Soybean antinutritional factors and their relative importance in limiting the use of soybean meal in salmonid diets

by

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Introduction

Salmonids (primarily Atlantic salmon and rainbow trout) are the most cultured family of carnivorous fishes in the world, with 2004 production values of approximately two million metric tonnes (mmt) valued at US $6.5 billion (FAO 2006). The salmonid industry accounts for approximately 3% of total global aquacultural production, but its value is nearly 10% of total global aquacultural value (FAO 2006). Over the past ten years, the industry has more than doubled production output. Norway and Chile are the two primary producing countries, accounting for approximately 60% of global salmonid production (FAO 2006).

Commercial feed formulations for salmonids currently incorporate 30 to 45% fish meal (New and Wijkstroem 2002). Global fish meal production typically ranges between 6-7 mmt annually (FAO 2006). The salmonid industry is utilizing about one third of the total amount of fish meal used in aquaculture feeds (Hardy 2002). However, recent declines in the harvest of fishes utilized to produce fish meal (primarily in Peru) lead to an historic high of $1,600 per metric tonne of fish meal in the summer of 2006 (Hardy 2006). As fish meal prices climb, production costs for salmonid farmers increase as well (New and Wijkstroem 2002). Additionally, as fish farmers increase production, the economic return per unit of production decreases. For Atlantic salmon, the return per unit has decreased by 20-40% since 1986-1987 (FAO 2006). If the salmonid industry is going to continue to grow, alternative proteins will need to be incorporated into their feed formulations.

As the salmonid industry incorporates changes to their feed formulations, they need to assure that these changes have a minimal impact on fillet quality. Salmonid fillets are an excellent source of high quality protein. In addition, they contain high amounts of the n-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Farmed salmonids contain slightly higher levels of EPA and DHA than wild harvested salmonids, with farmed rainbow trout containing 1.15 g EPA and DHA per 100 g serving, compared to 0.99 g EPA and DHA per 100 g serving of wild caught rainbow trout. Farmed Atlantic salmon contain 2.15 g EPA and DHA per 100 g serving, compared to 1.80 g EPA and DHA per 100 g serving of wild caught Atlantic salmon (all values are cooked edible portion) (USDA/ARS 2006). The Food and Drug Administration (FDA) and American Heart Association (AHA) recommend two meals (one meal is 170.1-226.8 g) of fish per week, especially fatty species such as salmonids, to maintain a healthy lifestyle. In 2004, the FDA allowed a qualified health claim for reduced risk of coronary heart disease on conventional foods that contain EPA and DHA (FDA 2004). As the salmonid feed industry transitions to alternative protein and lipid sources, EPA and DHA levels in the diet and fillet will need to be considered.

Soybean meal (SBM) is an alternative protein ingredient currently of great interest to the aquaculture industry. In 2005, global production of soybean seeds was estimated at 218 mmt, but more importantly, global production of SBM was estimated to be 145 mmt, more than 20 times greater than the average global production of fish meal (USDA 2006). SBM prices can vary, but typically cost around US $200 per metric tonne (www.aquafeed.com). Dehulled SBM contains approximately 48% crude protein, one of the highest protein contents of all plant-based protein ingredients (NRC 1993). When used as a primary protein source, the essential amino acid profile of SBM, with the exception of methionine, is adequate to meet the known requirements of salmonids (NRC 1993).

Despite the global availability and nutritional characteristics of SBM, it has not been incorporated into salmonid diets at high inclusion rates. SBM is typically incorporated at 10-30% of the diet depending on species and feed manufacturer (Hardy 2002; Pillay and Kutty 2005). The relatively high concentration of carbohydrates and presence of antinutritional factors (ANF) are believed to be the primary factors limiting the amount of SBM used in salmonid feeds (Francis et al. 2001; Hardy 2002). Lectins, oligosaccharides, saponins and trypsin inhibitors are all present in SBM and have the ability to act as ANF when fed to fish. Soy isoflavones (also called phytoestrogens) may also exert negative effects when fed to fish by exerting estrogenic effects; however, isoflavones may also have a beneficial effect by acting as an antioxidant. Researchers have attempted to identify the specific components of SBM that are limiting its use in salmonid diets; however, most of this research evaluated SBM that had been subjected to various extraction techniques that removed several potential ANF instead of evaluating purified ANF derived from SBM (Francis et al. 2001). Consequently, the specific antinutritive compounds present in SBM that are exerting negative effects on salmonids have not been identified.

In 2002, the United Soybean Board (USB) and the Indiana, Illinois and Ohio State Boards funded the Soy-in-Aquaculture (SIA) Managed Aquaculture Program. SIA was established to overcome the barriers limiting the amount of soy ingredients in aquaculture feeds. The initial SIA initiative contained two separate but related programs. The goal of the first component focused on increasing the amount of soy inclusion in feeds used by well-established, intensive aquaculture industries that were underutilizing soy in their feed formulations. Specific objectives were to systematically evaluate specific ANF and determine which ones were causing problems when fed to salmonids. This research was conducted at seven institutions, led by Purdue University (West Lafayette, IN, USA). The second program was international marketing led by the American Soybean Association/United States Soybean Export Council. The primary objective of that program was to increase usage of soy-based formulations by fish farmers in Southeast Asia and India. The remainder of this document will focus on the research portion of the SIA initiative.

SIA: Salmonids

During the first two years of SIA, seven universities were identified to address the inclusion of soy in salmonid diets. Researchers at each university agreed to evaluate one specific component that may be limiting soy use. The effects of feeding lectins, trypsin inhibitors, saponins and genistein (a soy isoflavone) were evaluated at Purdue University, Michigan State University, Ohio State University and the University of Wisconsin, respectively. Additionally, the University of Maine evaluated the effects of feeding genistein and SBM on fillet characteristics and consumer acceptance. The University of Idaho tested various extruder settings to establish conditions for extrusion of soy-based salmonid feeds and they formulated test diets and fed them to rainbow trout for use in the University of Maine evaluations. Finally, Kentucky State University conducted an economic analysis of the use of soy ingredients in salmonid diets.

Saponins

Saponins are glycosides of steroids or triterpenoids present in many plants including the soybean. The saponin content of SBM typically ranges from 0.43 to 0.83% (Ireland et al. 1986; Goda et al. 2002). Saponins are surface-active compounds that mediate membrane transport (Francis et al. 2001). When mixed with water they can become toxic to fish by causing damage to the gill epithelium through detergent action (Francis et al. 2001). However saponins of high purity show strong adjuvant activity, making them a safe and inexpensive compound for practical use as adjuvants in vaccines (Oda et al. 2003). The effects of dietary intake of soy saponins on fish have not been well established. Feeding saponin from Quillaja (0.15 and 0.30% of the diet) to Chinook salmon and rainbow trout caused a reduction in weight gain and significant intestinal damage similar to that caused by a purified alcohol extract from SBM (Bureau et al. 1998). The authors concluded that soy saponin was the component of the purified alcohol extract causing the adverse effects.

In the first of two studies conducted at Ohio State University, eight experimental diets were formulated to evaluate the effects of dietary soy saponins and other soy extracts on first-feeding rainbow trout. The experimental diets consisted of a control (casein-gelatin based) and seven others supplemented with soy protein concentrate (SPC), SBM after methanol/water extraction, soy-saponin extract (extracted with 80% methanol), soy-flavonoid extract (extracted with 40% methanol), SBM, SPC after methanol/water extraction and Quillaja-saponin extract (Table 1). Flavonoid and saponin content was quantified with HPLC using daidzein and soy saponin standards, respectively (Oleszek and Stochmal 2002). The initial feeding trial was conducted for eight weeks. Upon conclusion of the trial, samples of fish from each treatment were collected for histological evaluation of the digestive tract. Remaining fish were fed their respective diets for five additional weeks at which point an immune response trial was conducted. Half of the fish in each tank were injected with Furogen (AquaHealth Ltd.; aluminum phosphate adjuvanted A. salmonicida bacterin) and the other half with phosphate buffered saline. Fish were given a fin clip for identification purposes. Fish were sampled after two and four weeks to determine antibody response. The replacement of 50% of casein-gelatin protein source with SBM or SPC resulted in significant growth depression in first-feeding rainbow trout (Figure 1); however, there was a significant confounding effect of protein concentrations on growth (Figure 2) as the correlation coefficient between weight gain and protein content in the diet was 0.89 (if two diets, SBM after extraction and Quillaja saponin are excluded). In contrast to rainbow trout fed Quillaja saponins, rainbow trout fed soy saponins did not exhibit growth impairment (Figure 1). Four weeks after vaccination, fish fed soy saponin, SPC, Quillaja saponin and SBM exhibited significantly higher antibody levels than fish fed the control diet (Figure 3). Histological data indicated that rainbow trout fed SBM and SPC exhibited varying degrees of digestive tract abnormalities. Fish fed diets supplemented with SPC had large vacuoles in the enterocytes and their pancreases were surrounded by adipose tissue while the cytoplasm in the enterocytes of fish fed diets supplemented with SBM were filled with lipid vacuoles.

In the second study conducted at Ohio State, eight experimental diets were formulated and prepared to investigate the effects of dietary supplementation of soy fractions on physiological and nutritional performance of rainbow trout. The experimental diets consisted of a casein-gelatin based control (Cont) and seven others containing 50% replacement of casein by SBM or SBM after methanol/water extraction or supplemented with extracted fractions in an amount equal to that in 50% protein replacement; SBM, SBM after methanol/water extraction, SBM-carbohydrate extract, SBM-flavonoid extract, SBM-saponin extract, SBM-lipid extract and a negative control diet (Table 2). The feeding trial was conducted for 15 weeks. During that time, fish were weighed every two weeks (Figure 4). Replacement of 50% of casein-gelatin protein source with SBM or SBM after extraction resulted in significant growth depression in first feeding rainbow trout (Table 3). Supplementation with the carbohydrate fraction of SBM also resulted in decreased growth, though less marked. Soybean lipid extract did not significantly alter fish growth, nor did supplementation with soy saponin and flavonoid extracts. The researchers concluded that these studies indicated transport and metabolism of nutrients were affected by the presence of soy-based ingredients in diets, but that soy saponin was not the limiting factor. These results further suggest that soy saponins may have a beneficial effect when fed to rainbow trout by augmenting the immune response.

Lectins

Lectins are glycoproteins present in plant-based feed ingredients including SBM (Lajolo and Genovese 2002). Typical levels in SBM range between 0.01 to 0.2% (Russett 2002). Their effects on fish are not well known, but their antinutritive effects on rats have been well established. Lectins bind to the intestinal epithelium of rats and interfere with nutrient absorption (Lajolo and Genovese 2002). When included at 0.73 mg/g diet, soybean lectins have also been shown to depress nitrogen retention and increase nitrogen excretion via the urine, indicating interference with protein metabolism (Czerwinski et al. 2005). Additionally, soybean lectins reduce insulin production of rats given oral doses greater than 0.02 g/kg body weight (Bardocz et al. 1996).

