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Soybean lectins and trypsin inhibitors, but not oligosaccharides or the interactions of factors, impact weight gain of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Several compounds within soybean meal (SBM) have been implicated as potentially having antinutritional effects when fed to trout. We conducted an 8-week study designed to elucidate the antinutritional impacts and possible interactions of soybean lectins (SBA), trypsin inhibitors (TI) and oligosaccharides (OLIG) fed to rainbow trout. All three antinutritional factors (ANF) were included at levels corresponding to a diet containing 40% SBM. Eight purified diets were formulated consisting of a control (devoid of ANF), SBA, TI, OLIG, SBA + TI, SBA + OLIG, TI + OLIG and SBA + TI + OLIG. Feed consumption, weight gain, feed efficiency, specific growth rate (SGR), protein retention, and serum chemistry responses were evaluated using a 2^3 factorial design. Two-factor analysis detected significant main-effect reductions in weight gain and SGR of fish fed SBA or TI in comparison to fish fed neither ANF. However, there were no significant interactive effects of ANF. A significant increase in amylase and 1-h post-prandial insulin response as a result of feeding OLIG was also detected using one-factor analysis. Results of this study indicate that the amounts of SBA or TI present in a 40% SBM diet fed to rainbow trout are high enough to significantly decrease production parameters.

1. Introduction

The salmonid industry is one of the largest intensive aquaculture industries and one that demands a significant percentage of the global fish meal supplies (Tacon and Metian, 2008). The current cost and availability of fish meal led to several evaluations of alternative protein ingredients in diets for salmonids. Protein ingredients derived from soybeans have been one of the more commonly evaluated ingredients in both trout and salmon. Published data indicates solvent-extracted, dehulled soybean meal (SBM) can be used in diets for salmonids, but at relatively low levels (15–30% of the diet, Gomes et al., 1995; Olli et al., 1995; Kaushik et al., 1995; Storebakken et al., 2000; Francis et al., 2001). Chemical components of soybean seeds, termed antinutritional factors (ANF, Rackis, 1974), have been implicated as the cause of limited use. Soybean agglutinins (SBA), also known as lectins (Sharon and Lis, 2002), trypsin inhibitors (TI) and oligosaccharides (OLIG) are three components of soybeans that have been implicated as possible ANF when fed to salmonids (Francis et al., 2001).

Lectins are glycoproteins that bind to carbohydrates and glycoconjugates on the epithelial surface of the intestine, potentially interfering with nutrient absorption (Lajolo and Genovese, 2002). Salmonids fed soy products have exhibited intestinal damage similar to what would be expected from SBA consumption (van den Ingh et al., 1991; Burrells et al., 1999; Krogdahl et al., 2003). However, there have been few evaluations of purified sources of lectin. Hendricks et al. (1990) reported purified SBA binds *in vitro* to both proximal and distal preparations of the brush border membrane in Atlantic salmon. Buttle et al. (2001) reported significant histological changes in Atlantic salmon fed 3.5% dietary SBA, but only in the distal segments of the gastrointestinal tract. They also reported similar results in rainbow trout fed a diet containing 60% SBM, but did not evaluate a purified source of SBA in trout. Iwashita et al. (2008) fed trout purified diets containing 0.0075% SBA and clearly identified that ANF as contributing to enteritis in the distal segment of the gastrointestinal tract. However, there were no changes in weight gain in trout fed SBA. Subsequently, Iwashita et al. (2009) identified both saponin and SBA as causative agents in distal enteritis in trout fed purified diets. Processed SBM typically contains 10–200 mg/kg SBA (Russett, 2002); thus, the concentration evaluated in salmon was well above what would be expected in SBM, while the concentrations evaluated by Iwashita et al. (2008) in trout were within the range that would be expected.

There are two TI families of proteins in soybeans that inhibit either trypsin or trypsin and chymotrypsin activity in the gastrointestinal tract, thus interfering with protein digestion (Norton, 1991). TI concentrations in SBM range from 5 to 8 g/kg (Russett, 2002). Salmonids are sensitive to TI (Sandholm et al., 1976; Krogdahl et al., 1994). In studies with rainbow trout and Atlantic salmon, trypsin production increased as dietary soy inclusion increased, then decreased as dietary soy inclusion

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reached 20–25% of the diet (Krogdahl et al., 1994; Olli et al., 1994). Overwhelming the protein synthetic capabilities of the pancreas (pancreatic exhaustion) was suggested as the cause of the observed results.

