Effect of Feeding Soy Products with Varying Trypsin Inhibitor Activities on Growth of Shrimp[1]

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Six isonitrogenous, isocaloric diets containing commercially defatted, toasted and lightly toasted soy flours (SF) (diets 1 and 2) and four soy protein concentrates (SPC) (diets 3–6) as replacements for 40% of animal protein were fed to satiation to juvenile shrimp (*Penaeus vannamei*) for 10 weeks. The SPCs used in diets 3 and 5 were chemically modified products with reduced trypsin inhibitor (TI) content. The chemical modification of SF in diet 2, which resulted in an SPC for diet 3, and of SPC in diet 4 consisted of heating at 70°C for 1 hr with 50 mM $Na_2S_2O_5$, followed by dialysis to remove salt residues. To keep all diets isocaloric, cornstarch was added to replace the oligosaccharides lost during processing to an SPC. The TI contents, in mg TI/g diet, were 0.77, 6.14, 0.64, 1.40, 0.92 and 1.72 for diets 1–6, respectively. Shrimp fed lightly toasted SF had the highest weight gain, which was significantly higher than shrimp fed SPC diets 4, 5 and 6, but not significantly higher than shrimp fed diets 1 and 3. No significant difference was observed in survival rates. Shrimp fed diet 3 (with lowest TI) had the highest body percentages of crude protein, while toasted soy flour diet 1, also with low TI, had the lowest content of this constituent. In general, a high body protein reflects good health of the animal and excellent utilization of the feed. At the replacement levels of soy evaluated, TI content did not affect overall weight gain.

KEY WORDS: Chemical inactivation, growth, shrimp, soybean, trypsin inhibitors.

A goal of fish nutritionists in the semi-intensive and intensive culture systems of the aquaculture industry is to partially or entirely replace expensive, less readily available fish meal protein with a less expensive plant protein source. Among all the plant sources evaluated, soybean meal has been the most widely used. Soybean meal has a favorable amino acid profile as compared with other plant sources and is consistently available at a cost considerably less than fish meal or squid meal.

Feeding raw soybean meal to different species of animals inhibits growth, reduces protein digestibility, causes pancreatic enlargement, stimulates hypersecretion of pancreatic enzymes and causes adverse physiological effects (1–3). This has generally been attributed to the deleterious effects of trypsin inhibitors (TIs). In comparison with various mammalian species, trout proteases are the most sensitive among the pancreatic proteases to TIs, where inhibition of trout enzymes is 15 times greater than that of the same amount of human enzymes (4). The Kunitz type soy TI caused reduced intestinal trypsin activity when fed to rainbow trout and caused increased fecal excretion of protein and lipids (5).

Of the cultured fish and shellfish species, marine shrimp represent the largest and fastest growing aquaculture enterprise in the world (6). Little published information is available on the impact of TIs in soy feeds used in shrimp diets. Akiyama (7) reviewed growth studies in which soybean meal replaced fish meal in shrimp feeds. He indicated that marine shrimp digest soy protein with a TI content ranging from 1.6–3.7 mg/g diet very efficiently. Gates and Travis (8) found that the proteolytic enzymes isolated from the digestive gland of white shrimp (*Penaeus setiferus*) will readily complex with soybean TI. In the research of Van Wormhoudt *et al.* (9), a fish meal diet supplemented with 3.5% soy TI and 10% squid meal was fed to shrimp (*Penaeus japonicus*) for 28 days. Growth of shrimp in this time period was 123% for those fed fish meal alone, 142% for those fed the mixed diet containing TI and 217% for those fed combined fishmeal and squid meal. The added TI abolished the stimulating effect of the squid meal on growth but the combination of all three proteins still surpassed shrimp fed only fish meal. These authors found hypertrophy of the pancreas and hyperactivity of the hepatopancreas to generate trypsin in shrimp fed the TI-supplemented diet.

