Feeding Atlantic salmon *Salmo salar* L. soybean products: effects on disease resistance (furunculosis), and lysozyme and IgM levels in the intestinal mucosa

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Abstract

Two trials were initiated to investigate the consequences of including various soybean products in diets for Atlantic salmon *Salmo salar* L. on (1) mortality following infection by *Aeromonas salmonicida* ssp. *salmonicida* during a cohabitation challenge, and (2) the lysozyme and IgM content of the intestinal mucosa. Groups of salmon were fed control diets containing fishmeal as the sole protein source (Contr1 and Contr2, respectively), soy concentrate-containing diets (SoyConc1 and SoyConc2, respectively), or diets containing either solvent-extracted soybean meal (SoyMeal, trial 1) or soybean molasses (SoyMol, trial 2), an alcohol extract of soybean meal. Both SoyMeal and SoyMol caused enteritis-like changes in the distal intestine, which were not observed in fish fed the Contr1, Contr2, SoyConc1, or SoyConc2 diets. There were significant differences ($P < 0.05$) in mortality between feeding groups following the *A.s. salmonicida* challenge: these differences were greatest in fish fed SoyMeal (65.6%), least in fish fed SoyConc1 (60.5%), and intermediate in the fish fed the Contr1 diet (62.9%). The SoyMol diet caused significantly ($P < 0.0001$) increased levels of both lysozyme and IgM in the mid and distal intestinal mucosa. It is concluded that components of soybean meal and soybean molasses cause an inflammatory response in the distal intestine that may lead to increased susceptibility to furunculosis.

KEY WORDS: *Aeromonas salmonicida*, distal intestine, enteritis, nutrition, salmonid, soybean meal

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Introduction

Different soybean products have been tested for suitability in the search for an alternative, easily available and inexpensive protein source to feed salmonids raised on fish farms. From results of earlier studies (van den Ingh *et al.* 1991; Rumsey *et al.* 1994; Baeverfjord & Krogdahl 1996), it has been shown that feeding Atlantic salmon *Salmo salar* L. and rainbow trout *Oncorhynchus mykiss* standard, solvent-extracted soybean meal (SBM) causes morphological changes in the mucosa of the distal intestine. There is a shortening of the primary and secondary mucosal folds, with a profound widening of the lamina propria which becomes infiltrated with a mixed population of inflammatory cells such as lymphocytes, polymorphonuclear leukocytes, macrophages and eosinophilic granule cells. The number of normal supranuclear vacuoles in the enterocytes are dramatically decreased or even absent. The microvilli of the brush border membrane are shortened and there is an increased formation of microvillar vesicles. Weight gain, fecal dry matter, and protein and fat digestibility by salmonids on a toasted or solvent-extracted SBM diet are decreased (Olli & Krogdahl 1994; Olli *et al.* 1994; Rumsey *et al.* 1994). In the search for the causative agent(s) of this SBM-induced enteritis, the alcohol-extract of SBM (soybean molasses) has been found to cause the same changes (Olli & Krogdahl 1995; van den Ingh *et al.* 1996), although precisely which component is involved is not known. Correspondingly, alcohol-extracted soy concentrate has been found to be of high nutritional value in salmonid diets (Olli & Krogdahl 1994; Olli *et al.* 1994).

Over the last several years, fishmeal prices have increased, which has resulted in a sharp increase in the use of soybean products in salmonid rations. There is an urgent need for knowledge on health-related effects of SBM. Its effects on the

intestine's integrity and local immune response may influence its resistance to bacterial invasion, especially since cells of the specific and nonspecific immune response appear to be well represented in the widened lamina propria (Baeverfjord & Krogdahl 1996). The morphological alterations caused by soybean feeding indicate changes in cell turnover by affecting cell proliferation and apoptosis. The mucosal cells may therefore differ in maturation and hence cell membrane receptor glycosylation (Pusztai *et al.* 1995), which may in turn cause changes in the ability of microbes to colonize the intestinal epithelium (Pusztai *et al.* 1993).

It has been suggested that the disease-causing agent of furunculosis, *Aeromonas salmonicida* ssp. *salmonicida*, enters the organism through the intestine (Tatner *et al.* 1984; Bøgwald *et al.* 1994; Austin 1997). During a challenge trial in which experimentally infected cohabitants are used to transmit the disease, the bacteria were thought to gain entry into the gastrointestinal tract when feed pellets become coated with bacteria as they pass through the lipid-rich surface microlayer of water where pathogens accumulate (Enger *et al.* 1992).

