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Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*

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Abstract

The effect of partially replacing fish meal in seabass diets with different soybean meal (SBM) products on the growth and apparent protein digestibility was studied. Five isonitrogenous and isocaloric diets were formulated to contain all fish meal (control diet), 21.0% solvent extracted SBM, 27% extruded full-fat SBM, 28.5% steamed full-fat SBM and 27.5% soaked raw full-fat SBM as substitutes for 15% fish meal in the control diet. Each diet was fed to three replicate groups of juvenile seabass with an average initial weight of 1.26–1.37 g over a period of 10 weeks. The fish were contained in flow-through and aerated aquaria. Fish fed the control diet grew significantly better than fish fed extruded full-fat SBM, steamed full-fat SBM or soaked raw full-fat SBM diets, but not significantly different from that of fish fed the solvent-extracted SBM diet. Feed efficiency and survival did not differ significantly among treatments except for fish fed the soaked raw full-fat SBM diet. Fish fed the soaked raw full-fat SBM had significantly lower weight gain, feed efficiency, protein efficiency and survival. Fish fed this diet also showed some histological changes in their gastrointestinal tract. The apparent protein digestibility of diets containing all fish meal, solvent-extracted, extruded, steamed and soaked raw full-fat SBMs were 92.8, 94.2, 92.3, 94.4 and 73.7%, respectively. The lower growth obtained in fish fed the extruded or steamed full-fat SBM diets as compared to that of the control fish meal diet could not be attributed to protein digestibility, but it could be due to the lower feed intake during the first two weeks suggesting that palatability was possibly a factor. Soaked raw full-fat SBM is a poor protein source for seabass diet. Solvent-extracted SBM is more palatable for small seabass (1.30–3.50 g)

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than extruded or steamed full-fat SBM. Thus, this study suggests that, if palatability of the diet can be improved, 37.5% of protein from fish meal or 15% fish meal in diet for seabass can be replaced by solvent-extracted SBM, extruded full-fat SBM or steamed full-fat SBM. © 1998 Elsevier Science B.V.

Keywords: Soybean meal; Asian seabass; Plant protein; Protein digestibility; Histology

1. Introduction

Asian seabass have been cultured in many Asian countries such as Hong Kong, Indonesia, Malaysia, Philippines, Singapore, Taiwan and Thailand for some 20 years. It is a fast growing fish and can grow well in water of high turbidity and varying salinity. It has a white flesh which is preferred by consumers and usually has a good market price. However, as in most cultured high-valued species, feed constitutes a significant portion of the operating cost.

Fish meal has traditionally been a major ingredient in fish feeds because of its protein quality and palatability (Lovell, 1984). However, increasing demand, high cost and uncertain availability of fish meal (Barlow, 1989) have resulted in nutritionists studying alternative sources, particularly plant proteins to replace fish meal in the diet of a number of freshwater and marine fish species (Dabrowski et al., 1989; Olli et al., 1989; Wee and Shu, 1989; Shiau et al., 1990; Lovell, 1991; Quartararo et al., 1991; Watanabe et al., 1992; Pongmaneerat and Watanabe, 1993; Robinson and Li, 1994). Among plant proteins, soybean meal (SBM) is the most promising candidate for partial or total replacement of fish meal in fish diets. However, very little is known about the feasibility of using various soybean products as a source of dietary protein in feed formulations for seabass. This study was to ascertain whether commercially available solvent-extracted SBM, extruded full-fat SBM, farm-made steamed full-fat SBM, and soaked raw full-fat SBM could partially replace fish meal in practical diets for seabass.

2. Materials and methods

2.1. Soybean products

Soybean products evaluated were solvent-extracted SBM (obtained commercially); extruded full-fat SBM (obtained commercially); steamed full-fat SBM (whole raw soybeans steamed for 1 h, cooled, ground and dried in an oven at 50°C for 8 h then ground again); and soaked raw full-fat SBM (whole raw soybeans soaked in freshwater for 24 h, strained, ground and dried in an oven at 50°C for 8 h then ground again).

2.2. Experiment 1

Five isonitrogenous and isocaloric diets were formulated to contain 40% crude protein, 490 kcal of GE/100 g of diet and all the essential nutrients necessary for

satisfactory growth of seabass (Boonyaratpalin, 1991). Diet composition is shown in Table 1. The diets were processed by blending the dry ingredients into a homogenous mixture, then adding oil and 30% water. The moist feed mixture was passed through a meat grinder, broken into pellets, dried in an air-forced oven at 60°C for 4 h to reduce the moisture to approximately 10%. All feeds were packed in plastic bags and stored at room temperature.