Salmonids fed SBM-based diets have exhibited intestinal damage and reduced insulin levels indicative of lectins' antinutritional effects (Burrells et al. 1999; Buttle et al. 2001; Krogdahl et al. 2003); however, since purified sources were not evaluated, the cause of intestinal damage and reduced insulin levels cannot be solely attributed to dietary intake of soybean lectins. In vitro experiments have shown soybean lectins bind to brush border membrane extracts of Atlantic salmon (Hendricks et al. 1990). Also, intestinal damage was reported in Atlantic salmon fed soybean lectins at 3.5% of the diet (Buttle et al.

2001); however, this level is much higher than the amount of lectins found in processed SBM.

Research conducted at Purdue University as part of SIA was designed to determine if the amount of lectins present in SBM was enough to exert antinutritional effects on rainbow trout and Atlantic salmon. In the first study, purified diets were formulated to meet all the known rainbow trout nutritional requirements. Juvenile rainbow trout were fed six diets containing 43% protein and 15% lipid (Table 4). The amount of lectin incorporated into the diets corresponded to the range of lectin that would be found in rainbow trout diets containing 40% SBM. The amounts of purified soybean lectins included in the diets were 0 (Control), 4 (SBA4), 20 (SBA20), 40 (SBA40), 60 (SBA60) and 80 (SBA80) mg/kg diet. These inclusion levels were chosen because they were within the range found in SBM (i.e., 40% of 10 mg/kg = 4 mg/kg and 40% of 200 mg/kg = 80 mg/kg). The feeding trial was conducted for eight weeks. Upon completion of the trial, consumption and weight gain data were recorded, intestinal sections were removed and blood was collected. Fish fed all levels of lectins exhibited slightly lower weight gain than fish fed the control (Table 5). These values were not statistically significant, but over the course of an entire grow-out cycle they may become biologically significant. Evaluation of the intestinal sections indicated no structural damage as a result of feeding soybean lectins. Insulin levels were not affected by dietary treatment (data not shown).

The second salmonid SIA lectin study evaluated the effects of feeding soybean lectins to juvenile Atlantic salmon. The design of the experiment was similar to that used in the first study. Six purified diets containing 50% protein and 19% lipid were formulated to meet all known nutritional requirements of Atlantic salmon (Table 6). The amount of soybean lectin included in the diets corresponded to the range that would be found in Atlantic salmon diets containing 35% SBM. These amounts were 0 (Control), 3.5 (SBA3.5), 17.5 (SBA17.5), 35 (SBA35), 52.5 (SBA52.5) and 70 (SBA70) mg/kg diet. This trial was conducted for eight weeks and the same sampling procedures were used upon conclusion. There were no significant dietary effects on weight gain (Table 7) or intestinal structure of Atlantic salmon fed soybean lectins. Post-prandial insulin levels were lower in fish fed all levels of soybean lectins, but not significantly (Table 8).

While there were no significant effects observed in either of the initial salmonid SIA studies, the results seemed to indicate potential antinutritive effects. It was concluded that soybean lectin inclusion at the levels evaluated may become biologically significant over an entire grow-out cycle, or that the negative effects of feeding SBM-based feeds to salmonids may be a result of certain ANF combinations.

Trypsin Inhibitors

Trypsin inhibitors are proteins that interfere with nutrient absorption by reducing the activity of the proteolytic enzymes trypsin and chymotrypsin (Norton 1991). Trypsin inhibitor concentrations in SBM range from 0.5 to 0.8% (Russett 2002). The amount and activity of trypsin inhibitors in the diet has been shown to be inversely related to the availability of energy and protein (Sandholm et al. 1976; Krogdahl et al. 1994). When fed SBM diets, trypsin production of rainbow trout and Atlantic salmon increased until dietary inclusion rates exceeded 20-25%. As SBM inclusion rates increased beyond this range, trypsin production decreased, most likely an exhaustion of the pancreas' ability to produce trypsin (Krogdahl 1994; Olli et al. 1994; Krogdahl et al. 2003).

As part of SIA, three feeding trials were conducted at Michigan State University to evaluate the effects of soybean trypsin inhibitors on Atlantic salmon. In the first two studies, soybean trypsin inhibitors were added to purified diets at levels corresponding to the amount of trypsin inhibitors that would be present in diets containing 0-60% SBM. Five diets were formulated to contain the amount of trypsin inhibitors that would be present in 0, 15, 30, 45 and 60% SBM diets (the diets were similar in formulation to those in Table 6). In the first study, one year old Atlantic salmon were fed their respective dietary treatments for 21 days. At the conclusion of the trial, fecal matter and intestinal sections (small intestine, proximal and distal large intestine) were collected and analyzed for trypsin activity. A slight reduction in trypsin activity in the large intestines of Atlantic salmon was reported at dietary trypsin inhibitor concentrations that corresponded to 15% SBM in diets (Figure 5). In the second study, juvenile Atlantic salmon were fed their respective dietary treatments for eight weeks. At the conclusion of the study, weight gain and survival data were recorded and condition factor was calculated. Weight gain was not impacted by dietary trypsin inhibitor inclusion (Table 9). Condition factor was the lowest and mortalities the highest in fish fed the highest inclusion rates of trypsin inhibitors (Figures 6 and 7, respectively). The findings in these two studies were not statistically significant; however, soybean trypsin inhibitors may be a contributing factor affecting nutrient absorption when feeding diets containing 30-60% SBM to Atlantic salmon.

The final feeding trial conducted at Michigan State University was designed to develop a commercially acceptable Atlantic salmon feed formulation that incorporated higher SBM and lower fish meal inclusion rates. Diets were formulated to meet the known nutritional requirements of Atlantic salmon smolts. Diets were extruded by SIA collaborators at the University of Idaho based on the best available knowledge obtained from SIA research of SBM-based diet processing for rainbow trout. Fish were fed an open formula control and SBM test diets containing 5 – 30% SBM for 12 weeks (Table 10). Results indicated there were no differences in growth, protein and energy retention or feed conversion (Table 11). Also, intestinal trypsin activity was not affected by SBM inclusion rate. There was a significant decrease in whole body lipid content with increasing SBM inclusion (Table 12). Despite the lower whole body lipid concentration, the researchers concluded that the higher SBM diets did not have a negative impact on Atlantic salmon.

Interactions

In addition to lectins and trypsin inhibitors, the effects of feeding soy oligosaccharides needed to be evaluated. The total oligosaccharide content in SBM is 12-15%, with sucrose (6-7%), stachyose (5-6%) and raffinose (1-2%) being the primary oligosaccharides present (Francis et al. 2001; Russett 2002). Rainbow trout fed diets containing SPC with less than 2% soluble carbohydrates grew better than fish fed diets containing SBM with 16% soluble carbohydrates (Kaushik et al. 1995). However, the authors could not conclude that soy oligosaccharides were causing the observed differences in dietary effects because some other component of SBM that is not present in SPC may have been exerting antinutritive effects. Most ANF research, including the first series of SIA studies, either evaluated individual effects of single ANF or evaluated whole soy-based ingredients or isolated fractions that may have contained multiple ANF. These studies have not identified the possible interactive or cumulative effects of various ANF. In a study with chicks, soybean lectins accounted for approximately 15% of the total soybean antinutritional effects (Douglas et al. 1999). Lectins, trypsin inhibitors and oligosaccharides have all been implicated as exerting antinutritive effects on salmonids, but their relative contributions have not been identified. Atlantic salmon fed a SBM diet with reduced content of ANF (oligosaccharides, trypsin inhibitors, lectins and soy antigens) grew as well as fish fed a fish meal control, suggesting that some combination of various soy ANF is causing a reduction in nutrient availability (Refstie et al. 1998).

A third study was conducted at Purdue as part of SIA to evaluate any possible interactions between lectins, trypsin inhibitors and oligosaccharides. Purified diets containing 43% protein and 15% lipid were formulated to meet all known nutritional requirements of rainbow trout (Table 13). All three ANF were included at levels corresponding to amounts that would be present in a 40% SBM diet. The diets consisted of a control (devoid of ANF), and diets supplemented with either lectins (80 mg/kg), trypsin inhibitors (2.6 g/kg), oligosaccharides (25.3, 5.6 and 17.7 g/kg sucrose, raffinose and stachyose, respectively), lectins + trypsin inhibitors (same amounts as above), lectins

+ oligosaccharides (same amounts as above), trypsin inhibitors + oligosaccharides (same amounts as above) and lectins + trypsin inhibitors + oligosaccharides (same amounts as above). The feeding trial was conducted for eight weeks. Upon completion of the trial, consumption and weight gain data were recorded and blood was collected. Feed consumption, weight gain, feed efficiency, specific growth rate (SGR), protein retention, and serum chemistry responses were evaluated using a factorial design. One-factor analysis indicated a significant reduction in protein retention in trout fed diets containing trypsin inhibitors (39.1% protein retention in fish fed diets devoid of trypsin inhibitors versus 36.7% protein retention in fish fed diets supplemented with trypsin inhibitors). Two-factor analysis of the effects of feeding lectins and trypsin inhibitors detected significant main-effect reductions in weight gain and SGR of fish fed lectins or trypsin inhibitors in comparison to fish fed neither ANF (Table 14). Fish fed both lectins and trypsin inhibitors did not exhibit significantly different weight gain or SGR from those fed only lectins or trypsin inhibitors or from fish fed neither ANF. The effects on serum chemistry values were similar to the effects on weight gain. Alanine aminotransferase and amylase activities were increased in fish fed only lectins or trypsin inhibitors, but not in fish fed both or neither ANF (Table 15). These increases were indicative of liver and pancreatic damage (Steiner 2003; Melotti et al. 2004). These results were similar to mammalian studies in which it was concluded that pancreatic damage and increased enzymatic activity as a result of feeding soybean lectins and trypsin inhibitors were contributing to the antinutritive effects of SBM (Hajós et al. 1995). The only significant effects as a result of feeding rainbow trout oligosaccharides were an increase in amylase activity and one-hour post prandial insulin levels (Table 16). These increases may have been a result of differences in digestibilities of the various carbohydrate ingredients (dextrin versus oligosaccharides). Dextrin digestibility values in salmonids have been reported to vary from 37-77% (Singh and Nose 1967; Storebakken et al. 1998), while sucrose and raffinose values have been reported to range from 85-99% and 54-71%, respectively (Refstie et al. 2005). While not statistically significant, serum glucose levels were slightly higher in fish fed diets containing oligosaccharides compared to those fed diets devoid of oligosaccharides (70.4 and 77.0 mg/dL, respectively). It is likely that the higher digestibility of sucrose and possibly raffinose resulted in higher blood glucose levels, resulting in an increased insulin response of fish fed diets containing oligosaccharides. There were no significant interactions as a result of feeding lectins, trypsin inhibitors or oligosaccharides on rainbow trout. Results of this study indicate that the average amounts of lectins and trypsin inhibitors present in a 40% SBM diet fed to rainbow trout are high enough to significantly decrease production parameters. Because of the reductions in weight gain, SGR and protein retention, combined with the increases in enzymatic activities indicative of liver and pancreas disease, lectins and trypsin inhibitors should be removed or inactivated from SBM.

Genistein

Isoflavones are phenolic compounds present in soybeans and SBM. The total isoflavone content of SBM ranges from 0.25 to 0.42% (Pelissero et al. 1992; Goda et al. 2002). Isoflavones are sometimes referred to as phytoestrogens because they can exert estrogenic effects when consumed (Farnsworth et al. 1975a,b). In studies specifically focusing on soy isoflavones' effects on fishes, they have been reported to exert estrogenic effects by inducing vitellogenesis which, in turn, can have stimulatory or suppressive actions on growth and reproduction (Pelissero et al. 1991a,b; Ko et al. 1999; Pelissero et al. 2001).

