data were subjected to a two-way ANOVA, including the fixed effect of diet and the random effect of tank within diet. Differences between treatments were considered significant at the $P < 0.05$ level, and are presented as means ± SEM (where $n = 2$, individual values are given).

Results

There was no mortality in the growth or metabolism trials. In the growth trial, mean feed consumption in the 12 tanks completing the experiment was 0.71 ± 0.01% BW day^{-1} in the first and 0.55% BW day^{-1} in the second period and was not significantly affected by dietary treatment ($P > 0.05$). In addition, there were no significant effects of soybean inclusion on final weight, SGR (range, 0.8–1.1% day^{-1}), or feed efficiency (1.3–1.6 g wet gain g dry feed^{-1}) (Table 2). There was no dietary effect on dressing percentage, but the hepatosomatic index of the fish fed with 36% FFSM diet was lower ($P < 0.05$) in other groups (Table 2).

Although fat, protein and energy concentrations in the fish increased during the trial, these did not differ significantly among diets (Table 3). Retention of dietary nitrogen and energy was within the range 50–55% and neither was significantly affected by diet (Table 3).

During period 1, the tanks of fish subsequently used in the metabolism trial showed feed intake levels within 7% of the mean of the other four tanks fed with same diet. However, during period 2, feed intake was 22% lower in the fish in the

Table 2 Feed consumption, growth, feed efficiency, hepatosomatic index and dressing percentage of Atlantic halibut[1] fed with experimental diets in the growth trial (mean ± SEM, $n = 4$)

Soybean inclusion level (%)	Feed consumption (% BW day^{-1})	Final body weight (g)	Specific growth rate (% day^{-1})	Feed efficiency[2]	Hepatosomatic index (%)	Dressing percentage (%)
0	0.59 ± 0.02	314.8 ± 9.6	0.93 ± 0.05	1.51 ± 0.02	2.24 ± 0.12[a]	95.0 ± 0.2
18	0.61 ± 0.02	310.7 ± 9.1	0.95 ± 0.05	1.49 ± 0.05	2.21 ± 0.20[a]	95.2 ± 0.3
36	0.65 ± 0.02	325.4 ± 7.4	0.97 ± 0.02	1.44 ± 0.04	1.67 ± 0.13[b]	95.6 ± 0.2

[1] Initial body weight, 169 ± 36 g (SD) ($n = 324$); initial hepatosomatic index and dressing percentage, 1.71 ± 0.4 and 94.9 ± 1.0%, respectively ($n = 12$).
[2] Wet weight gain (g) × dry feed consumed (g)$^{-1}$.
Values not sharing a common superscript are significantly different ($P < 0.05$).

Table 3 Whole body chemical composition and nitrogen and energy retention of Atlantic halibut fed with experimental diets in the growth trial (mean ± SEM, $n = 4$)

	Whole body chemical composition						
Soybean inclusion level (%)	Dry matter (g kg^{-1})	Protein (g kg^{-1})	Fat (g kg^{-1})	Ash (g kg^{-1})	Energy (MJ kg^{-1})	Nitrogen retention (%)	Energy retention (%)
Initial	288	157	90	27	7.68		
0	304 ± 2	161 ± 1	112 ± 1	26 ± 0.1	8.27 ± 0.06	51.9 ± 1.2	54.9 ± 1.0
18	304 ± 1	163 ± 1	109 ± 2	26 ± 0.6	8.27 ± 0.11	51.2 ± 1.5	54.2 ± 0.5
36	305 ± 2	165 ± 2	113 ± 4	25 ± 0.5	8.28 ± 0.07	50.7 ± 1.4	51.7 ± 1.6

metabolism trial (0.43% BW day^{-1}) than in the main trial (0.55% BW day^{-1}). No significant differences were found in oxygen consumption or ammonia excretion rates between fish fed with control and 36% FFSM diets (Table 4).

No differences in morphology were observed between dietary treatments for any of the intestinal regions examined and no pathological reactions were identified. Intestinal morphology corresponded with the description given by Murray *et al.* (1996), with the possible exception of the region of the pyloric caecae. In the present study, the enterocytes of the pyloric caeca were more vacuolated than corresponding cells shown in a study by Murray and co-workers (Murray *et al.* 1996).