The aim of this study was to investigate whether the SBM-induced enteritis would affect Atlantic salmon's susceptibility to infection. In the first of two feeding trials, mortality following infection by *A.s.* ssp. *salmonicida* during a cohabitation challenge trial is reported in salmon fed diets containing solvent-extracted SBM and soy concentrate, and compared with fish fed a control diet containing fishmeal as the sole protein source. The subsequent feeding trial was initiated to investigate lysozyme and immunoglobulin (IgM) content of the mid and distal intestinal mucosa in salmon fed soybean molasses and soy concentrate compared with those fed a control diet.

Materials and methods

Challenge experiment

The challenge experiment was carried out at Vikan AkvaVet, Norway, and consisted of three consecutive periods: 4 weeks of acclimatization, 3 weeks of feeding the experimental diets, and finally, a 4-week challenge period.

Fish, feeds and feeding: Unvaccinated, seawater-adapted Atlantic salmon smolts (mean weight 37 g) of a farmed stock originating from the national salmon breeding programme (Norwegian Sunndalsøra breed) were used (Gjedrem *et al.* 1991). The fish had been transferred from freshwater and allowed a 4-week period of gradual acclimatization to seawater, and to their new environment before initiation of the trial. The average water temperature during acclimatization was 10.5 °C and average salinity was 25 g L^{-1}. The fish were hand-fed to satiety with an extruded commercial diet (Skretting AS, Stavanger, Norway) prior to the experimental period. The feeding experiment was initiated by distributing the fish into three 1-m^2 tanks (225 fish in each tank). At the same time, the fish were marked with fin notches for identification by feed group.

Three diets were developed for the trial (Skretting AS): one control diet in which fishmeal was the sole protein source (Contr1), one containing soy concentrate (SoyConc1) and one containing solvent-extracted SBM (SoyMeal). The two soy-containing rations were formulated so that the soy proteins made up 30% of the total protein. The vitamin and mineral premix was formulated and added to the diets to meet or exceed the known nutrient requirements of salmonids (National Research Council 1993). See Table 1 for diet compositions. The diets were extruded at a pellet size of 3 mm. One ration was designated for each of the three tanks. The fish were fed to satiety twice daily (0800–0900 h and 1200–1500 h) for 21 days. The amounts of feed given were registered.

The design of the challenge study (see below), in which one-third of the fish from each feeding group was allocated each of three replicate contagion tanks, made replication of feeding groups unnecessary. Tank variation was included in the 'error' of the challenge study. Moreover, it has been reported that the morphological changes in the distal intestine caused by dietary SBM hardly vary between replicate tanks (Baeverfjord & Krogdahl 1996). The variance of the study was low, and differences in mortality less than 2.4% were significant (see Results). It was therefore not considered necessary to estimate tank effects between feeding groups to increase accuracy.

Sampling for morphological studies: To monitor the development of morphological changes caused by the soybean-containing diets, samples of the distal intestine from five anaesthetized fish from each feeding group were collected at the end of the feeding trial, and at the end of the challenge period 28 days later (a total of 30 fish). The intestines were removed immediately, opened longitudinally and rinsed with cold (4 °C) saline. Samples were taken from the mid and distal intestine for light microscopy and fixed in 10% buffered formalin, pH 7.2, at room temperature. After fixation in formalin, the samples were routinely dehydrated and embedded in paraffin. Sections 5 μm thick were stained with haematoxylin and eosin (H&E). A microscopic, qualitative evaluation of the mucosa of the distal intestine from

Table 1 Formulation of the experimental diets in g kg^{-1}

	Challenge experiment			Mucosal immunological parameters		
Ingredients (g kg^{-1})	Contr1	SoyConc1	SoyMeal	Contr2	SoyConc2	SoyMol
Fishmeal[1]	560	392	364	619	387	345
Extracted SBM[2]	0	0	24.0	—	—	—
Soy concentrate[3]	0	178	0	0	222	198
Soybean molasses[4]	—	—	—	0	0	108
Fish oil	179	175	161	150	167	149
Wheat	117	114	105	200	193	172
Methionine[5]	—	—	—	1.5	1.4	1.3
Suprex[6]	102	100	92	—	—	—
Binder[7]	26	25	23	20	19	17
Vitamin C[8]	1	1	1	—	—	—
Vitamin/mineral premix[9]	10	10	9	10	10	10
Pigment mix 1%[10]	0.5	0.5	0.5	—	—	—
Yttrium	—	—	—	0.1	0.1	0.1
Sum	1000	1000	1000	1000	1000	1000