As part of SIA, the University of Wisconsin conducted nutritional research evaluating the effects of feeding the soy isoflavone genistein on growth, development and reproduction of rainbow trout and Atlantic salmon. In the first study, rainbow trout were fed a commercial control diet and three diets supplemented with soy genistein (500, 1,000 and 3,000 mg/kg) for a one year period (all diets supplied by Nelson and Sons, Inc., UT). Throughout the trial, genistein concentration had no effect on weight gain of the fish (Figure 8). Serum levels of vitellogenin, measured at four times during the treatment period, were higher in genistein-treated fish (all doses) than controls. There were no observed effects of genistein on serum levels of reproductive hormones, morphology and histology of the gonads (both sexes), egg production, fertility rates, fry size nor viability. Accordingly, the genistein content of soy products should not be a factor in determining their incorporation into diets for rainbow trout.

In the second study, Atlantic salmon parr were fed a control diet, three diets supplemented with soy genistein (500, 1,000 and 3,000 mg/kg) and a fifth diet supplemented with estradiol-17β (E_2) at 20 mg/kg. The feeding trial was conducted for six months. Genistein supplementation had no effect on growth or feed conversion. At the end of the feeding trial, serum levels of vitellogenin were higher in the E_2- and genistein-fed fish than control-fed fish. E_2 and genistein at all doses tested inhibited the smoltification process, as measured by 24-hour seawater challenge tests. These results indicate that genistein at levels found in SBM inhibits smoltification and that further research should be conducted to determine the effects of high SBM diets on the smoltification process of Atlantic salmon.

In addition to the work conducted at the University of Wisconsin, researchers at the University of Maine performed quality evaluations on the fillets from the rainbow trout fed the three levels of soy genistein supplementation. Genistein was extracted from fillets to test if feeding diets supplemented with genistein resulted in fillet deposition. Trout fed all levels of genistein supplementation (500, 1,000 and 3,000 mg/kg) had accumulation in the fillets ranging from 2.02 to 5.48 pmol/mg. Fillets from fish fed the genistein supplemented diets were tested for lipid oxidation using the thiobarbituric acid reactive substances (TBARS) method. Fillets from fish fed the genistein supplemented diets had significantly lower TBARS levels than fillets from fish fed the control (Figure 9); indicating there was less lipid oxidation as a result of feeding genistein. Dietary genistein had no result on fillet color (using tristimulus L, a, b measurements), and triangle tests using an untrained panel detected no differences in fillet quality or taste (D’Souza et al. 2005). These results indicate that soy genistein may help prolong product shelf-life by acting as an antioxidant and that there are no detectable differences in fillet quality as a result of feeding genistein to rainbow trout.

In a similar study, fillets from rainbow trout fed a fish meal control diet and two diets with SBM inclusion (20 and 40% SBM) were evaluated to determine if SBM inclusion had an effect on fillet quality. Diets for these fish were formulated and manufactured by researchers at the University of Idaho. Trout were reared for a total of six months at the Idaho facility. Rainbow trout fed the fish meal control (374 g/fish) and diet containing 20% SBM (352 g/fish) were significantly larger than the fish fed the diet containing 40% SBM (289 g/fish). Upon completion of the feeding trial, fillets were collected and shipped to the University of Maine. Fillets from fish fed the 40% SBM diet had significantly lower TBARS levels than fish fed the 20% SBM and fish meal control diets (Figure 10); indicating there was less lipid oxidation in fillets of fish fed the 40% SBM diet. Fillets from the fish fed the 40% SBM diet were significantly lighter in color than fish fed the other diets. Panelists were able to visually identify differences in fillets from soy-fed fish; however, panelists indicated no differences in acceptability of baked rainbow trout fillets from any of the dietary treatments (D'Souza et al. 2006).

Extrusion Processing

Extrusion cooking technology is a process used to manufacture feeds by exposing them to a combination of moisture, pressure, temperature and mechanical shear (Barrows and Hardy 2001). Additional processing of SBM can potentially reduce the antinutritive effects of lectins and trypsin inhibitors. Aqueous heat treatment for 2 – 15 minutes at 100°C abolishes the antinutritive effects of lectins, most likely by inhibiting its carbohydrate binding ability (Bender and Reaidi 1982; Fish and Thompson 1991; Grant

1991); however, care must be taken to assure that nutrient digestibilities (primarily essential amino acids) are not impacted by excessive heat treatment (Cheng and Hardy 2003; Deng et al. 2005). The use of extrusion cooking has lead to higher digestibility values of plant protein ingredients (Srihara and Alexander 1984; Hakansson et al. 1987).

SIA research conducted by the University of Idaho and the United States Department of Agriculture Agricultural Research Service (Hagerman Fish Culture Experiment Station) evaluated the effects of heating and extrusion processing to determine optimum conditions for manufacturing high-SBM rainbow trout diets. A base diet was formulated to meet or exceed all known rainbow trout nutritional requirements (Table 17). The basal diet was subjected to two levels of pre-cooking (the SBM was either pre-cooked or not), extrusion temperature (93 or 127°C) and retention time in the extruder barrel (18 or 37 seconds), for a total of eight experimental diets. The feeding trial was conducted for 12 weeks. Pre-cooking of the SBM had no effect on weight gain or feed conversion of the fish; however, it did increase the digestibility of organic matter, energy and carbohydrate. The extruder temperature and retention time in the barrel had significant effects on feed conversion and weight gain, respectively. The higher barrel temperature resulted in the lowest feed conversion and the shorter retention time resulted in the highest weight gain (Table 18). Weight gain of the fish was lower than other studies evaluating lower percentage inclusion of SBM, but still over 400% over 12 weeks. However, the lower growth rates were expected since a 52% SBM diet was being evaluated. These results indicate that a higher extrusion temperature (127°C) and lower barrel retention time (18 seconds) may be the best extrusion conditions for high-SBM salmonid diets (Barrows et al. 2007).

Economic Analysis of Soy Inclusion in Salmonid Diets

Traditional salmonid diets have been composed of fish and terrestrial animal proteins, but issues related to environmental sustainability, feed ingredient availability and production costs have propelled feed manufacturers to consider inclusion of plant proteins into salmonid diets. There has been considerable research published on the nutritional benefits and limitations of soybean based feed ingredients and their role in salmonid feeds; however, very little has been done to evaluate the economic value of soybean feed ingredients in salmon and trout feed production. Global salmonid feed production, in recent years, has been estimated to range between 1.6 and 2 mmt. If soybean products were included at 25% of the diets, by weight, it would imply an annual global demand for 0.4-0.5 mmt of soy products for salmonid aquaculture.

Researchers at Kentucky State University, as part of SIA, developed least-cost models for Atlantic salmon and rainbow trout diets and evaluated the cost of utilizing SBM, full-fat SBM (FF-SBM), and SPC at different inclusion rates. The model also evaluated a demand curve for soybean feed products for the U. S. salmon and trout aquaculture industries. The general concept of the least-cost model is to develop a diet by minimizing the cost of the ingredient mix while meeting all the nutritional requirements of the animal. The feed ingredients used in this model have been fed to salmonids with success.

This includes SBM, FF-SBM, SPC, ground wheat, corn gluten meal, distiller's grains, blood meal, low temperature fish meal, menhaden fish meal, poultry feather meal, poultry by-product meal, fish oil, U. S. Fish and Wildlife vitamin and mineral premix, vitamin C, and a carotenoid such as Astaxanthin. Additional potential ingredients include dicalcium phosphate, choline chloride, lecithin, L-methionine, and L-lysine. The nutritional requirements of salmon and trout were modeled via constraints satisfying 1) minimum digestible protein, energy and lipid levels, 2) maximum crude fiber and ash content of the diet, 3) minimum essential amino acid levels, 4) minimum vitamin levels, and 5) minimum and maximum mineral levels. In general, the results indicated that if feed ingredient prices followed common market trends (based on 2004 feed ingredient prices), soy products were utilized at rates of 15 and 25% in Atlantic salmon and rainbow trout diets, respectively. The diet models developed showed that it was possible to include soy products at much higher rates in salmonid diets, but these high rates did not represent least-cost solutions (Table 19). SBM demand curves identified the change in the economically efficient inclusion rates with respect to SBM prices (Figures 11 and 12). These figures also identified upper limits on SBM prices, beyond which SBM inclusion becomes cost-prohibitive. Using Least-cost models (updated with 2006 feed ingredient prices) soy inclusion for rainbow trout was calculated to be greater than 20% of a diet containing as much as 38% protein (Figure 13) and greater than 25% of a diet containing as much as 39% protein for Atlantic salmon (Figure 14). When protein levels were increased above 38 and 39% of the diet (for rainbow trout and Atlantic salmon, respectively) the least-cost formulations contained higher proportions of other animal-based ingredients (mainly poultry-based meals). While these models indicated the least-cost formulations for rainbow trout and Atlantic salmon, they did not take into account palatability of the diet.

Conclusions

The results of the SIA research have begun to elucidate which factors present in SBM have been acting as ANF when fed to salmonids. The amount of saponins present in SBM does not appear to be exerting antinutritive effects on salmonids. In fact, soy saponins may have a beneficiary effect by boosting the immune response when fed to fish. Trypsin inhibitors and lectins at the amounts present in dehulled, solvent-extracted SBM, exerted antinutritive effects, as fish in the SIA studies exhibited reduced weight gain, changes in enzymatic activities and a reduction in protein retention (effect of feeding trypsin inhibitors only). Both of these ANF are heat sensitive, so further conditioning during the extrusion process may be enough to inactivate lectins and trypsin inhibitors. Additionally, there are null strains of soybeans that are devoid of lectins and trypsin inhibitors; however, the soybean processing industry would have to process these null lines specifically for use in aquaculture diets which might add some expense. There were no observed negative effects as a result of feeding soy genistein to rainbow trout. Lipid oxidation of rainbow trout fillets from fish fed diets supplemented with soy genistein and high levels of SBM (40% of the diet) was reduced in comparison to fillets from trout fed a control. Color tests revealed that fillets from rainbow trout fed SBM were lighter in color than those from fish fed a fish meal control, but consumer preference tests revealed no differences between the fillets from fish fed the SBM and fish meal diets. Smoltification of Atlantic salmon was inhibited when fed diets supplemented with soy genistein, but no other negative effects were observed. Further studies should be conducted to determine the effects of higher SBM inclusion rates in diets for Atlantic salmon smolts. Extrusion cooking technology can be used to further improve performance of salmonids fed high SBM diets. Shorter barrel retention times and higher temperatures produced optimum results when extruding 52% SBM diets for rainbow trout. Least-cost modeling indicated that soy products should be utilized at rates of 15 and 25% in Atlantic salmon and rainbow trout diets, respectively. While it was possible to achieve higher soy inclusion rates, given current feed ingredient prices, these did not represent least-cost solutions.

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Table 1: Composition of diets fed to juvenile rainbow trout during year one feeding study conducted at Ohio State University to evaluate the effects of soy saponins and other soy extracts.