Thirty day-old, hatchery reared seabass (*Lates calcarifer*) fry obtained from the National Institute of Coastal Aquaculture were acclimated to laboratory conditions and fed a commercial shrimp pelleted feed until individual weights reached approximately 1.3 g. Selected individuals were randomly distributed into 15, 45-l aquaria at the rate of 20 fish per aquarium. The aquaria were equipped with a flow-through water system and continuous aeration. Fish in three aquaria were assigned to each of the experimental diets and fed to satiation twice daily. Bi-weekly feed consumption was recorded. Total weight and number of fish in each aquarium were determined every 2 weeks. From week five to 10, the aquaria were cleaned 1 h after the last feeding to remove remaining diet or feces every day. The feces collector designed by the Tokyo University of Fisheries (Ogino et al., 1973) was installed instead of the normal siphoning column.

Table 1
Composition of diets containing various soybean products as substitutes for 15% fish meal

Ingredient	Percent in diet				
	1	2	3	4	5
Fish meal	40	25	25	25	25
Solvent extracted SBM	–	21.0	–	–	–
Extruded full-fat SBM	–	–	27.0	–	–
Steamed full-fat SBM	–	–	–	28.5	–
Soaked raw full-fat SBM	–	–	–	–	27.5
Oil[a]	8.0	9.0	5.5	3.5	3.5
Rice flour	13.48	6.48	3.98	4.48	5.48
Shrimp head meal	10	10	10	10	10
Wheat gluten	8	8	8	8	8
Wheat flour	15	15	15	15	15
Vitamin mix[b]	1.2	1.2	1.2	1.2	1.2
Mineral mix[c]	3.8	3.8	3.8	3.8	3.8
BHT	0.02	0.02	0.02	0.02	0.02
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Determined nutrients (% dry matter basis)					
Crude protein	39.51	39.64	39.03	40.31	39.66
Crude fat	14.25	13.24	14.99	13.76	13.82
Ash	14.14	13.65	13.15	13.17	12.44
Chromic oxide	0.55	0.57	0.56	0.54	0.57

[a] Mixture of three parts fish oil and two parts soybean oil.
[b] Vitamin mix (mg/kg diet): thiamin HCl, 60; riboflavin, 100; pyridoxine HCl, 40; choline chloride, 5000; niacin, 400; Ca-pantothenate, 100; ascorbic acid, 500; para-amino benzoic acid, 50; inositol, 2000; biotin, 6; folic acid, 15; vitamin B_{12}, 0.1; menadione, 50; tocopheryl acetate, 100; vitamin A, 1.38; vitamin D3, 0.02.
[c] Mineral mix (g/kg diet) $CaHPO_4$, 8; $NaH_2PO_4 \cdot 2H_20$, 15; KH_2PO_4, 10; KCl, 5.

Each morning, the feces collector was released for feces collections. The feces were separated from the water and stored in a freezer before freeze-drying. The experimental feed and pooled feces samples were analyzed for protein (AOAC, 1980) and chromium (Furukawa and Tsukahara, 1966). The apparent digestibility of dietary protein was calculated according to Cho et al. (1985). At the end of the experiment, three fish from each aquarium were anesthetized with 100 mg/l of tricane methane sulfonate (MS-222). Tissues from internal organs such as the liver, kidney, anterior and posterior intestine were dissected and fixed in Bouin's fixative for 24 h. They were processed using standard histological methods (Humason, 1972). Samples were processed through an alcohol series and embedded in paraplast. Sections of 3–4 μm in thickness were cut and stained with hematoxylin and eosin. The sections were observed under a light microscope. Three sections of each organ per fish were examined for any pathological changes.

2.3. Experiment 2

To better understand the nutritive value of the experimental soybean products, the apparent protein digestibility and urease activities were studied. The apparent protein digestibility was determined by using reference and test diets (Wilson and Poe, 1985b). The composition of the reference diet is shown in Table 2. The test diets consisted of 70% reference diet and 30% of the test ingredient. Chromic oxide (0.5%) was used as an indicator. Seabass with an average body weight of about 10 g were stocked in 45-l aquarium at the rate of 25 fish each. Three replicate aquaria were randomly assigned to each of the experimental diets. Fish were conditioned to both aquarium conditions and the dietary regime for 5 days before feces collection began. Fish were fed to satiation twice daily. Feces was collected for 2 weeks and kept in a freezer before freeze-drying. Determination of protein and chromium contents in feed and feces was done as previously described. The apparent digestibility of soybean protein was calculated according to Cho et al. (1985). Urease activities were measured by the rise in pH (AOCS, 1993).

Table 2
Composition of the reference diet used for digestibility study in experiment 2

Ingredient	Percent
Fishmeal	40.00
Shrimp head meal	10.00
Wheat gluten	8.00
Wheat flour	15.00
Rice flour	12.98
Fish oil	8.00
BHT	0.02
Cr_2O_3 (50%)	1.00
Vitamin mixture[a]	1.20
Mineral mixture[a]	3.80

[a] See Table 1.