[1]Norse-LT, low-temperature dried fishmeal (Norsildmel, Bergen, Norway).
[2]Solvent-extracted, toasted soybean meal (Denofa, Fredrikstad, Norway).
[3]Soycomil (Loders Croklaan, Wormerveer, Holland).
[4]Alcohol-extract of soybean meal (Central Soya Aarhus, Aarhus, Denmark).
[5]DL-methionine (Degussa, Hanau, Germany).
[6]Suprex extruded wheat (Codrico, Rotterdam, Holland).
[7]Edelbind (Lyckeby Stärkelsen, Kristianstad, Sweden).
[8]Trovi-C (Hoffmann-La Roche, Basle, Switzerland).
[9]Commercially available premix (BASF, Ludwigshafen, Germany).
[10]Carophyll Pink, 1% astaxanthin formulation (Hoffmann-La Roche, Basle, Switzerland).

the groups fed the SoyConc1 and SoyMeal diets was performed and compared with the morphological features of the Contr1 fed group at both points of time.

Challenge: After 3 weeks of feeding, the fish were redistributed into three contagion tanks for the challenge trial: 70 fish from each of the three feeding groups in each tank. The fish were fed a common commercial diet (3 mm pellets, Skretting AS) to satiety during this part of the experiment. The 'infection source' were fish (cohabitants) of the same population as the fish in the feeding trial. The number of cohabitants chosen corresponded to 10% of the number of fish in each contagion tank. They were anaesthetized, marked with fin notches and given an intraperitoneal injection of 0.1 mL of a solution (0.1 mol L^{-1} phosphate buffered saline, pH 7.2, 0.85% NaCl) containing *A.s.* ssp. *salmonicida* in a concentration of 100 000 CFU (colony-forming units) mL^{-1}. Prior to injection with the infectious agent and transfer to the contagion tanks, the cohabitants had been fed the commercial diet (3 mm pellets) while the fish in the feeding trial had received the experimental diets. The infected cohabitants were introduced into the contagion tanks in random order. Within 4–6 days these cohabitants died (100%). The number of fish from each feeding group that subsequently died was registered and samples were taken from 30% of these fish and all the infected cohabitants for bacteriological examination. For this purpose, the head kidney was sampled and placed on trypticase yeast agar (TYA) for incubation at 22 °C for 2–4 days. The final identification of the bacteria was done by species-specific antiserum (BIONOR Aqua Mono-As, Skien, Norway).

Mucosal immunological parameters

Lysozyme and IgM content of the mucosa of the distal intestine of Atlantic salmon were analysed following a second feeding experiment carried out at AKVAFORSK, Sunndalsøra, Norway. In this study, the alcohol extract of SBM (soybean molasses) was added to the one experimental diet (SoyMol) rather than solvent-extracted SBM. The fish were fed the experimental diets for 3 weeks.

Fish, feeds and feeding: Atlantic salmon of the Sunndalsøra breed, average weight 620 g, were kept in nine 1-m^2 salt water tanks (salinity 32–33 g L^{-1}). Each tank contained 15 fish. The average water temperature was 8 °C during the experiment. The diet compositions of the three rations are given in Table 1 (Contr2, SoyConc2 and SoyMol) and were

produced by Skretting AS. The vitamin and mineral premix was formulated and added to the diets to meet or exceed the known nutrient requirements of salmonids (National Research Council 1993). The diets were cold-pelleted. Each diet was fed to fish in three randomly selected tanks, according to the expected growth rate. The feed requirement was considered to be 1 kg kg^{-1} weight gain. Feed was supplied automatically every 15 min throughout the experiment until termination after 3 weeks.

Sampling for morphological studies: At the termination of the experiment, five fish from each tank were anaesthetized and sections of the distal intestine were prepared for microscopy and qualitatively evaluated as described in the challenge experiment. The mucosal layer of the intestinal wall of the mid-intestine and the remainder of distal intestine was sampled for analysis of lysozyme and IgM content.