Use of a predominately soy-based shrimp feed is limited by the lack of knowledge available on shrimp nutrition. According to Lim and Dominy (10), shrimp (*Penaeus vannamei*) fed diets in which up to 40% of marine animal protein is replaced with toasted, defatted soy flour showed weight gains comparable to shrimp fed 100% marine animal protein diets. However, when soy flour diets totally replaced the marine animal protein, growth response and nutritive value of the diet decreased significantly. This decrease was partly attributed to rejection of the feed by shrimp. Similar findings were observed by Piedad-Pascual *et al.* (11), who replaced up to 55% of a crude, animal-based protein diet with toasted, defatted soybean meal to feed shrimp (*Penaeus monodon*).

Basic to the incorporation of even larger amounts of soy as a replacement of animal proteins, more research is needed to better understand the utilization of various soy products by shrimp. Our major objective was to determine the growth response of shrimp (*P. vannamei*) fed various diets containing soy products and to determine whether the endogenous TIs in soybeans adversely affect growth.

EXPERIMENTAL PROCEDURES

Materials. Soy products evaluated consisted of a commercially toasted, dehulled, defatted soy flour (TSF) a commercial, lightly toasted, defatted soy flour, Soyafluff (SF), a soy protein concentrate prepared by an acid-washing process, Promax 70 (SPC-A); and a soy protein concentrate prepared by an ethanol-washing procedure, Promosoy 100, (SPC-E), supplied by Central Soya Company, Inc. (Fort Wayne, IN). Nitrogen solubility index (NSI) of these products were <10, 55, 28 and 9, respectively. To chemically inactivate soybean protease inhibitors, SF and SPC-A were each treated with 50 mM sodium metabisulfite ($Na_2S_2O_5$) according to the procedure of Sessa and Nelsen (12), as outlined in Figure 1. The three-day dialysis against water removed low molecular weight constituents, including oligosaccharides (see analyses in Results and Dis-

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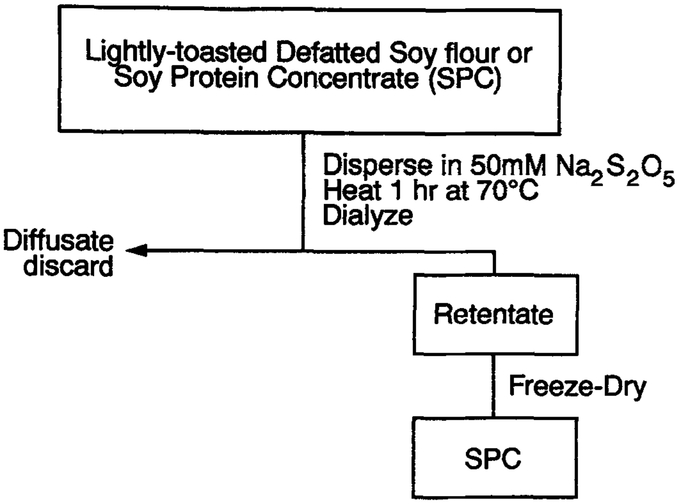

FIG. 1. Processing scheme for chemical inactivation of soybean trypsin inhibitors.

cussion section). Therefore, the SF treatment generated a soy protein concentrate based on protein and carbohydrate contents. NSI of the treated SF and SPC-A samples were 17 and 12, respectively.

Six isonitrogenous, isocaloric diets containing TSF and SF (diets 1 and 2) and four SPCs (diets 3–6) were each used as a replacement for 40% of animal protein, which consisted of 42.8% anchovy fish meal, 41.3% shrimp head meal and 15.9% squid meal. The soy protein concentrates used in diets 3 and 5 were chemically modified (see Fig. 1) SF and SPC-A and are designated as MSF and MSPC-A, respectively. Designation of diets 1–6, therefore, are TSF, SF, MSF, SPC-A, MSPC-A and SPC-E. Cornstarch was added to replace the oligosaccharides lost during processing.

Methods. All diets were calculated to contain approximately 35% crude protein and 3450 kcal/kg metabolizable energy and were formulated and prepared in accordance with the procedure of Lim and Dominy (10). Composition of the experimental soy-based diets is given in Table 1. The feeding experiments were performed on juvenile *P. vannamei* stocked at a density of 15 shrimp to each of 24 flow-through (0.85 L/min) 55-L glass aquaria, where each diet was fed to shrimp to satiation in four replicate aquaria six times daily for 10 weeks (10).