Ingredients % of diet	Control	SBM	SBM after extraction	SBM-Saponins	SBM-Flavonoids	SPC	SPC after extraction	Saponin Quillaja
Casein	40.00	20.00	20.00	40.00	40.00	20.00	20.00	40.00
Gelatin	8.00	4.00	4.00	8.00	8.00	4.00	4.00	8.00
SBM	-	44.00	-	-	-	-	-	-
SBM after extraction	-	-	44.00	-	-	-	-	-
SBM-Saponin	-	-	-	0.171	-	-	-	-
SBM-Flavonoids	-	-	-	-	0.277		-	-
SPC	-	-	-	-	-	32.00		-
SPC-after extraction	-	-	-	-	-	-	32.00	
Quillaja-Saponin								0.171
Dextrin	21.25	1.05	1.05	21.08	20.97	13.05	13.05	21.08
Fish protein concentrate	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Cod liver oil	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Vitamin mix	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Mineral mix	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CMC	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Arg	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Met	0.40	0.60	0.60	0.40	0.40	0.60	0.60	0.40
L-Lys	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline-Cl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 2: Composition of diets used in the year two feeding study conducted at Ohio State University. Each experimental diet was formulated to have 50% protein replacement or supplementation with the desired component in an amount equivalent to the amount in 50% protein replacement with SBM.

	Control	SBM	SBM-after extraction	SBM-carbohydrates	SBM-flavonoids	SBM-saponins	SBM-lipid	Negative Control
Casein	30.00	15.00	15.00	30.00	30.00	30.00	30.00	15.00
Casein-hydrolysate	6.00	3.00	3.00	6.00	6.00	6.00	6.00	3.00
Gelatin	6.00	3.00	3.00	6.00	6.00	6.00	6.00	3.00
SBM	0.00	44.00	0.00	0.00	0.00	0.00	0.00	0.00
SBM-after extraction	0.00	0.00	33.50	0.00	0.00	0.00	0.00	0.00
SBM-Carbohydrates	0.00	0.00	0.00	7.48	0.00	0.00	0.00	0.00
SBM-Flavonoids	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00
SBM-Saponin	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
SBM-Lipid	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.00
Dextrin	25.25	0.95	11.05	17.77	24.93	25.01	24.90	47.10
Fish protein concentrate	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lecithin	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Cod liver oil	13.00	13.00	13.00	13.00	13.00	13.00	13.00	13.00
Vitamin mix	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Mineral mix	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
CMC	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Arg	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.25
L-Lys	0.80	1.50	1.80	0.80	0.80	0.80	0.80	0.40
L-Met	0.40	1.00	1.10	0.40	0.40	0.40	0.40	0.20
Choline-Cl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3: Mean individual weight of rainbow trout after 15 week feeding trial conducted at Ohio State University to determine the effects of feeding soy saponins and other soy extracts. Extracted based on water/methanol method as described by Oleszek and Stochmal (2002). Different superscripts denote significant differences.

	Mean Individual Fish Weight (g)
Control	6.4 ± 0.3^{a}
SBM	3.8 ± 0.2^{d}
SBM-after extraction	3.5 ± 0.2^{d}
SBM-carbohydrates	4.4 ± 0.2^{c}
SBM-flavonoids	5.5 ± 0.2^{b}
SBM-saponins	5.8 ± 0.2^{ab}
SBM-lipids	6.2 ± 0.2^{a}
Negative Control	2.0 ± 0.1^{e}

Table 4: Composition of basal diet fed to juvenile rainbow trout at Purdue University. Soybean lectins (SBA) were included at 0, 4, 20, 40, 60 or 80 mg/kg diet.

Ingredient (%)	Control
Casein	44.0
Gelatin	4.0
L-Arginine	1.0
L-Methionine	0.5
Dextrin	15.0
α-Cellulose	6.3
CMC	2.0
Mineral Premix	8.0
Vitamin Premix	3.0
Stay-C	0.1
Choline-Cl	0.1
Lecithin	0.5
Krill Meal	0.5
Menhaden Oil	15.0
SBA (mg/kg)	Variable

Table 5: Mean feed consumption (FC), weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and percent survival of rainbow trout fed graded levels of soybean lectins (SBA) at Purdue University. Values with differing superscripts in the same column are significantly different ($P \leq 0.05$).

Diet	FC (g/fish)	WG (g/fish)	FE[1]	SGR[2]	Survival (%)
Control	11.44^a	10.71	0.91	1.28	84
SBA4	10.40^a	8.30	0.80	0.97	93
SBA20	9.73^a	6.71	0.69	0.93	91
SBA40	10.95^a	9.33	0.85	1.08	89
SBA60	7.98^b	6.01	0.76	0.83	64
SBA80	9.67^a	7.57	0.78	0.95	82
Pooled SEM	0.187	0.707	0.068	0.141	4.53
P-value	0.0006	0.15	0.45	0.36	0.19

[1] FE: g of weight gain/g of feed consumed.

[2] SGR: (ln final body weight – ln initial body weight)/days*100.

Table 6: Composition of basal diet fed to juvenile Atlantic salmon at Purdue University. Soybean lectins (SBA) were included at 0, 3.5, 17.5, 35, 52.5 or 70 mg/kg diet.

Ingredient (%)	Control
Casein	46.9
Gelatin	11.0
L-Methionine	0.5
Dextrin	13.0
α-Cellulose	1.05
CMC	2.0
Mineral Premix	3.3
Vitamin Premix	2.0
Stay-C	0.35
Choline-Cl	0.4
Lecithin	0.5
Menhaden Oil	19.0
SBA (mg/kg)	Variable

Table 7: Mean feed consumption (FC), weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and survival of Atlantic salmon fed graded levels of soybean lectins (SBA) at Purdue University.

Diet	FC (g/fish)	WG (g/fish)	FE[1]	SGR[2]	Survival (%)
Control	21.03	23.13	1.10	1.55	92.7
SBA3.5	19.53	19.93	1.03	1.48	83.3
SBA17.5	18.50	21.43	1.16	1.54	86.1
SBA35	20.60	23.37	1.14	1.58	88.9
SBA52.5	19.83	22.30	1.10	1.51	88.9
SBA70	17.87	21.03	1.18	1.48	91.7
Pooled SEM	1.185	2.235	0.080	0.111	6.21
P-value	0.437	0.875	0.824	0.983	0.919

[1] FE: g of weight gain/g of feed consumed.

[2] SGR: (ln final body weight – ln initial body weight)/days*100.

Table 8: Mean baseline (48 hour starved fish) and one- and three-hour post-prandial serum insulin levels (pmol/L) of Atlantic salmon fed graded levels of soybean lectins (SBA) at Purdue University.

Diet	Baseline	One-hour	Three-hour
Control	34.0	53.0	47.7
SBA3.5	37.3	42.3	39.0
SBA17.5	33.3	50.3	43.0
SBA35	28.0	46.0	43.3
SBA52.5	36.3	48.0	42.3
SBA70	32.7	43.3	42.7
Pooled SEM	4.405	4.130	4.166
P-value	0.732	0.465	0.810

Table 9: Mean weight gain (WG) of Atlantic salmon fed a commercial control and trypsin inhibitor test diets equivalent to diets containing 0, 15, 30, 45, and 60% SBM at Michigan State University.

Diet	WG (g/fish)
Control	21.92
0.0 g/kg trypsin inhibitor (0% SBM equivalent)	24.24
0.97 g/kg trypsin inhibitor (15% SBM equivalent)	26.76
1.95 g/kg trypsin inhibitor (30% SBM equivalent)	26.09
2.92 g/kg trypsin inhibitor (45% SBM equivalent)	27.37
3.90 g/kg trypsin inhibitor (60% SBM equivalent)	26.55

Table 10: Composition of diets fed to Atlantic salmon at Michigan State University. Diets consisted of a control (MNR-98HS) and varying levels of SBM and fish meal (FM). MNR-98HS is an open feed formulation.

Ingredients (%)	MNR-98HS	SBM5/FM30	SBM20/FM30	SBM20/FM24	SBM25/FM30	SBM30/FM30	SBM30/FM24
Wheat gluten meal	--	7.0	7.0	7.0	7.0	7.0	10.0
SBM	--	5.0	20.0	20.0	25.0	30.0	30.0
Fish meal, anchovy	30.0	30.0	30.0	24.0	30.0	30.0	24.0
Blood meal	7.0	7.0	5.95	5.7	4.75	3.3	3.85
Poultry by-product meal	6.0	6.0	5.0	4.9	3.94	2.9	3.08
Whey	9.0	9.0	5.5	4.1	4.4	2.66	2.1
Brewers yeast	5.0	5.0	3.0	2.29	2.5	1.6	1.3
Corn gluten meal	25.0	14.3	5.92	13.5	4.3	3.9	6.0
Lysine HCl	0.5	--	0.07	0.16	0.1	0.2	0.3
Vitamin premix	1.0	0.3	0.3	0.3	0.3	0.3	0.3
Mineral premix	0.5	0.2	0.2	0.24	0.2	0.22	0.24
Fish oil, menhaden	16.0	15.14	15.8	16.19	16.2	16.5	17.0
L-Methionine	--	--	0.11	0.08	0.13	0.15	0.15
Dicalcium Phosphate	--	0.16	0.25	0.64	0.28	0.37	0.78
Choline chloride	--	0.6	0.6	0.6	0.6	0.6	0.6
Stay-C	--	0.3	0.3	0.3	0.3	0.3	0.3

Table 11: Mean final weight, specific growth rate (SGR), condition factor (k), feed conversion rate (FCR), protein efficiency ratio (PER) and apparent protein retention (APR) in smolting Atlantic salmon fed diets containing varying levels of SBM and fish meal (FM) at Michigan State University. Mean standard errors are in parentheses. MNR-98HS is an open formula control diet. Values in each row awarded common superscripts are not significantly different (P>0.05).

Ingredients	MNR-98HS	SBM5/ FM30	SBM20/ FM30	SBM20/ FM24	SBM25/ FM30	SBM30/ FM30	SBM30/ FM24
Final weight	81.39	81.13	76.32	82.24	82.03	82.53	82.50
(g)	(2.64)	(0.50)	(0.56)	(0.90)	(2.28)	(3.07)	(1.18)
SGR[1]	1.94	1.94	1.88	1.92	1.93	1.94	1.93
	(0.02)	(0.01)	(0.02)	(0.02)	(0.03)	(0.02)	(0.03)
K[2]	0.0107^{a}	0.0105^{ab}	0.0100^{b}	0.0106^{a}	0.0102^{ab}	0.0102^{ab}	0.0105^{ab}
	(0.0002)	(0.0003)	(0.0002)	(0.0001)	(0.0001)	(0.0002)	(0.0001)
FCR[3]	0.823	0.789	0.797	0.788	0.789	0.780	0.803
	(0.027)	(0.028)	(0.032)	(0.007)	(0.002)	(0.013)	(0.04)
PER[4]	2.20	2.27	2.26	2.31	2.29	2.32	2.27
	(0.07)	(0.08)	(0.09)	(0.02)	(0.01)	(0.04)	(0.11)

[1] SGR: (ln final body weight – ln initial body weight)/days*100.