The total OLIG content in SBM is 12–15%, with sucrose (6–7%), stachyose (5–6%) and raffinose (1–2%) being the primary OLIG present (Francis et al., 2001; Russett, 2002). The effects of OLIG on fish have not been well established. Kaushik et al. (1995) reported that rainbow trout fed a soy protein concentrate with less than 2% soluble carbohydrates exhibited production characteristics similar to fish fed a fish meal control, whereas trout fed a SBM diet with 18% OLIG gained less weight. Extraction of OLIG from lupin meal and enzyme pre-treatment of OLIG containing lupin meal increased the nutritional value of diets fed to rainbow trout (Glencross et al., 2003). However, there have been no formal evaluations of the constituent components of OLIG in diets fed to trout. There have also been no formal evaluations of interactive effects of ANF in salmonids, despite the fact that interactions have been identified in other animals (Douglas et al., 1999).

Refstie et al. (1998) reported that weight gain in Atlantic salmon fed a SBM containing reduced concentrations of TI, SBA and OLIG was not significantly different from fish fed a fish meal control diet. However, the study was not designed to evaluate interactive effects of ANF. Knudsen et al. (2008), using a crude ethanol extract from soybeans, concluded that both soy saponins and an unidentified compound were likely contributors to enteritis in salmon. The published data on soybean meal use in trout clearly indicates negative effects at moderate to high levels of inclusion in diets, yet the factor(s) causing this limitation remain unclear and there are no indicators of those effects.

The goal of this study was to take a more rigorous approach in experimental dietary formulation using purified ANF and test the hypotheses that: (1) soybean ANF were not causing impacts on weight gain of trout; and, (2) interactive effects of soybean ANF were not influencing weight gain in trout.

2. Materials and methods

Vitamins, casein, gelatin, dextrin, carboxymethylcellulose (CMC), crystalline L amino acids, cellulose and TI (Kunitz, 96%) were obtained from US Biochemical (Cleveland, OH, USA). Menhaden oil and ascorbic acid (C-PP) were obtained from Omega Protein (Reedville, VA, USA) and Roche Inc. (Nutley, NJ, USA), respectively. Reagent grade minerals, sucrose, stachyose and raffinose were obtained from Sigma Chemical (St Louis, MO, USA). Choline chloride and soybean lecithin were obtained from Bio-Serv (Frenchtown, NJ, USA). SBA (99%) was obtained from Vector Laboratories (Burlingame, CA, USA).

Eight purified diets (Control, SBA, TI, OLIG, SBA + TI, SBA + OLIG, TI + OLIG and SBA + TI + OLIG) were formulated to supply 43.0 g protein, 15.0 g lipid and 10–15.0 g carbohydrate/100 g diet. All diets contained (g/kg) casein, 440.0; gelatin, 40.0; L-arginine, 10, L-methionine, 5.0; dextrin, 101.4–150.0; α-cellulose, 90.4–93.0; carboxymethylcellulose, 20.0; mineral premix 60.0; vitamin premix, 30.0; ascorbyl polyphosphate, 1.0; choline-Cl, 1.0; lecithin, 5.0; and, menhaden oil, 145.0. Diets were formulated to meet the known nutritional requirements of rainbow trout (NRC, 1993; Hardy, 2002). Vitamin and mineral premixes were added at previously reported levels for a nutritionally complete diet (Twibell et al., 2000). TI and OLIG were added at the median concentrations that would be expected in diets containing 40% SBM (2.6 g TI/kg diet, and 25.3 g sucrose/kg diet, 5.6 g raffinose/kg diet, and 17.7 g stachyose/kg diet, respectively), and SBA was added at the maximum concentrations expected in diets containing 40% SBM (80 mg SBA/kg diet, Table 1). TI and OLIG were substituted into diets replacing cellulose and dextrin, respectively. SBA was not substituted in replacement of another ingredient because of the low concentration in the diets (80 mg/kg). Diets were mixed, neutralized with saturated NaOH, pelleted, dried and stored using previously reported methods (Twibell et al., 2000).