Analyses. All diets were analyzed for moisture, protein, fat, fiber, ash, phosphorus, potassium, calcium, TI and urease by approved AACC methods (13). Mineral analyses were performed with a Varian AA6 atomic absorption spectrophotometer (Varian Associates, Palo Alto, CA). Available lysine was analyzed by the method of Carpenter (14). Residual sulfite was assayed enzymatically with sulfite oxidase and nicotinamide adenine dinucleotide peroxidase according to the procedure outlined by Sessa *et al.* (15). All analyses were done in triplicate.

Body moisture content and composition of shrimp fed the soy-based diets were determined in triplicate by methods described by Lim and Dominy (10). Except for the diet compositions, all data were subjected to analyses of variance and Tukey's test to determine the differences between the treatment means (16). Results were considered significant at the 0.05 probability level.

RESULTS AND DISCUSSION

Analyses. An analysis of each of the six soy-based shrimp diets is given in Table 2. In diets 3 and 5, designated MSF and MSPC-A, the $Na_2S_2O_5$ treatment reduced the TI activity of SF diet by 89.6% and the activity of the SPC-A diet by 27.8%. The range of TI in the diets varied from 0.64 mg/g diet for MSF to 6.14 mg/g diet for SF. This range was much broader than that in a previous study (7).

Based on a soy product composition study, the crude carbohydrate content of MSF was measured as 12.8% with glucose as a standard. This compared favorably with the soy protein concentrates—16.9% for SPC-A, 14.9% for MSPC-A and 12.2% for SPC-E. Also, the crude protein

TABLE 1

Composition of Experimental Diets Containing Different Soy Products

	Percent in diet ("as is")[a]					
Ingredient	1. TSF	2. SF	3. MSF	4. SPC-A	5. MSPC-A	6. SPC-E
Soy product	28.00	26.34	21.37	21.04	20.52	20.33
Animal protein[b]	39.70	39.70	39.70	39.70	39.70	39.70
Cornstarch	—	0.09	3.49	4.46	4.38	5.66
Soy oil	1.42	1.47	1.54	1.56	1.49	1.60
Dipotassium phosphate	0.92	1.22	2.44	2.44	2.61	1.68
Dicalcium phosphate	3.41	3.14	2.65	2.65	2.81	2.92
Calcium carbonate	—	0.28	0.46	0.44	0.39	0.21
Celite (filler)	—	1.21	1.80	1.16	1.55	1.35
Others[c]	26.55	26.55	26.55	26.55	26.55	26.55

[a]Diet symbols: Toasted soy flour, TSF; lightly-toasted soy flour, SF; $Na_2S_2O_5$-modified soy flour, MSF; acid-washed soy protein concentrate, SPC-A; $Na_2S_2O_5$-modified SPC-A, MSPC-A; and ethanol-washed soy protein concentrate, SPC-E.

[b]Contains fish meal, 42.8%; shrimp waste meal, 41.3%; and squid meal, 15.9%.

[c]Contains wheat flour, 15.25 g; fish oil, 3.0 g; Kelvis (binder), 2.5 g sodium; hexametaphosphate, 1.0 g; cholesterol, 0.5 g; soybean lecithin, 1.0 g; vitamin mix, 2.0 g; and mineral mix, 1.0 g; each/100 g of diet. Mineral mix, modified BT_m salt mixture plus the following minerals (mg/kg diet): Zinc, 3.15; iodine, 4.8; selenium, 0.4; cobalt, 0.4. Vitamin mixture (mg/kg diet): Vitamins A, 80,000IU; D_3, 2000IU; E, 500IU; K, 20; niacin, 300; riboflavin, 60; pyridoxine, 57; thiamin, 60; pantothenic acid, 147; biotin, 2; folic acid, 20; B_{12}, 0.1; choline chloride, 3000; inositol, 200; and C, 500.