[2] k: weight / length^3

[3] FCR: g feed consumed/g weight gain

[4] PER: g weight gain/g protein consumed

Table 12: Body composition (%) analysis of smolting Atlantic salmon fed diets containing varying levels of SBM and fish meal (FM) at Michigan State University. Mean standard errors are in parentheses. MNR-98HS is an open formula control diet. Values in each row awarded common superscripts are not significantly different (P>0.05).

Ingredients	MNR-98HS	SBM5/FM30	SBM20/FM30	SBM20/FM24	SBM25/FM30	SBM30/FM30	SBM30/FM24
Dry matter	28.82^{a}	27.89^{b}	28.47^{ab}	28.42^{ab}	28.48^{ab}	28.40^{ab}	28.32^{ab}
	(0.10)	(0.26)	(0.20)	(0.16)	(0.14)	(0.07)	(0.25)
Protein	49.45^{ab}	49.16^{b}	50.22^{ab}	50.82^{ab}	51.50^{a}	51.41^{a}	50.54^{ab}
	(0.83)	(0.36)	(0.45)	(1.03)	(0.12)	(0.84)	(0.31)
Fat	22.04^{a}	21.85^{a}	16.06^{b}	15.82^{bc}	15.04^{bcd}	12.95^{d}	13.20^{cd}
	(0.75)	(0.56)	(0.70)	(1.39)	(0.57)	(0.77)	(0.46)
Ash	6.73^{bc}	7.17^{a}	6.97^{abc}	6.94^{abc}	7.00^{ab}	6.57^{c}	6.79^{abc}
	(0.11)	(0.06)	(0.25)	(0.02)	(0.14)	(0.04)	(0.09)

Table 13: Composition of diets fed to juvenile rainbow trout at Purdue University. All ingredients are given as a percent of the diet except soybean lectin (SBA).

Ingredient (%)	Diet 1 Control	Diet 2 SBA	Diet 3 TI	Diet 4 OLIG	Diet 5 SBA + TI	Diet 6 SBA + OLIG	Diet 7 TI + OLIG	Diet 8 SBA + TI + OLIG
Casein	44.0	44.0	44.0	44.0	44.0	44.0	44.0	44.0
Gelatin	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
L-Arginine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dextrin	15.0	15.0	15.0	10.14	15.0	10.14	10.14	10.14
α-Cellulose	9.3	9.3	9.04	9.3	9.04	9.3	9.04	9.04
CMC	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Min. Premix	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Vit. Premix	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Stay-C	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline-Cl	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Menhaden Oil	14.5	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Trypsin inhibitor	--	--	0.26	--	0.26	--	0.26	0.26
Sucrose	--	--	--	2.53	--	2.53	2.53	2.53
Raffinose	--	--	--	0.56	--	0.56	0.56	0.56
Stachyose	--	--	--	1.77	--	1.77	1.77	1.77
SBA (mg/kg)	--	80.0	--	--	80.0	80.0	--	80.0

Table 14: Two-factor analysis of mean feed consumption (FC), weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein retention (PR) of rainbow trout fed soybean lectin (SBA) and trypsin inhibitor (TI) at Purdue University. Presence of SBA and TI is indicated by +. Values with corresponding superscript in the same column are not significantly different ($P > 0.05$).

Dietary ANF presence Treatments	SBA	TI	FC^1	WG^1	FE^2	SGR^3	PR^4
Diets 1 and 4	-	-	19.12	20.42^a	1.07	2.97^a	39.5
Diets 3 and 7	-	+	17.80	18.05^b	1.02	2.77^b	36.7
Diets 2 and 6	+	-	16.73	17.63^b	1.05	2.74^b	38.6
Diets 5 and 8	+	+	18.52	19.33^{ab}	1.04	2.89^{ab}	36.6
Pooled SEM			1.550	2.030	0.021	0.176	0.383
P-value			0.029*	0.002	0.498	0.001	0.725

*Tukey adjustment for multiple comparisons of means was unable to distinguish differences.

[1] FC and WG: g/fish.

[2] FE: g of weight gain/g of feed consumed.

[3] SGR: (ln final body weight – ln initial body weight)/days*100.

[4] PR: (g of final body weight*percent crude protein – g of initial body weight*percent crude protein)/g of feed consumed*percent crude protein*100.

Table 15: Two-factor analysis of mean serum chemistry values for alanine aminotransferase (ALT, U/L) and amylase (AMY, U/L) of rainbow trout fed soybean agglutinin (SBA) and trypsin inhibitor (TI) at Purdue University. Presence of SBA and TI is indicated by +.

Dietary ANF presence Treatments	SBA	TI	ALT	AMY
Diets 1 and 4	-	-	9.7	50.3
Diets 3 and 7	-	+	14.2	76.2
Diets 2 and 6	+	-	13.2	72.2
Diets 5 and 8	+	+	8.1	63.2
Pooled SEM			3.96	16.94
P-value			0.03*	0.04*

*Tukey adjustment for multiple comparisons of means was unable to distinguish differences.

Table 16: One-factor analysis of mean baseline (24 hour starved) and one-hour post-prandial serum insulin (pmol/L) and amylase (U/L) levels of rainbow trout fed oligosaccharides (OLIG) at Purdue University. Presence of OLIG is indicated by +. Values with differing superscripts in the same column are significantly different ($P \leq 0.05$).

Dietary ANF presence		Baseline insulin	One-hour post-prandial	Amylase
Treatments	OLIG			
Diets 1, 2, 3 and 5	-	20.6	25.0^{a}	56.9^{a}
Diets 4, 6, 7 and 8	+	25.2	31.6^{b}	74.1^{b}
Pooled SEM		3.03	4.66	11.80
P-value		0.38	0.05	0.04

Table 17: Composition of diets fed to 40 g rainbow trout at the University of Idaho.

Ingredient	%
Fish meal	24.2
Solvent-extracted SBM	52.5
Wheat flour	7.3
Menhaden oil	9.1
Menhaden oil, top dressing	6.0
Vitamin premix	0.6
Mineral premix	0.1
Stay-C	0.2

Table 18: The effect of processing conditions on growth and feed conversion ratio of rainbow trout fed SBM based feeds at the University of Idaho.

Temperature, °C	Extrusion time, seconds	Weight gain, g/fish	FCR
93	18	193.3	1.08
93	36	191.5	1.07
127	18	201.5	1.05
127	36	187.2	1.03
P-value of model effects		0.05	0.09
P-value of temperature effects		0.58	0.01
P-value of time effects		0.03	0.50
P-value of temperature*time interactive effects		0.09	0.65

Table 19: Maximum inclusion rates (and corresponding ingredient cost) of soy feed products in nutritionally complete diets for Atlantic salmon and rainbow trout based on research conducted at Kentucky State University. Inclusion rates are in as-fed percentages. Feed ingredient costs are in 2004 U.S. dollars per metric tonne.

	Atlantic salmon				Rainbow trout			
	SBM	FF-SBM	SPC	Combined	SBM	FF-SBM	SPC	Combined
Spring 2004 prices	29.3 (510)	29.2 (503)	62.3 (1,724)	62.3 (1,724)	29.6 (607)	35.4 (583)	60.3 (1,677)	60.3 (1676)
Summer 2004 prices	29.3 (543)	29.2 (537)	62.3 (1,727)	62.3 (1,727)	29.6 (622)	35.4 (601)	60.3 (1,678)	60.3 (1676)
Fall 2004 prices	29.3 (440)	29.0 (431)	62.3 (1,726)	62.3 (1,726)	29.6 (507)	35.4 (473)	60.3 (1,677)	60.3 (1676)

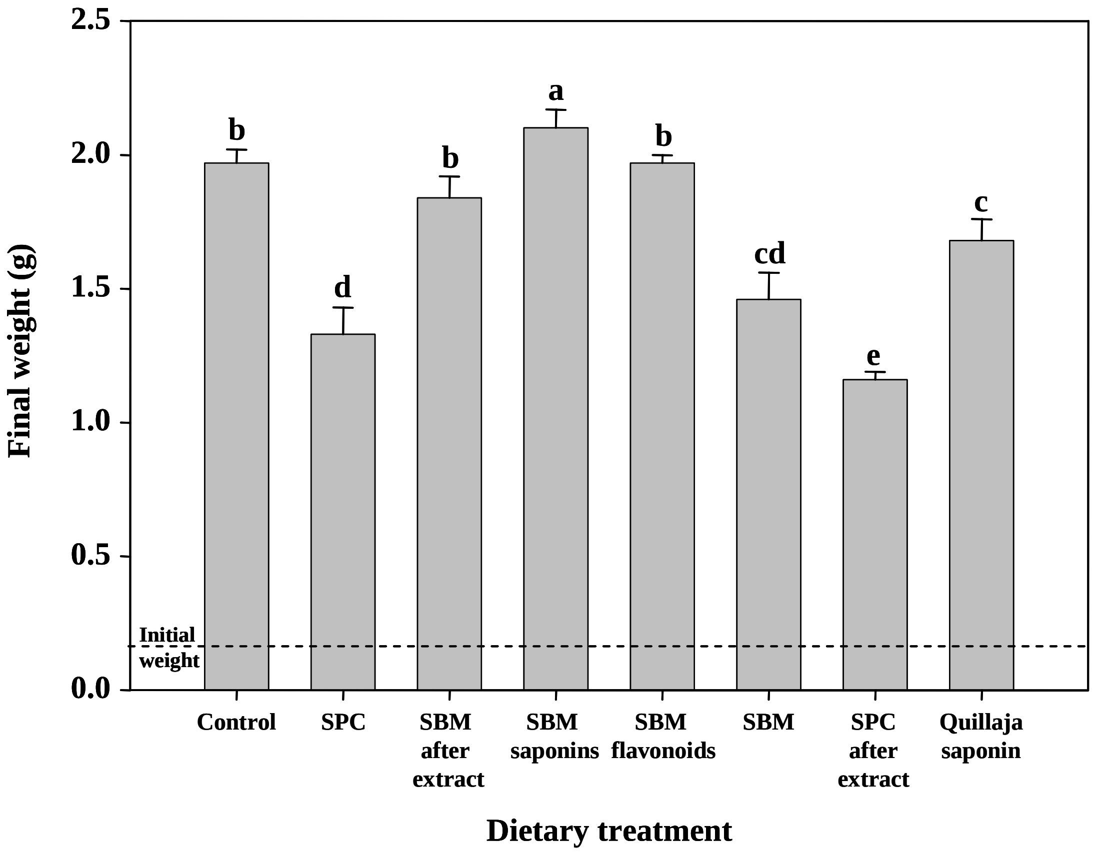

Figure 1: Final weights of rainbow trout after eight weeks of feeding experimental diets in year one study at Ohio State University.