Assays of lysozyme activity: Lysozyme activity was determined as described by Røed *et al.* (1993) using a *Micrococcus* lysoplate assay developed by Osserman & Lawlor (1966), and a modified Gram-staining preservation technique developed by Lie *et al.* (1986). Briefly, agarose gels (1%) containing 50 μg mg^{-1} *Micrococcus lysodeikticus* were used. Intestinal mucosa homogenate, diluted 1:10 in 0.06 mol L^{-1} NaH_2PO_4/$NaHPO_4$-buffer (pH 6.6), was placed in wells in the agarose and incubated at 20 °C for 20 h. Methyl violet and Lugol solutions (1 g iodine, 2 g potassium iodide in 100 mL distilled water) were used to develop the unstained lysed zones. The diameter of the lysed zone was measured and quantified relative to the lysozyme activity in a standard salmon serum sample.

IgM level: The total amount of IgM was determined as described by Lund *et al.* (1995): enzyme-linked immunosorbent assay (ELISA) plates were coated with rabbit antisalmon Ig diluted 1:6000 in 0.05 mol L^{-1} carbonate buffer (pH 9.6). The plates were rinsed and incubated with rinsing buffer containing 1% bovine serum albumin (BSA) for 45 min at 37 °C. After a further rinse, mucosa homogenate (diluted 1:20 in phosphate buffer containing 0.5 mL L^{-1} Tween 20 and 4% horse serum) was added to the wells and incubated overnight at 4 °C. The plates were then rinsed and incubated with a monoclonal antibody raised against salmon IgM, diluted in rinsing buffer, for 1 h at room temperature. After rinsing, peroxidase-labelled antimouse Ig (Amersham, Uppsala, Sweden) diluted 1:3000 in rinsing buffer, was added and rinsed. The substrate *o*-phenylenediamine was added and the plates were incubated for 10 min at room temperature, followed by addition of 100 μL 1 mol L^{-1} H_2SO_4. The optical density was read spectrophotometrically at 492 nm.

Statistical evaluation

The number of fish sampled and the number of replicate tanks used in each of the two experiments were chosen based on earlier, comparable studies that have shown that these numbers supply statistically adequate accuracy. The results of the experiments were evaluated using analysis of variance with or without interaction included in the model, as appropriate for the experimental design in the two experiments (General Linear Model, SAS 1989). The model for the challenge trial was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \eta_k + e_{ijk}$$

where μ = total average, α_i = fixed effect of day, β_j = fixed effect of tank, η_k = fixed effect of diet, and e_{ijk} = error. The model used for the lysozyme and IgM content of the intestinal mucosa was:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

where μ = total average, α_i = fixed effect of diet, β_j = fixed effect of intestinal region $(\alpha\beta)_{ij}$ = interaction between diet and intestinal region, and e_{ij} = error.

Results

In both feeding trials, the distal intestine from the fish fed Contr1, Contr2, SoyConc1 and SoyConc2 appeared normal, whereas the fish fed SoyMeal or SoyMol displayed all the characteristic changes in the distal intestine described and illustrated by Baeverfjord & Krogdahl (1996): i.e. shortened mucosal folds, increased cellularity of the widened lamina propria and decreased vacuolization of the enterocytes.

Challenge experiment

The fish appeared to consume 1% of their body weight after an initial lag phase during the first 2 weeks of the acclimatization period. The mortality before the challenge was 4.7%. The majority of the fish that died did so within the first week of the acclimatization period. This was most likely due to the stress induced by seawater acclimatization and handling (netting and marking for identification) during transfer to the feeding tanks, since examination for bacteria in these individuals did not reveal the presence of specific pathogens. The 3 weeks of feeding the experimental diets progressed without complications. In the challenge trial, the body weights of the five fish from each feeding group, sampled for morphological studies after the 3-week feeding period and 4-week challenge period, revealed no significant effect of diet.

