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Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar)* and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*)

Marije Booman[a,1], Ian Forster[b], John C. Vederas[c], David B. Groman[d], Simon R.M. Jones[a,*]

[a] Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia V9T 6N7, Canada
[b] Centre for Aquaculture and Environmental Research, Fisheries and Oceans Canada, West Vancouver, British Columbia V7V 1N6, Canada
[c] Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada
[d] Aquatic Diagnostic Services, Atlantic Veterinary College, Charlottetown, Prince Edward Island C1A 4P3, Canada

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ABSTRACT

To improve sustainability in the aquaculture industry plant meals are increasingly used to replace fish meal in fish feed. Solvent-extracted soybean meal (SBM) is an attractive protein source for fish feed because of its high protein content, favorable amino acid profile, and low cost. In Atlantic salmon (*Salmo salar*), SBM at low levels causes soybean meal-induced enteritis (SBMIE). Few studies have been done with SBM in Pacific salmon, and none of those have included intestinal inflammation analysis. To gain more insight into salmonid responses to SBM, we assessed and compared the effects of SBM on intestinal morphology, inflammation and microbiome composition of Chinook salmon (*Oncorhynchus tshawytscha*), pink salmon (*O. gorbuscha*) and Atlantic salmon (*Salmo salar*).

Atlantic, Chinook and pink salmon were fed for three weeks on a diet with 20% inclusion of SBM, or a control diet with fish meal. After one week on the SBM diet, Atlantic and Chinook salmon showed increased submucosa thickness in the distal intestine compared to the fish fed on the fishmeal diet. Intestinal inflammation in these species increased over time, with a further increase in submucosa thickness coincident with an infiltration of eosinophilic granular and mononuclear leucocytes. After 3 weeks on the SBM diet, intestinal inflammation was most severe in Chinook salmon. In contrast, pink salmon only showed a slight increase in submucosa thickness after three weeks on the SBM diet, and no significant increase in inflammatory cell infiltrate. Sequence-based analysis of the intestinal microbiome showed a significant difference in overall microbiome composition between species, but did not show an effect of the SBM diet on microbiome diversity or composition in any of the three salmon species. In conclusion, SBM-fed Chinook salmon were more susceptible to SBMIE than Atlantic salmon whereas pink salmon were not susceptible to SBMIE at the levels of SBM tested.

1. Introduction

Fish meal is an important source for protein in aquaculture feeds, especially for carnivorous species like salmon. Most fish meal is sourced from wild oil-rich fish, which may not be sustainable for those stocks with limited abundance. Therefore alternative sources of protein such as plant meals are being considered. Despite the widespread availability of crop-based plant meals, these protein sources can contain anti-nutritional factors such as fibers, indigestible sugars, and chemicals that adversely affect feed intake, palatability and nutrient digestibility (Krogdahl et al., 2010). In addition, some anti-nutritional factors are inflammatory with the potential to elicit enteritis. Furthermore, in some aquaculture species, plant-based diets may induce changes in intestinal microbiota composition (Desai et al., 2012; Green et al., 2013; Navarrete et al., 2013), which can alter intestinal health including the feed digestibility and intestinal immunity (Nayak, 2010).

Solvent-extracted soybean meal (SBM) is an attractive protein source for fish feed because of its high protein content, favorable amino acid profile, and low cost. In Atlantic salmon (*Salmo salar*), SBM can be included in the diet at low levels without significant negative effects on feed intake or fish growth (Krogdahl et al., 2003; Romarheim et al., 2013). However, a 20% inclusion rate causes shortening of the mucosal

* Corresponding author.
E-mail addresses: mbooman@aquatechcenter.com (M. Booman), ian.forster@dfo-mpo.gc.ca (I. Forster), john.vederas@ualberta.ca (J.C. Vederas), groman@upei.ca (D.B. Groman), Simon.jones@dfo-mpo.gc.ca (S.R.M. Jones).
[1] Present address: Centre for Aquaculture Technologies, Souris, Prince Edward Island, C0A 2B0, Canada.

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folds, thickening of the lamina propria and submucosa, and disappearance of supranuclear vacuoles in the enterocytes of the distal intestine, accompanied by an infiltration of inflammatory cells; a process known as soybean meal-induced enteritis (SBMIE) (Baeverfjord and Krogdahl, 1996; Urán et al., 2009). Recovery from SBMIE is spontaneous in some species whereas in Atlantic salmon, recovery occurs after the fish are returned to a fishmeal-based diet (Baeverfjord and Krogdahl, 1996; Urán et al., 2008). The mechanism of SBMIE is not well understood, but saponins may play a role (Knudsen et al., 2008; Krogdahl et al., 2015).

Few studies have examined the effects of SBM in diets for Pacific salmon. Inclusion of most soy products in diets for Chinook salmon (*Oncorhynchus tshawytscha*) severely reduced feed intake (Bureau et al., 1998; Fowler, 1980; Hajen et al., 1993), and the products were deemed unacceptable with no further analyses undertaken. However, most of these studies used either soybean products containing high levels of saponins, or solvent-extracted soybean meal at relatively high inclusion levels and none included analyses of intestinal morphology and inflammation. To gain more insight into salmonid responses to SBM, we compared Chinook and pink salmon (*O. gorbuscha*) with Atlantic salmon, a well-established SBMIE model organism, fed an experimental soybean meal diet. The objectives of the study were to assess the effects of SBM on intestinal morphology and inflammation and to investigate the effect of an SBM diet on the intestinal microbiome.