The data from each experiment were subjected to analysis of variance and Duncan's multiple range test to determine if the differences among means were statistically significant (Steel and Torrie, 1980).

3. Results

The growth response and feed utilization efficiency data are presented in Table 3. Fish fed diets 1, 2, 3 and 4 had similar growth patterns throughout the 10-week period, although fish fed the control diet (diet 1) was significantly larger ($P < 0.05$) than those fed diets 3 and 4. Weight gain of fish fed diet 2 was not significantly different ($P > 0.05$) from those of fish fed diets 1, 3 and 4. The growth rate of fish fed the soaked raw full-fat SBM diet (diet 5) was consistently lower throughout the 10-week period. By the end of week 2, fish fed this diet weighed significantly less than those fed other diets. By the end of week 10, fish fed diet 5 gained only 624.4% whereas the fish fed other diets had 2200% or higher increase in weight.

Total feed intake data reflected the weight gain data. Feed intake for fish fed the fish meal diet (diet 1) was highest although not significantly different ($P > 0.05$) from those of fish fed diets 2 and 3. Fish fed diet 5 consumed significantly ($P < 0.05$) less feed than fish fed the other diets. Total feed intake was not significantly different among fish fed diet 2, 3 and 4 ($P > 0.05$). During the first two weeks, feed intake for fish fed diet 3 and 4 was significantly lower than fish feed diet 1 ($P < 0.05$).

Fish fed diet 5 had the lowest ($P < 0.05$) survival. Mortality was observed after 2 weeks of feeding and increased gradually until the end of week 8. No mortality was observed in fish fed diets 1, 2, 3 and 4. Feed efficiency ratio (FER) and apparent protein digestibility followed the same trend and were lowest ($P < 0.05$) for diet 5. No significant differences ($P > 0.05$) were observed among the FER and the apparent

Table 3
Weight gain, feed efficiency, protein efficiency, apparent protein digestibility and feed intake of seabass fed diets containing different soybean meal products for 10 weeks[1]

	Diet				
	1	2	3	4	5
Initial weight (g)	1.27	1.27	1.26	1.26	1.26
Final weight (g)	34.91^{c}	$31.85^{b,c}$	29.22^{b}	29.07^{b}	9.19^{a}
Final weight gain (%)	2641.7^{c}	$2402.3^{b,c}$	2213.3^{b}	2201.4^{b}	624.4^{a}
Feed efficiency	1.18^{b}	1.18^{b}	1.09^{b}	1.13^{b}	0.53^{a}
Protein efficiency ratio	2.98^{c}	2.98^{c}	2.78^{b}	2.79^{b}	1.32^{a}
Survival (%)	100^{b}	100^{b}	100^{b}	100^{b}	87.5^{a}
Total feed intake (g/fish)	28.60^{c}	$25.86^{b,c}$	$25.73^{b,c}$	24.68^{b}	14.33^{a}
Feed intake-first 2 weeks (g/fish)	2.29^{d}	$2.14^{c,d}$	2.05^{c}	1.67^{b}	1.09^{a}
Protein digestibility (%)	92.8^{b}	94.2^{b}	92.3^{b}	94.4^{b}	73.7^{a}

[1]Means within the same row having different superscript are significantly different ($P < 0.05$).

Table 4
Urease activity, and protein digestibility of the tested soybean products[1]

Soybean product	Urease activity (pH change)	Protein digestibility (%)
Solvent extracted	0.061[b]	90.4[b]
Extruded full-fat	0.079[b]	93.2[b]
Steamed full-fat	0.000[b]	93.5[b]
Soaked raw full-fat	1.800[a]	21.4[a]

[1]Means within the same column having different superscript are significantly different at $P < 0.05$.

protein digestibility values of diets 1, 2, 3 and 4. The apparent protein digestibility values of diets 1, 2, 3 and 4 were high and ranged from 92.3% for diet 3 to 94.4% for diet 4.

Urease activities of the SBM products varied from a 0 to 1.8 pH unit change. Urease activities of solvent-extracted SBM and extruded full-fat SBM were 0.061 and 0.079, respectively. No pH change was detected in steamed full-fat SBM, while soaked raw full-fat SBM had the highest urease activity of 1.8.

The protein digestibility of the different SBM products varied from 21.4 to 93.5% (Table 4). Extruded full-fat SBM and steamed full-fat SBM had the highest values of 93.2 and 93.5%, respectively, which were not significantly different ($P > 0.05$) from the value of 90.4% for solvent-extracted SBM. The protein digestibility of soaked raw full-fat SBM was only 21.4% and significantly lower than the other SBM products ($P < 0.05$).