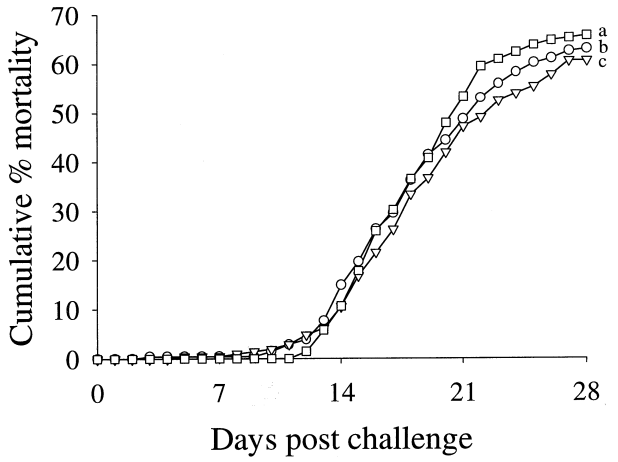

Figure 1 Cumulative percentage mortality of Atlantic salmon over a 28-day period following cohabitant challenge with *Aeromonas salmonicida* ssp. *salmonicida*. The fish were fed a control (Contr1, ○), soy concentrate (SoyConc1, ▽), or solvent-extracted soybean meal diet (SoyMeal, □) for 3 weeks prior to initiation of the challenge period. Different letters (a, b, c) signify significant differences ($P < 0.05$).

After the challenge period, during which all fish were fed a commercial diet for 28 days, qualitative evaluation of sections from the distal intestine revealed that the intestines of fish formerly fed SoyMeal had recovered in four of the five fish examined. The absorptive vacuoles of the mucosa had reappeared, and the mucosal folds appeared to have gained in length. In the one remaining fish, the typical SBM-induced changes were still present. No morphological differences were identified between fish fed the Contr1 and SoyConc1 diets.

Figure 1 shows the mean cumulative percentage mortality after challenge from each ration group. By the end of the trial, there was significantly ($P < 0.05$) higher mortality in the groups fed the SoyMeal diet (65.6%) and significantly lower mortality in the groups fed SoyConc1 (60.5%), compared with the groups that received Contr1 (62.9%). The experiment revealed a significant ($P < 0.05$) general tank effect.

The bacteriology report from necropsies performed on the dead fish after the challenge showed that all but four salmon were infected with *A. s. salmonicida*. Of the four fish with negative findings for the pathogen, three died on days 2, 4, and 8 postchallenge, i.e. before the incubation period of 10–12 days had elapsed.

Mucosal immunological parameters

The lysozyme activities in the mucosa of the mid and distal intestine of the fish fed the three different diets are shown in

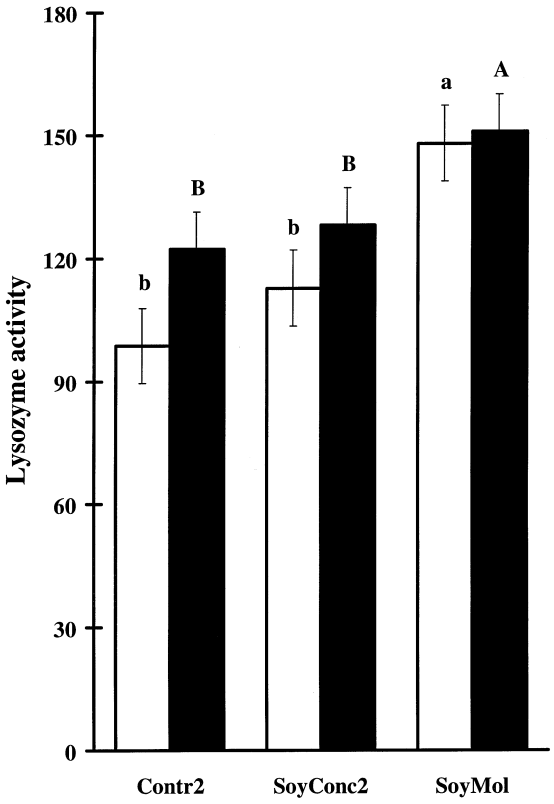

Figure 2 Lysozyme activity in mid (□) and distal (■) intestinal mucosa of Atlantic salmon fed a control (Contr2), soy concentrate (SoyConc2), or alcohol extract of soybean meal (SoyMol) diet. The lysozyme activity is expressed relative to an external standard (salmon serum sample). Data are presented as means ± SEM. Different capital and lower case letters signify significant differences ($P < 0.05$) when data were split according to intestinal region.

Fig. 2. Lysozyme activity was significantly higher in the intestinal mucosa of the salmon fed the SoyMol diet compared with the groups fed the Contr2 or SoyConc2 diets ($P < 0.0001$). The lysozyme activity had a tendency of being higher in the distal intestine than the mid-intestine ($P = 0.1433$). There was, however, a significant interaction between diet and intestinal region ($P = 0.0173$), showing a more marked increase in diet effect (SoyMol) on lysozyme activity in the mid-intestine than the distal intestine.