2. Materials and methods

2.1. Fish husbandry

Juvenile Atlantic salmon were obtained from a commercial hatchery on Vancouver Island and held at the Pacific Biological Station (PBS) in Nanaimo, BC, Canada. Pink salmon were obtained as swim-up fry from Quinsam River Hatchery on Vancouver Island, BC and were reared at the PBS. Chinook salmon were obtained from a commercial hatchery on Vancouver Island, BC and held at the Centre for Aquaculture & Environmental Research (CAER) in West Vancouver, BC. One week before the start of the experiment, fish were stocked in their experimental tanks at 50 fish per tank, 4 tanks per species. Pink salmon (initial mean weight of 166 g) and Atlantic salmon (535 g) were maintained in 850 L and 1900 L tanks respectively, and Chinook salmon (140 g) were maintained in 650 L tanks. All tanks were supplied with flow-through sea water with mean salinity at PBS of 29.6 ppt (range 28.0–29.9 ppt) and at CAER of 30.2 ppt (range 27.9–31.0 ppt) and an ambient temperature at PBS of 9.4 °C (range 9.1–9.8 °C) and at CAER of 9.0 °C (range 8.7–9.8 °C) under natural photoperiod. These trials were conducted during February and March 2016 and approved by the Pacific Regional Animal Care Committee (AUP #15-015), according to Canadian Council of Animal Care guidelines.

2.2. Experimental diets and feeding experiment

Two diets were produced: a control diet containing fish meal (FM) and a test diet containing 200 g/kg solvent-extracted soybean meal (SBM). These diets were formulated to be isonitrogenous and isolipidic (Table 1). Diets were produced by blending dry ingredients in a mixer (Model M-802, The Hobart Manufacturing Co., Troy, Ohio, USA) for 15 min with a portion of the oil added to the mash. Hot water (ca. 85 °C) was added to the mash at 10% by weight, further mixed for 15 min and passed through a pellet mill (Model CL3, California Pellet Mill Co, San Francisco, California, USA). The pellets were spread onto screens, sieved to remove fines and dried with gentle heating (30–40 °C) until moisture content was approximately 8%. The pelleted diets were then top-coated with fish oil and stored in plastic bags in a humidity-controlled room at 4–5 °C. Two pellet sizes were produced: 3 mm for pink and Chinook salmon, and 4 mm for Atlantic salmon. For each species, fish in two tanks received the FM control diet while those in the remaining two tanks received the SBM test diet. All fish were fed a daily ration at 1% biomass, adjusted weekly, for 3 weeks. Pink and Atlantic salmon were fed half their ration by hand in the morning, and the remainder on automated feeders. Chinook salmon were fed by hand equally in the morning and afternoon.

Table 1
Formulations of fish meal (FM) and solvent-extracted soybean meal (SBM) diets used in this study.

Ingredients and composition	FM diet (g/kg)	SBM diet (g/kg)
Fishmeal	500.0	378.8
Soybean meal	0.0	200.0
Whole wheat flour	298.0	211.4
Fish oil	190.0	197.8
Vitamin/mineral premix	2.0	2.0
Permapell	10.0	10.0
Crude protein	372.4	372.4
Lipid	230.5	230.5

2.3. Sampling

Four fish were haphazardly sampled from each tank for histology at weeks 1, 2, and 3. Each fish was euthanized with an overdose of tricaine methane sulphonate (TMS, 400 mg/L). A 2.5 cm section of the distal intestine was removed, contents rinsed out with 10% neutral buffered formalin (NBF), and the tissue fixed in NBF for 48 h. At week 3, two additional fish per tank were euthanized and sampled for intestinal microbiome analysis. Each fish was wiped with ethanol, and the combined mid- and distal intestine including digesta was aseptically removed and flash-frozen in liquid nitrogen.

2.4. Histology

2.4.1. Slide preparation

For samples collected in weeks 1, 2 and 3, eight fish per diet per species were processed further for histology. Each NBF-fixed intestine sample was cut into 5 equal-sized pieces, dehydrated in an alcohol gradient, cleared in two changes of xylene and sequentially embedded into a single paraffin block. For each fish, 5 μm-thick cross-sections of all five pieces of intestine were mounted onto a glass slide, stained with haematoxylin and eosin and sealed under a coverslip.

2.4.2. Quantitative and semi-quantitative measurements

Histological features of the intestine were viewed at a total magnification of 125 × and images captured using a QImaging digital Camera (QImaging, Surrey, BC, Canada). The thickness of the submucosa (SM), i.e. the distance between the base of the villus and the stratum compactum, was measured at four points on the intestinal wall where these features were clearly visible, preferably at 0, 90, 180 and 270 degrees as observed in the microscopic field of view of each intestinal cross-section. Twenty such measurements were used to calculate the mean SM per fish.

For semi-quantitative measurements, an Inflammation Score (IS) of 0 to 4 was assigned to each intestinal cross-section according to the following criteria:

0: Normal background leucocyte infiltrate
1: Minor increase in infiltrate – primarily by eosinophilic granular leucocytes (EGL)
2: Mild increase in infiltrate – EGL and/or mononuclear leucocytes
3: Moderate increase in infiltrate – EGL and mononuclear leucocytes
4: Heavy increase in infiltrate – EGL and mononuclear leucocytes.

For each fish, the individual cross-sectional scores were used to