Table 1
Dietary treatments fed to juvenile rainbow trout to evaluate the effects of purified soybean agglutinin (SBA, g/kg diet), trypsin inhibitor (TI, g/kg diet) and oligosaccharides (OLIGO, mg/kg diet).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
TI	–	–	2.6	–	2.6	–	2.6	2.6
Sucrose	–	–	–	25.3	–	25.3	25.3	25.3
Raffinose	–	–	–	5.6	–	5.6	5.6	5.6
Stachyose	–	–	–	17.7	–	17.7	17.7	17.7
SBA	–	80.0	–	–	80.0	80.0	–	80.0

Rainbow trout juveniles (London strain) were obtained from the Ohio Department of Natural Resources (London State Fish Hatchery, London, Ohio, USA) and transported to the Purdue University Aquaculture Research Facility. Fingerlings were quarantined in a 1700-L holding tank for two weeks and fed a commercial diet (42% crude protein, 14% lipid, Nelson and Sons, Inc., Murray, Utah, USA). Transport, quarantine and experimental procedures followed Purdue Animal Care and Use Committee requirements.

A recirculating system containing 110-L glass aquaria was used for the feeding trial. Black polyethylene was wrapped around the sides and bottom of each tank to prevent fish in separate tanks from interacting and to diminish disturbance from activity in the laboratory. Plastic egg crate was placed on the tops of each aquarium to prevent escape. The diurnal light/dark cycle was 16 h light/8 h dark.

Water quality was monitored daily. Dissolved oxygen concentrations and temperature were measured using a dissolved oxygen meter (YSI, Inc., Yellow Springs, OH, USA), pH was monitored with a HACH Sension 156 meter (HACH Chemical, Co., Loveland, CO, USA) and ammonia-N, nitrite-N and alkalinity concentrations were measured with a HACH DREL 1-C Water Quality Test Kit (HACH Chemical, Co., Loveland, CO, USA). Temperature was maintained at 14 ± 2 °C. Dissolved oxygen was ≥8.0 mg/L throughout the feeding trial, pH was ≥7.5, alkalinity was ≥150 mg/L, ammonia-N (unionized) did not exceed 0.0125 mg/L, and nitrite-N did not exceed 1.0 mg/L.

Groups of 12 fish were randomly stocked into 24 aquaria. Fish were allowed to acclimate to system conditions for 1 week, during which they were fed the same commercial diet used during quarantine and acclimation (Nelson and Sons, Murray, UT, USA). Dietary treatments were randomly assigned to triplicate groups of fish and all fish were fed their respective experimental diets for an additional week. Following the acclimation period, the number of fish in each aquarium was reduced to 10. Six fish were collected and stored at −80 °C. Average fish weight was 5.3 g. Fish were fed to apparent satiation twice daily for 53 days. Upon completion of the feeding trial, feed was withheld for 1 day. All fish were weighed the following day and five were returned to their previously assigned tanks. The remaining fish were euthanized using MS-222 (Argent Chemical Laboratories, Inc., Redmond, WA, USA). Fish that had been returned to their tanks were fed for an additional 2 weeks prior to blood sampling.

Blood was collected from three of the euthanized fish per replicate and pooled. Samples were chilled on ice, then centrifuged for 20 min at 4000 × *g*. Serum was collected and stored at −80 °C. Clinical chemistry variables (albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), total bilirubin (TB), calcium (Ca), phosphorus (P), creatinine (CRE), glucose (GLU), sodium (Na), potassium (K), total protein (TP), and globulin (GLOB)) were analyzed with a VetScan analyzer using the comprehensive diagnostic rotor (Abaxis, Inc., Union City, CA, USA).

The two remaining euthanized fish from each replicate were dried for 24 h at 100 °C using a Shel Lab FX14 oven (Sheldon Manufacturing, Inc., Cornelius, OR, USA) and ground to a fine powder. Whole-body crude protein concentration of initial and final fish was determined by AOAC (2006) method 990.03.