Figure 3 shows that the SoyMol diet caused significantly increased IgM content compared with levels found in the intestinal mucosa of fish fed the Contr2 or SoyConc2 diets ($P < 0.0001$). Moreover, the difference between intestinal regions was significant ($P < 0.0001$). The interaction between diet and region was also significant ($P < 0.0001$): feeding the SoyConc2 diet caused clearly significant increases in IgM levels in the mid intestine ($P < 0.0001$) compared

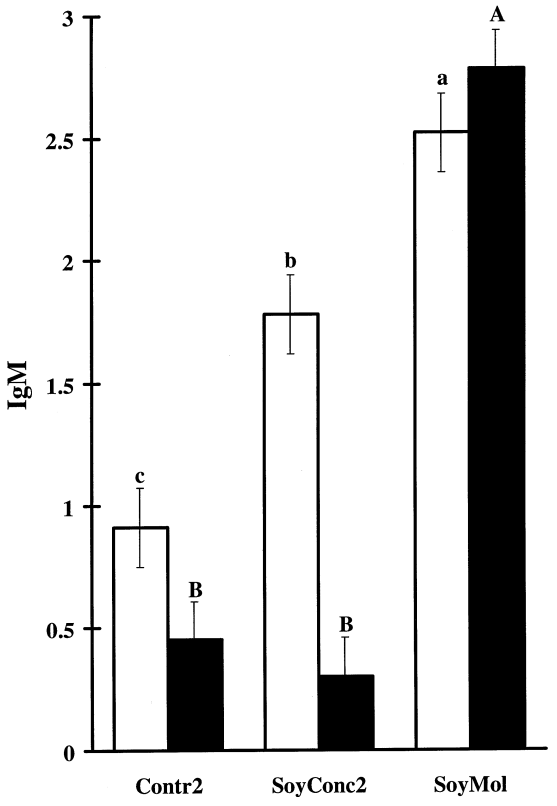

Figure 3 IgM level in mid (□) and distal (■) intestinal mucosa of Atlantic salmon fed a control (Contr2), soy concentrate (SoyConc2), or alcohol extract of soybean meal (SoyMol) diet. The IgM level is expressed as the optical density of the 1:20 mucosal homogenate dilution. Data are presented as means ± SEM. Different capital and lower case letters signify significant differences ($P < 0.05$) when data were split according to intestinal region.

with Contr2, whereas there was no significant difference between these diets in the distal intestine.

Discussion

Both solvent-extracted SBM and soybean molasses cause morphological changes in the distal intestine of Atlantic salmon, as described in this and earlier reports (van den Ingh *et al.* 1991, 1996; Baeverfjord & Krogdahl 1996). These changes, in addition to the increased lysozyme and IgM content measured in the intestinal mucosa of fish fed the soybean molasses diet, are signs of an inflammatory response that has previously been classified as a subacute enteritis (Baeverfjord & Krogdahl 1996), perhaps as a result of a hypersensitivity reaction (Pedersen 1989; Rumsey *et al.* 1994; Baeverfjord & Krogdahl 1996). The results from the challenge trial suggest that the enteritis-like changes brought on by prefeeding solvent-extracted SBM, may be a factor that led to increased mortality in these fish compared with salmon fed the control or soy concentrate-containing diets. However, although statistically significant, the differences in cumulative mortality were small, and the practical consequences are difficult to evaluate. The question arises whether the differences between feeding groups may have been greater if the fish had continued on the experimental diets during the challenge period. Recovery of the enteritis-afflicted intestine following reintroduction of the commercial diet at the initiation of the challenge period may have reduced the differences in mortality between feeding groups. A larger-scale investigation in which replicate tanks of fish that are continuously fed the experimental diets throughout the challenge period would be necessary to explore further the consequences of SBM feeding for disease susceptibility.

The SBM-induced enteritis may affect the integrity of the epithelial barrier of the distal intestine of Atlantic salmon, as has been described for antigen-induced enteropathies in other animals and man (reviewed by Madara *et al.* 1992). This may allow bacteria or other antigens to pass through the intestinal wall. There is a need to establish whether SBM-induced enteritis of the distal intestine of salmonids facilitates entry of pathogens or antigens through the intestinal mucosa; immunohistochemical techniques or electron microscopy may be useful for this purpose.