Table 4 Oxygen consumption and ammonia excretion of duplicate groups of halibut fed with experimental diets in the metabolism tanks during Period 2. The values are daily means ± SEM of measurements done on each tank of fish on 15–16 of the last 17 days of the feeding period for oxygen and on seven of the last 9 days of the feeding period for ammonia

Soybean inclusion level (%)	Replicate	Oxygen consumption (g O_2 day^{-1} kg^{-1})	Ammonia excretion (mg N day^{-1} kg^{-1})
0	1	2.52 ± 0.10	92.6 ± 8.8
0	2	2.57 ± 0.06	72.6 ± 4.3
36	1	2.64 ± 0.09	76.3 ± 7.0
36	2	2.64 ± 0.06	67.4 ± 3.9

Discussion

The growth of the halibut during the 2-month trial was similar to that recorded previously for similar fish sizes and water temperatures (Hjertnes *et al.* 1993; Helland & Grisdale-Helland 1998; Stefánsson *et al.* 2000), and was not affected by the replacement of the fish meal, fish oil and wheat, and the small quantity of cellulose and lignin, with FFSM. This contrasts with the findings of Oliva-Teles *et al.* (1994) in a study with rainbow trout. They reported that replacement of brown fish meal and dextrin by 30% FFSM resulted in improvements in growth, protein digestibility and nitrogen retention. Differences in the results between these trials may have arisen because of the quality of the protein in the respective fish and soybean meals used, as has been seen elsewhere (Anderson *et al.* 1993, 1995; Romero *et al.* 1994; Aksnes & Mundheim 1997; Refstie *et al.* 1998). Nitrogen retention by the rainbow trout used by Oliva-Teles *et al.* (1994) improved from 24.6 to 26.7%, which is much lower than those found with halibut in the present study. The high nitrogen retention by halibut indicates that protein quality was good in the three sources: fish meal, FFSM and wheat and the results confirm earlier work showing high utilization efficiency of LT-fish meal protein by halibut (Helland & Grisdale-Helland 1998; Berge *et al.* 1999).

The higher hepatosomatic index in the fish fed with control and 18% FFSM diets compared with the 36% FFSM diet

suggests that the level of absorbed carbohydrate decreased with increasing FFSM and decreasing wheat, as expected based on the lower content of starch in soybeans than in wheat (Bach Knudsen 1997).

Oxygen consumption and ammonia excretion were not affected by the inclusion of 36% FFSM, supporting the results of the growth study. Variation was high in the ammonia excretion data though, most likely caused by the day-to-day variation in feed intake and emphasizes the need for frequent measurements throughout the trial period. When oxygen consumption is calculated on a metabolic weight basis using the weight coefficient 0.78 determined for plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) at 10 °C (Fonds *et al*. 1992), there was no difference between our results and those of Hallaråker *et al*. (1995) for 80 g halibut held in 10 °C water (both 1.9 g O_2 day^{-1} $kg^{-0.78}$), but greater than that calculated from the results of Jonassen *et al*. (2000) for 80 g halibut held in 12 °C water (1.6 g O_2 day^{-1} $kg^{-0.78}$). Ammonia production of the fish fed with control diet in the present trial (57 mg NH_3-N day^{-1} $kg^{-0.71}$) was lower than that reported by Jonassen *et al*. (2000) (86 mg NH_3-N day^{-1} $kg^{-0.71}$), on a metabolic weight basis (using $b = 0.71$ found for plaice at 10 °C; Jobling 1981). The composition of the control diet used in the present trial and the commercial diet used by Jonassen *et al*. (2000) were similar, but because of the difference in fish sizes used in these two trials, the effect of feed intake level on the rates of oxygen consumption and ammonia excretion cannot be determined.

Atlantic salmon and rainbow trout may develop inflammatory reactions of the distal intestine when fed diets containing soybean meal (van den Ingh *et al*. 1991, 1996; Rumsey *et al*. 1994) and these may be hypersensitivity or allergic reactions (Baeverfjord & Krogdahl 1996). Interspecies variation in sensitivity has been demonstrated between Atlantic salmon and rainbow trout (Refstie *et al*. 2000) and we do not know to what extent halibut is sensitive to soy antigens even though no negative effects were observed in the present study. Intestinal reactions have been observed in Atlantic salmon fed solvent extracted soybean meals (Baeverfjord & Krogdahl 1996) and van den Ingh *et al*. (1991) reported intestinal pathology in Atlantic salmon fed with diets containing 30% FFSM, whereas an absence of such reactions in Atlantic salmon fed with 7–9% FFSM has also been noted (Refstie *et al*. 2001).

The results of this study indicate that up to 36% FFSM may be added to diets for Atlantic halibut without affecting growth, feed efficiency or intestinal morphology. Further studies are necessary though, on the effects of other soybean products on halibut intestine and, in addition, the sensory quality of halibut.

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