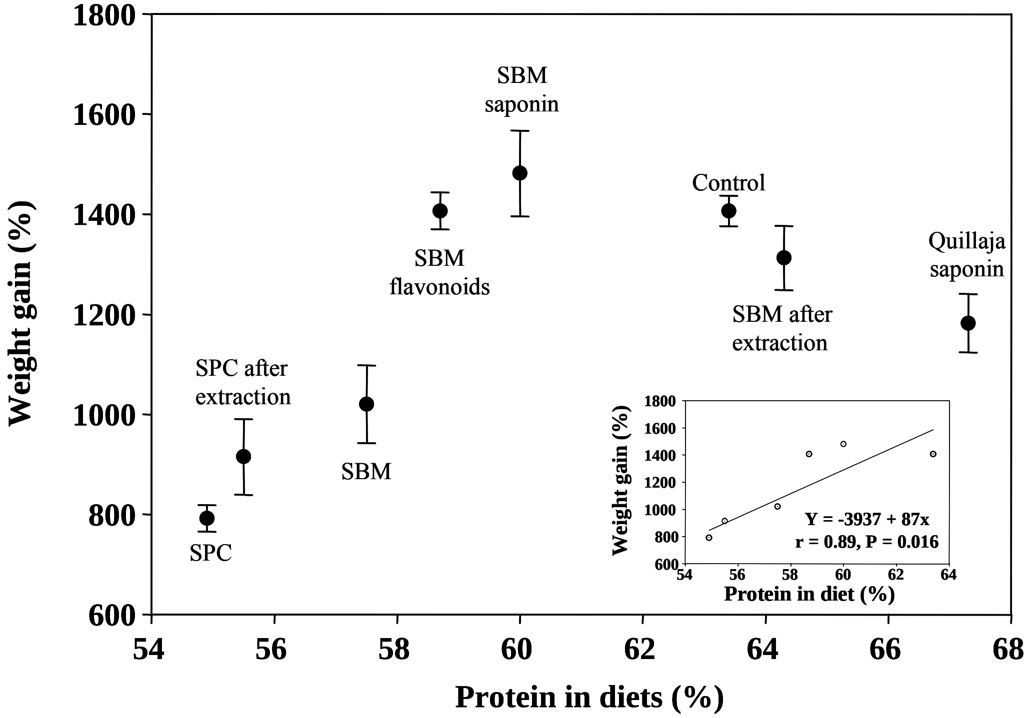

Figure 2: Relationship between protein or lipid content in experimental diets and the final weight gain of rainbow trout juveniles after eight weeks of feeding in year one study at Ohio State University.

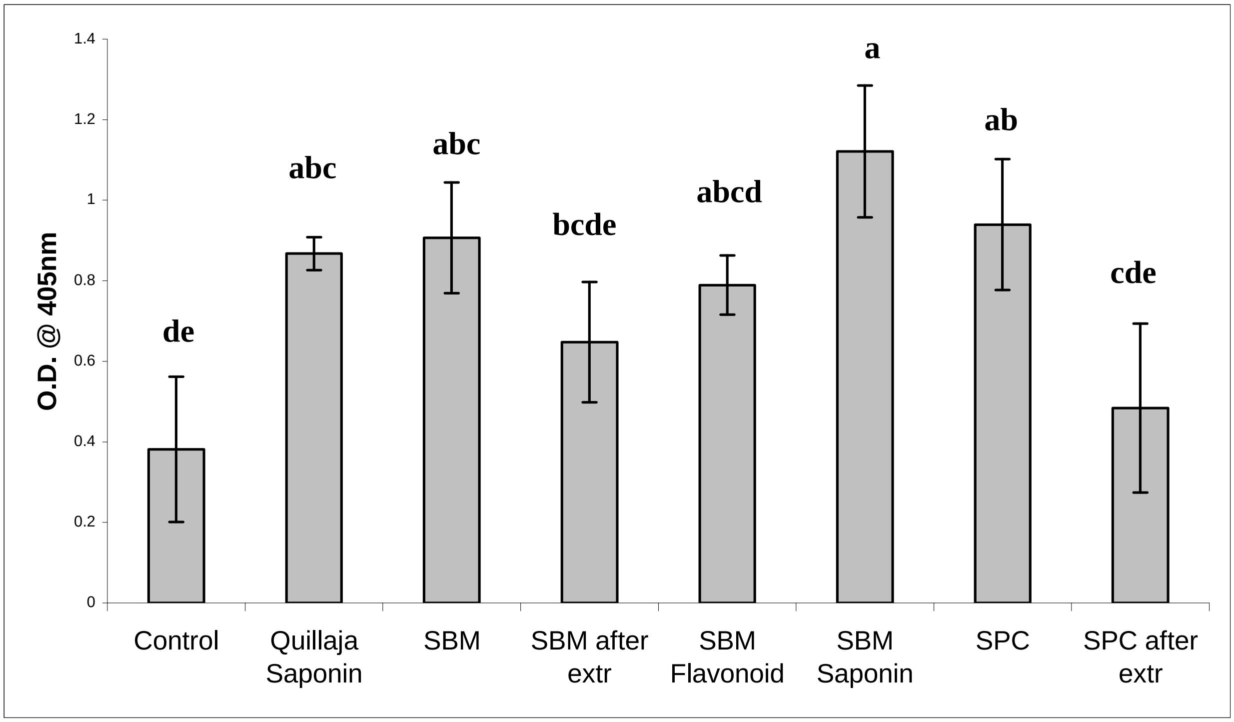

Figure 3: Specific antibody levels of rainbow trout against Aeromonas salmonicida four weeks after vaccination in year one feeding study at Ohio State University. Different subscripts denote significant differences.

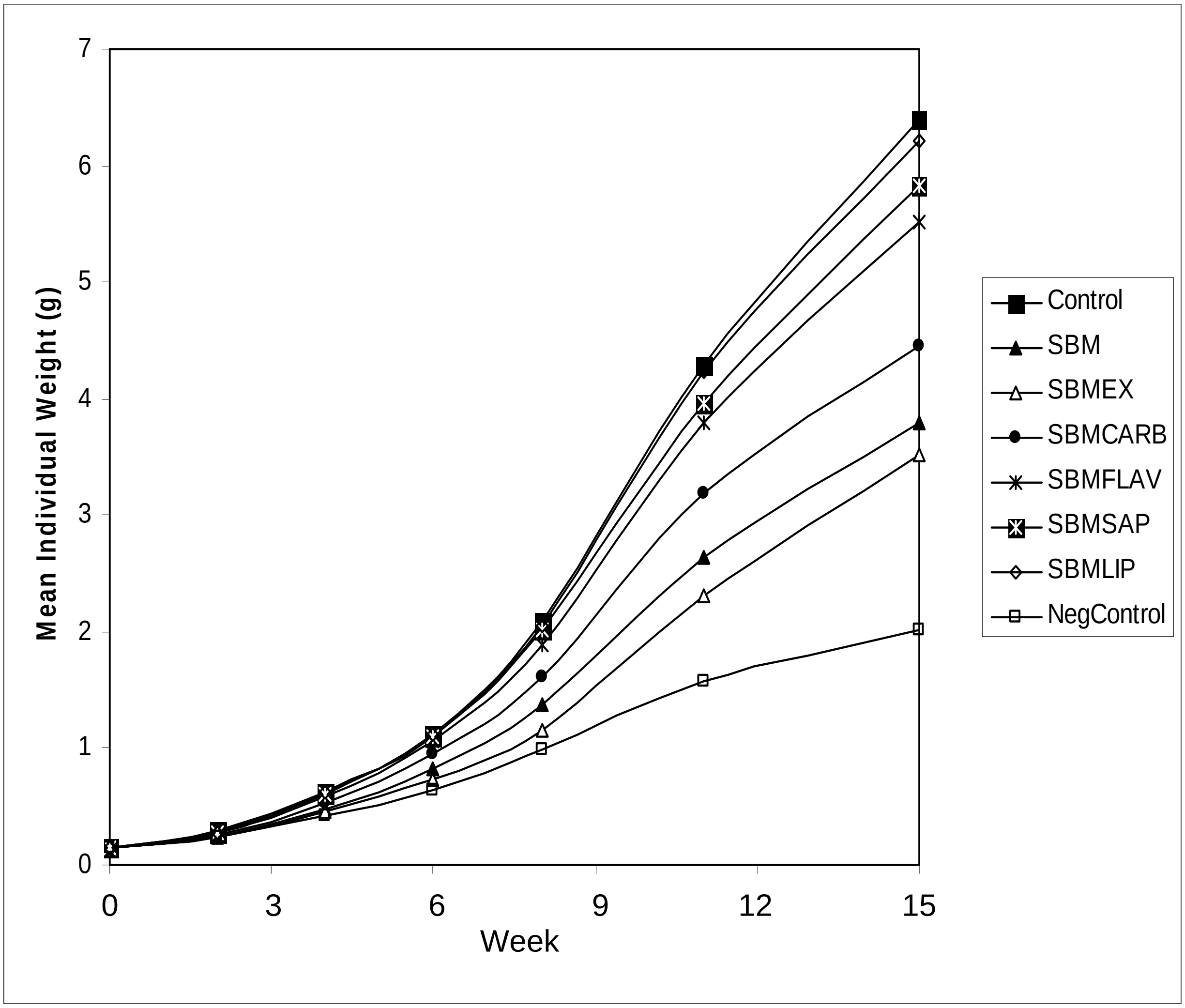

Figure 4: Mean Individual weights of rainbow trout over 15 weeks of feeding in year two study at Ohio State University. Feeds consisted of a Control, diets supplemented with SBM, SBM after extraction (SBMEX), SBM carbohydrates (SBMCARB), SBM flavonoids (SBMFLAV), SBM saponins (SBMSAP), SBM lipids (SBMLIP) and a negative control (NegControl).

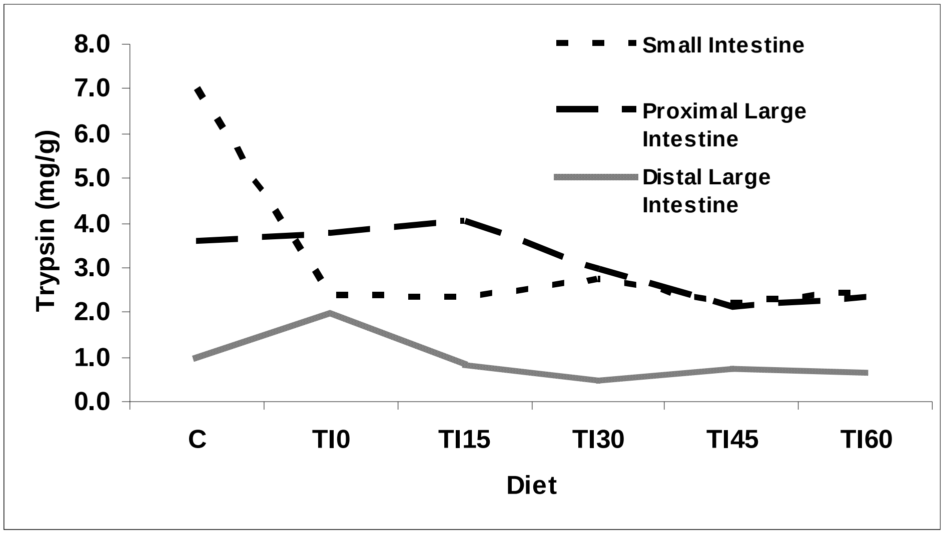

Figure 5: Residual trypsin concentrations from intestinal samples of fish fed varying levels of soybean trypsin inhibitors (TI) at Michigan State University. C = commercial control, TI0 = 0.0 g/kg TI (0% SBM equivalent), TI15 = 0.97 g/kg TI (15% SBM equivalent), TI30 = 1.95 g/kg TI (30% SBM equivalent), TI45 = 2.92 g/kg TI (45% SBM equivalent), TI60 = 3.90 g/kg TI (60% SBM equivalent).