The general systemic health of the SBM-fed salmon may also have been compromised by the SBM. As mentioned earlier, full-fat and solvent-extracted SBM, as well as alcohol-soluble components of SBM fed to salmonids cause diarrhoea, as well as reduced nutrient digestibility and growth at higher inclusion levels. This may have systemically weakened the fish, made them more susceptible to the disease-causing effects of the furunculosis bacteria, and led to higher mortality rates in the SoyMeal group.

The significantly increased lysozyme activity measured in the intestinal mucosa of fish fed soybean molasses may indicate that there were increased levels of activated macrophages and/or eosinophilic granule cells (Walker & Sanderson 1992; Sveinbjørnsson *et al.* 1996). These results appear to support the report by Baeverfjord & Krogdahl (1996) indicating elevated numbers of these immune cells infiltrating the lamina propria of SBM-fed Atlantic salmon. Although soybean molasses appeared to cause increased levels of IgM in the mid and distal intestinal mucosa homogenate, we were unable to distinguish with the materials and methods used in this study, whether this IgM was located in the mucus, epithelial cell layer or subepithelial tissue of the intestine, whether the IgM was of specific or nonspecific nature relative

to SBM components, or whether it was related to specific immune cells (secretory vs. bound to IgM-positive cells). Further study is needed to elucidate the location and properties of the IgM. Interestingly, fish fed soy concentrate as well as soybean molasses had significantly higher IgM levels in their mid-intestinal tissue compared with the fish fed the control diet. This implies two possibilities: (1) that both soy products may have an immunogenic effect in this region which is reportedly morphologically unaffected by SBM (van den Ingh *et al*. 1991; Baeverfjord & Krogdahl 1996), and (2) that the midintestine, along with the distal region, has local immunological significance.

Although direct cause-and-effect relationships between the results of the two experiments cannot be made, it is tempting to speculate based on earlier reports on the interactions between *A.s. salmonicida* and immunological factors. The activation of the nonspecific immune response has been considered pivotal in resisting *A. salmonicida* infection by numerous authors (Olivier *et al*. 1985; Grinde 1989; Møyner *et al*. 1993; Lamas & Ellis 1994; Ellis 1997), and cells and factors of the nonspecific immune response appear to be well represented in the intestinal mucosa of extracted SBM and soybean molasses-fed, as well as soy concentrate-fed salmon (present study; van den Ingh *et al*. 1991, 1996; Baeverfjord & Krogdahl 1996). Of particular interest are the soy concentrate-fed salmon that had relatively high IgM levels in the mid intestinal tissue (Fig. 3) combined with the significantly lowest mortality in the challenge experiment (Fig. 1). Michel *et al*. (1990) reported increased resistance of rainbow trout to furunculosis in the absence of *A. s. salmonicida*-specific antibodies. The induction of increased levels of polyspecific antibodies, thought to serve as opsonins, permitted increased phagocytosis of the pathogen by macrophages. Furthermore, Rumsey *et al*. (1994) found that feeding toasted SBM and soy protein concentrate to rainbow trout influenced systemic, nonspecific defence mechanisms. Plasma immunoglobulin levels, among other parameters — number of circulating leukocytes, production of oxidative radicals (O_2^-, OH^-) by activated neutrophils and other phagocytic cells, and macrophage function — were all significantly elevated compared with trout fed fishmeal as the sole protein source. The authors suggested that this systemic stimulation of immune parameters could provide the fish with some protection against disease, since another study by the same group showed that various immunostimulants (other than SBM and soy protein concentrate) added to rainbow trout feed elevated the same immune parameters and conferred heightened resistance to *A. salmonicida* challenge (Siwicki *et al*. 1994). Based on these reports, the results from the present studies on Atlantic salmon suggest that the local immune response (IgM) in the mid-intestinal mucosa to soy concentrate (SoyConc2) feeding may augment a possible systemic effect and confer heightened resistance to the furunculosis bacteria, leading to the lower mortality observed in the SoyConc1 group compared with both the Contr1 and SoyMeal groups during the challenge trial. In the distal intestine, the elevated lysozyme and IgM levels are most likely only signs of the inflammation and do not convey protection against disease. This is suggested by the higher mortality in the SoyMeal-fed group following *A.s. salmonicida* challenge. Further study is needed to investigate how different soybean products affect the salmon intestine's epithelial integrity and local immune response, and how these may influence disease resistance.

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