TABLE 2

Analysis of Soy-Based Shrimp Feed

	Percent in diet ("as is")[a]					
Analysis	1. TSF	2. SF	3. MSF	4. SPC-A	5. MSPC-A	6. SPC-E
Moisture	4.78	4.81	4.32	4.96	5.00	3.70
Crude protein	36.16	36.75	37.44	36.38	37.44	36.63
Crude fat	9.45	9.17	8.84	9.21	8.79	8.74
Crude fiber	6.76	7.02	7.46	7.83	7.69	7.77
Ash	17.03	16.94	16.88	17.00	15.53	16.88
Phosphorus	2.46	2.39	2.51	2.54	2.01	2.41
Calcium	4.25	4.05	4.15	3.90	3.55	4.35
Potassium	1.45	1.40	1.60	1.60	1.45	1.51
Available lysine	4.97	4.93	4.99	5.19	4.94	5.11
TI, mg/g	0.70	6.14	0.64	1.44	1.04	1.77
Urease, pH units	0.02	0.49	0.17	0.02	0.01	0.00

[a]As in Table 1.

TABLE 3

Growth Response and Feed Utilization of Shrimp Fed Soy-Based Diets

	Diet[a]					
Meal values	1. TSF	2. SF	3. MSF	4. SPC-A	5. MSPC-A	6. SPC-E
Weight gain (g)[b]	6.32[de]	6.56[d]	6.28[de]	5.68[ef]	5.38[f]	5.49[f]
Survival (%)[b]	93.4[d]	90.4[d]	86.7[d]	83.6[d]	91.7[d]	90.0[d]
Feed conversion[b,c]	1.83[de]	1.68[d]	1.96[de]	2.11[de]	2.38[e]	2.28[e]

[a]As in Table 1.

[b]Row values followed by the same letter are not significantly different ($P \leqslant 0.05$).

[c]As g air-dried weight of feed/g wet weight gain.

content was 63.56% for MSF *vs.* 64.56%, 66.18% and 66.81% for SPC-A, MSPC-A and SPC-E, respectively. Therefore, the processing scheme (Fig. 1) used in this study not only inactivated the TIs but also removed the oligosaccharides to generate a low TI soy protein concentrate.

The other uncontrolled variables were urease and available lysine. Metabisulfite treatment of SF decreased the level of urease from 0.49 to 0.17 in the mixed diet, which represents a 65% reduction, while the same treatment gave a 50% reduction in the SPC-A diet. Therefore, moderate heating of soy products in combination with sodium metabisulfite effectively reduced urease. Available lysine tended to be higher in the two SPCs, SPC-A and SPC-E, for diets 4 and 6. Values for available lysine in TSF and MSF did not differ from that in SF. Therefore, neither toasting nor treatment of SF with $Na_2S_2O_5$ affected availability of lysine. The cause for the 5% decrease in available lysine for MSPC-A of diet 5 is unexplainable.

Growth response. The growth response and feed utilization of shrimp fed the soy-based diets are given in Table 3. Shrimp fed lightly toasted SF had the highest weight gain in grams, 6.56 ± 0.34, which was significantly ($P < 0.05$) higher than shrimp fed SPC diets 4 (5.68 ± 0.34), 5 (5.38 ± 0.52) and 6 (5.49 ± 0.26), but not significantly higher than shrimp fed diets 1 (6.32 ± 0.36) and 3 (6.28 ± 0.64). No significant difference was observed for survival rate among shrimp fed the six diets. At the replacement levels of soy evaluated, TI content did not affect overall weight gain. Also, the commercial soy protein concentrates used in diets 4 and 6 and the chemically modified concentrate used in diet 5 were not as well utilized as all other soy-based diets evaluated.

Body composition. Body composition studies are performed in order to evaluate the overall health of the animal and assess its ability to utilize the feed. Body composition of shrimp fed the six soy-based diets are given in Table 4. Body moisture percentage was highest ($P < 0.05$) for shrimp fed SPC-E diet 6, while moisture percentages of shrimp fed all other diets were essentially the same. The reason for this observation is unexplainable. Shrimp fed MSF diet 3, with lowest TI content of all six diets, had highest body percentages of protein. In general, a high body protein reflects good health and excellent utilization of the feed. The protein content of the shrimp fed TSF diet 1 was significantly lower than that of the shrimp fed all other diets. Inactivation of TI with low to moderate heat may prove more beneficial for shrimp growth than the high heats used in the toasting procedure. Akiyama (7) observed that soybean meal extruded at 140°C gave slightly lower feed conversion than meal extruded at 170°C. However, no body composition data were given, so we cannot conclude whether the lower heat used in processing soy will reflect in better utilization of soy by the shrimp. Heat processing of soy needs to be further investigated to determine the extent needed to achieve best overall feed digestibility and utilization.