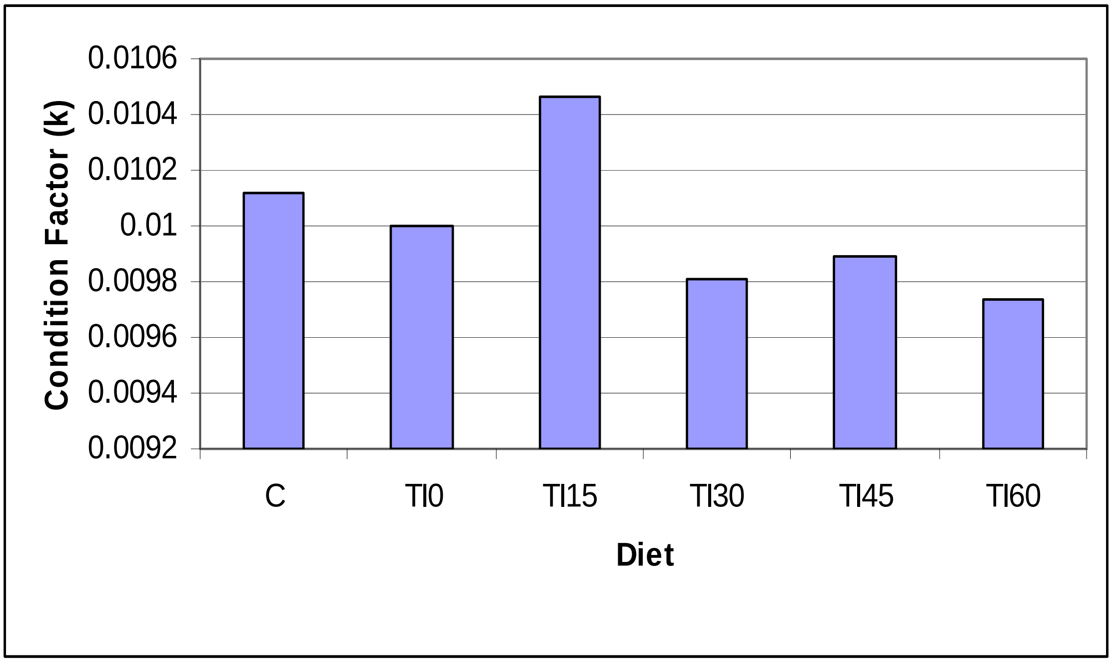

Figure 6: Condition factor (k = weight / length^3) for juvenile Atlantic salmon fed varying levels of soybean trypsin inhibitors at Michigan State University. C = commercial control, TI0 = 0.0 g/kg TI (0% SBM equivalent), TI15 = 0.97 g/kg TI (15% SBM equivalent), TI30 = 1.95 g/kg TI (30% SBM equivalent), TI45 = 2.92 g/kg TI (45% SBM equivalent), TI60 = 3.90 g/kg TI (60% SBM equivalent).

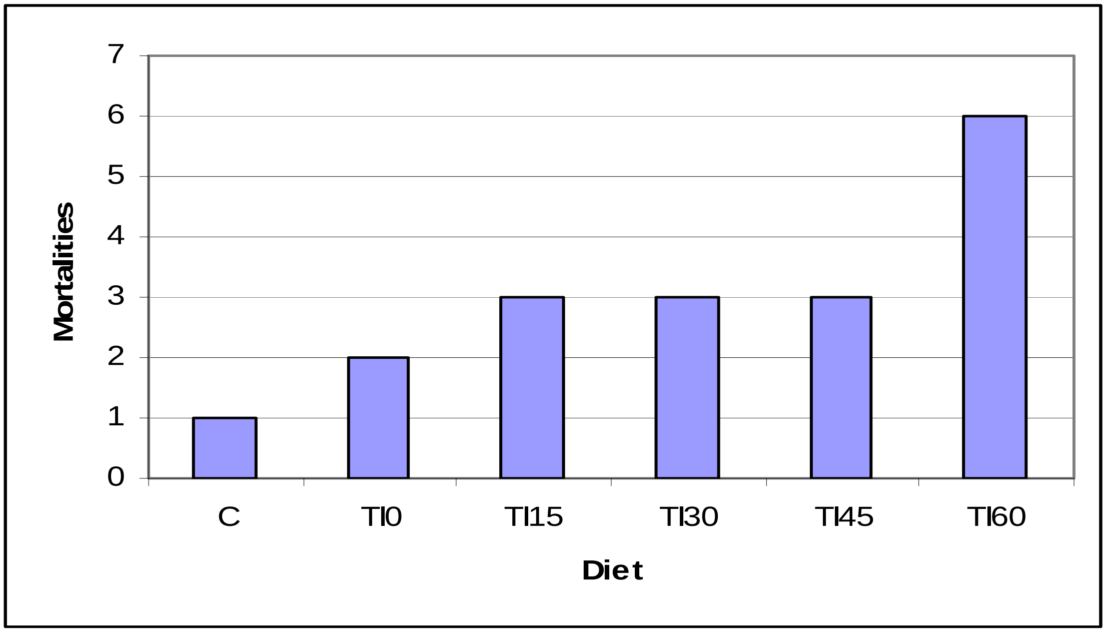

Figure 7: Mortalities of juvenile Atlantic salmon fed varying levels of soybean trypsin inhibitors at Michigan State University. C = commercial control, TI0 = 0.0 g/kg TI (0% SBM equivalent), TI15 = 0.97 g/kg TI (15% SBM equivalent), TI30 = 1.95 g/kg TI (30% SBM equivalent), TI45 = 2.92 g/kg TI (45% SBM equivalent), TI60 = 3.90 g/kg TI (60% SBM equivalent).

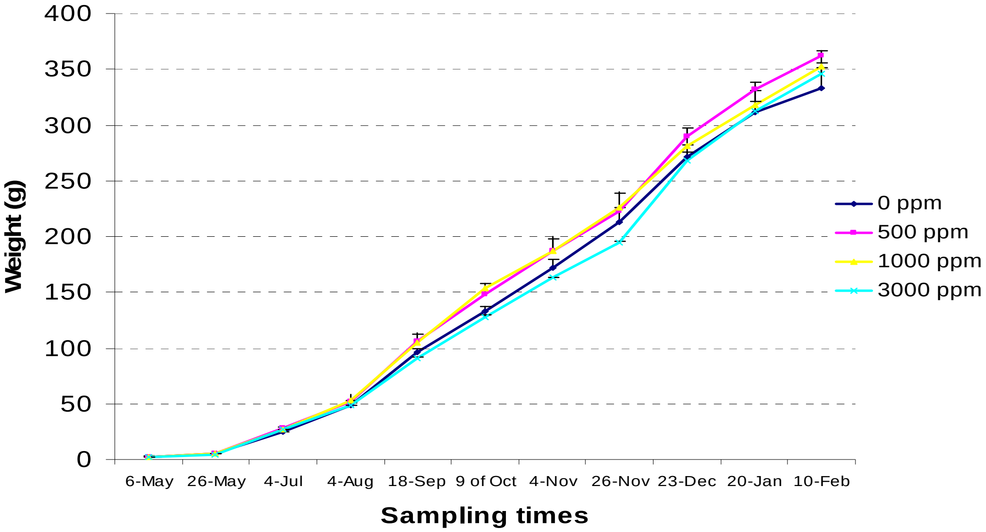

Figure 8: Average weight (g) of rainbow trout fed soy genistein at the University of Wisconsin. Data are based on three tanks per treatment.

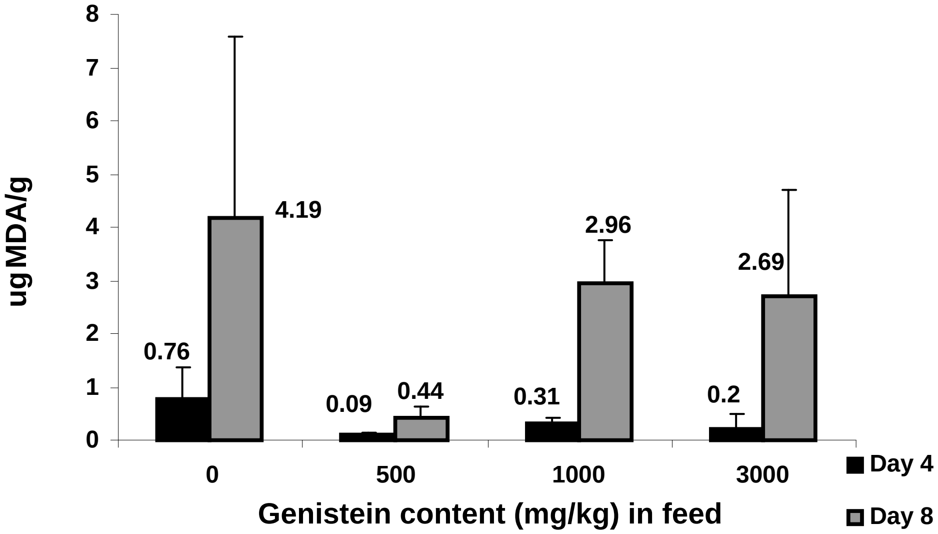

Figure 9: TBARS levels during refrigerated storage in fillets from rainbow trout fed varying levels of soy genistein at the University of Wisconsin. Analysis conducted by researchers at the University of Maine.

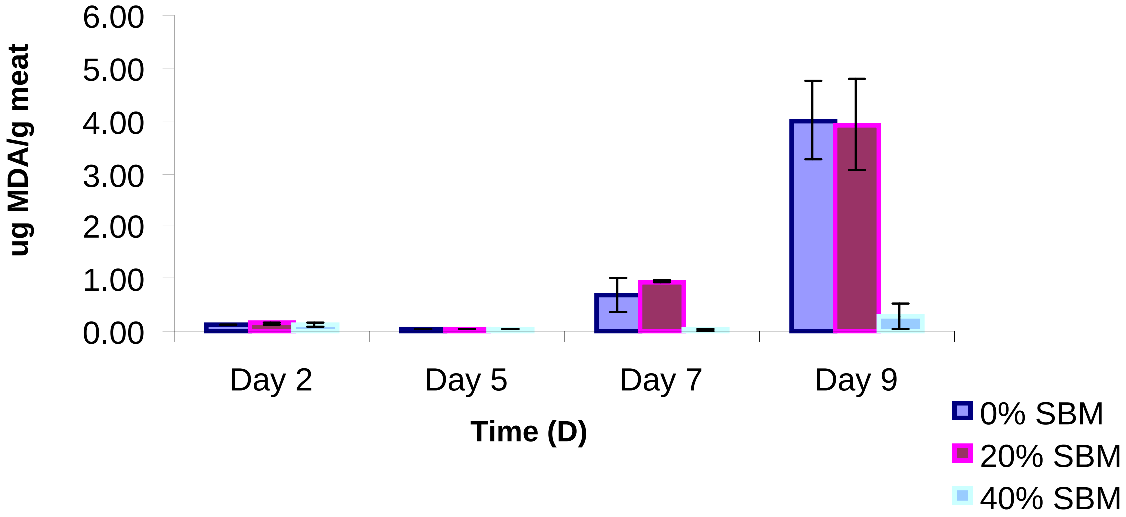

Figure 10: TBARS levels during refrigerated storage in fillets from rainbow trout fed varying levels of SBM at the University of Idaho. Analysis conducted by researchers at the University of Maine.