Many fish species besides trout (4) are sensitive to TIs in soybeans, including catfish, Nile tilapia and carp. Wilson and Poe (17) showed affects of feeding soy meals with varying trypsin inhibitor activities on growth of fingerling channel catfish. They observed that catfish weight

TABLE 4

Body Composition (Dry Matter) of Shrimp Fed Soy-Based Diets

	Percent nutrient[b]			
Diet[a]	Moisture	Protein	Fat	Ash
1. TSF	73.9[e]	75.6[g]	5.3[e]	14.4[d]
2. SF	74.7[de]	77.1[f]	5.2[f]	13.2[e]
3. MSF	73.7[e]	77.8[d]	5.4[d]	13.3[e]
4. SPC-A	74.2[e]	77.4[e]	4.5[h]	12.6[f]
5. MSPC-A	73.8[c]	77.3[ef]	4.6[g]	14.3[d]
6. SPC-E	75.2[d]	77.0[f]	5.4[d]	14.3[d]

[a]As in Table 1.

[b]Column values followed by the same letter are not significantly different ($P \leqslant 0.05$).

gains were best when TI content of soy was limited to 2.2 mg TI/g diet when fed a 25% crude protein diet and to 3.2 mg TI/g diet for a 35% crude protein diet. Amounts above those values yielded reduced weight gains. The best growth rate was achieved with soy products where about 85% of the TI was destroyed. Wee and Shu (18) showed that TI levels in soybeans higher than 1.6 mg/g diet retard growth of Nile tilapia, but that these fish grew well on a diet with 0.6 mg TI/g. The best growth rate was achieved by feeding soy products where 80% of the TI was inactivated. Abel *et al.* (19) found that either thermic or hydrothermic treatments of full-fat soybeans proved to effectively reduce TI activities. Yet, carp fed these treated products at best showed only 60–65% of the potential growth when compared to a fish meal control diet. These authors observed increased levels of body fat in the carp fed diets containing full-fat soybeans. Supplementation of the soy-based feeds with methionine, lysine and threonine improved the nutritional quality of the soy-based carp feeds. Shrimp apparently have the capacity to compensate for TI activity (9) *via* a gastrointestinal hormone action that generates gastrin-like peptides to stimulate higher trypsin production. Excessive heating can adversely affect the protein nutritive value of fish feeds (20). The excessive heat can cause formation of new, enzyme-resistant linkages within the protein molecule, which reduces its digestibility and the biological availability of some of the constituent amino acids (21). The available lysine values found in our current investigation (Table 2) do not reflect reduction based on heat treatment. The moderate-heat $Na_2S_2O_5$ treatment described in this study and elsewhere (12,15) may prove to be more beneficial for use of soy in feeds for the more sensitive fish species (4,17–19). Future investigation will involve evaluating soy-based feeds for shrimp that are produced from $Na_2S_2O_5$-treated whole beans.

Little information is available on the effects of soy oligosaccharides on fish and shrimp. Arnesen *et al.* (22) attributed the restriction in utilization of nutrients by Atlantic salmon to soy carbohydrates. However, Van den Ingh *et al.* (23) found that addition of soybean oligosaccharides to a fish meal-based diet did not cause morphological changes in the intestinal tract in Atlantic salmon. The only indication of diminished function of the distal intestine in the fish fed diets with full-fat soybean meal was the increased water content of the feces. The results in our current feeding study with soy protein concentrates did not demonstrate that soy oligosaccharides are a problem. The soy flour samples with higher content of soy oligosaccharides appeared to give higher weight gains than the concentrates. To better establish this point we will investigate the specific oligosaccharide contents of the shrimp feeds in our future investigations.

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