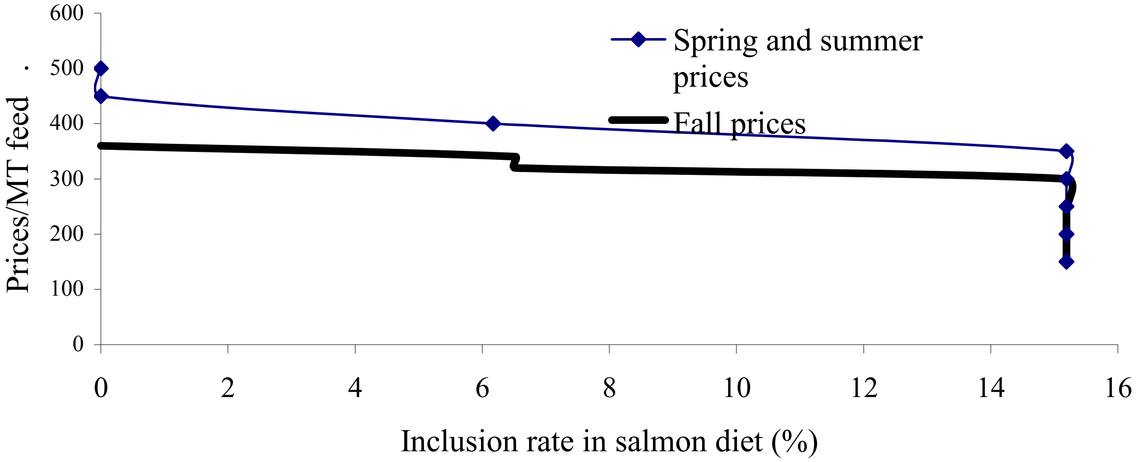

Figure 11: Normative demand function, as developed by researchers at Kentucky State University, for soybean meal used in least-cost Atlantic salmon diets with respect to other feed ingredient prices for spring, summer, and fall of 2004.

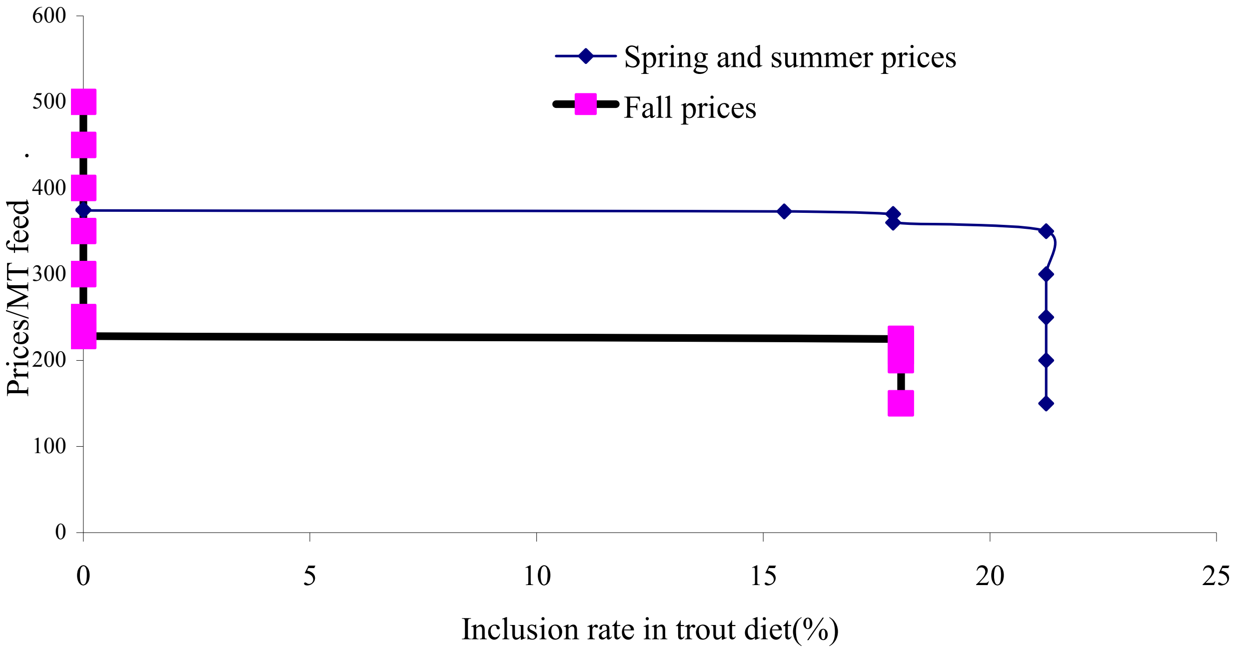

Figure 12: Normative demand function, as developed by researchers at Kentucky State University, for soybean meal used in least-cost rainbow trout diets with respect to other feed ingredient prices for spring, summer, and fall of 2004.

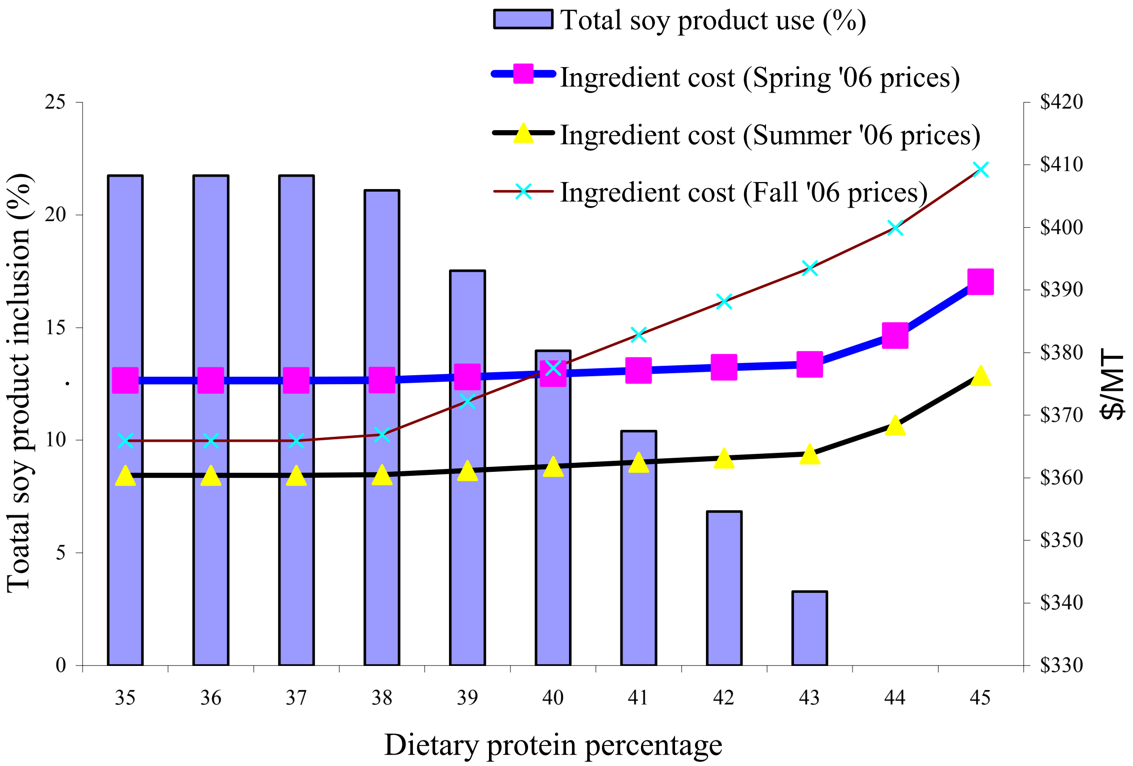

Figure 13: Effects of changing minimum protein requirements for rainbow trout diets on least-cost feed composition and ingredient cost ($/MT) with respect to 2006 prices.

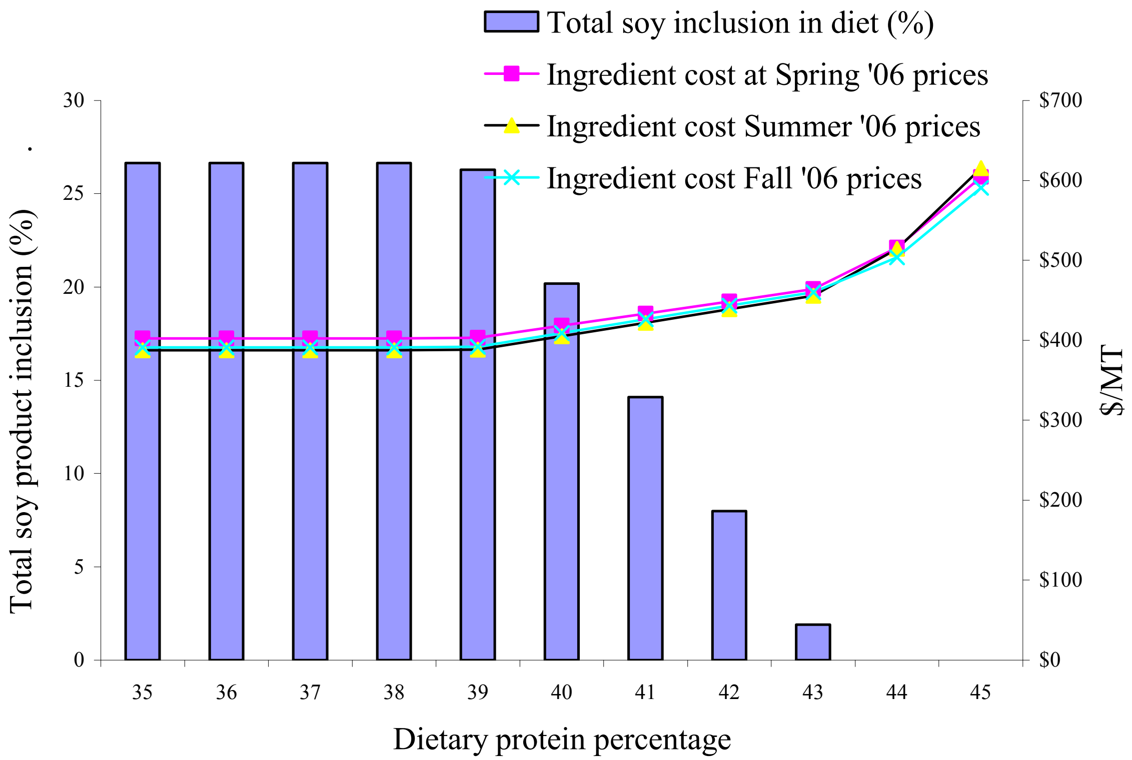

Figure 14: Effects of changing minimum protein requirements for Atlantic salmon diets on least-cost feed composition and ingredient cost ($/MT) with respect to 2006 prices.

List of suggested readings can be found at:

http://www.agbios.com/cstudies.php?book=FSA&ev=GTS&chapter=Preface

http://www.soyaqua.org

http://www.soymeal.org