Histological studies showed that the digestive tract and glands of fish fed the all fish meal diet had normal structure. The liver and pancreas showed normal structure (Fig. 1). Fish fed diets containing solvent-extracted SBM, extruded full-fat SBM and steamed full-fat SBM showed normal liver and pancreatic tissues as in the control. Fish fed the soaked raw full-fat SBM diet exhibited atrophic changes and loss of normal structure of the pancreatic tissue. Decreasing zymogen granules in the cytoplasm of acinar cells of the pancreas was also observed (Fig. 2).

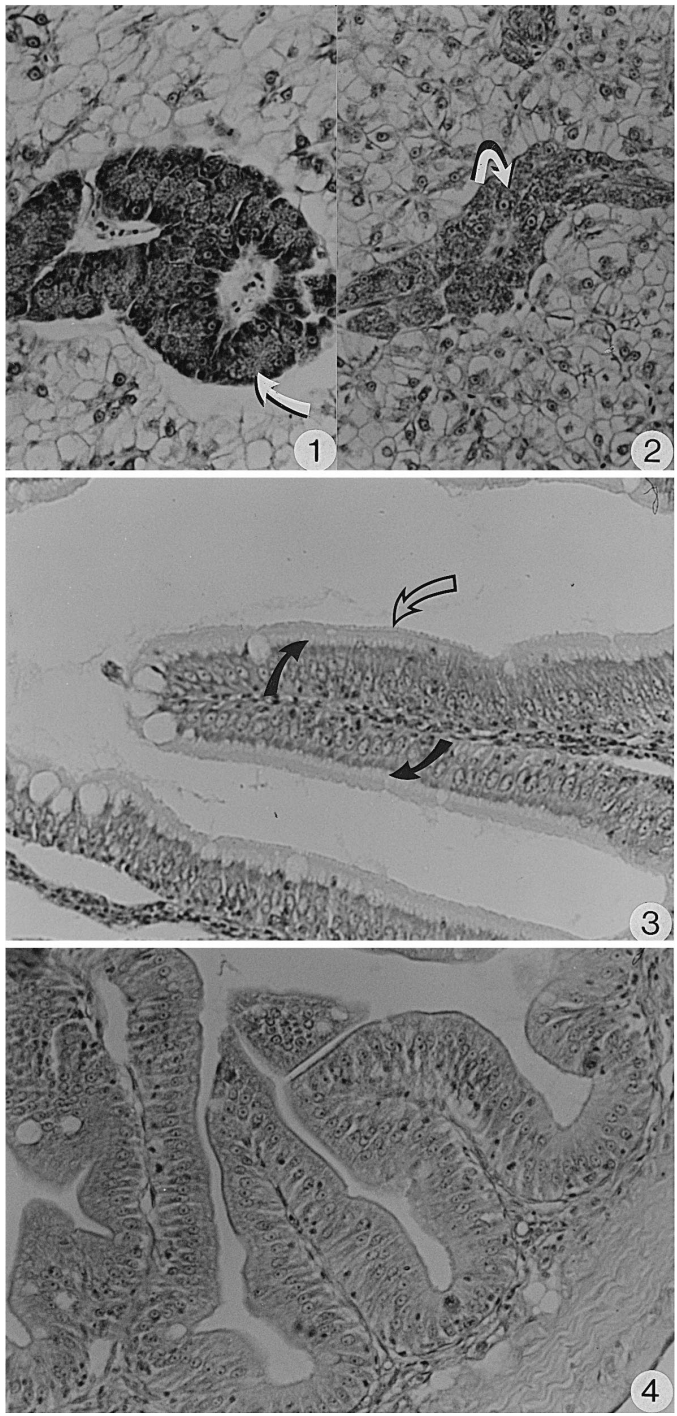
The anterior intestinal tissue of fish fed the all fish meal diet was normal. The mucosal epithelium had well-developed enterocytes with prominent microvilli, and a

Fig. 1. Seabass (*Lates calcarifer*) fed the all-fish meal diet. The pancreatic tissue shows normal cell structure and zymogen granules are clearly defined in the cytoplasm of acinar cells (arrow). (H&E, 390×).

Fig. 2. Seabass fed the diet containing soaked full-fat SBM. The pancreatic tissue exhibit atrophic changes, loss of normal cell structure and zymogen granules were poorly defined in the cytoplasm of acinar cell. (H&E, 390×).

Fig. 3. Seabass fed the all-fish meal diet. The mucosal epithelium of the anterior intestine showing normal structure. Note well developed enterocytes, containing numerous absorptive vacuoles (black arrow). Microvilli are prominent and clearly observed (clear arrow). (H&E, 390×).

Fig. 4. Seabass fed the diet containing soaked full-fat SBM. Number of absorptive vacuoles in the mucosal epithelium of the anterior intestine is decreased. Microvilli are not clearly observed. (H&E, 390×).


1
2
3
4

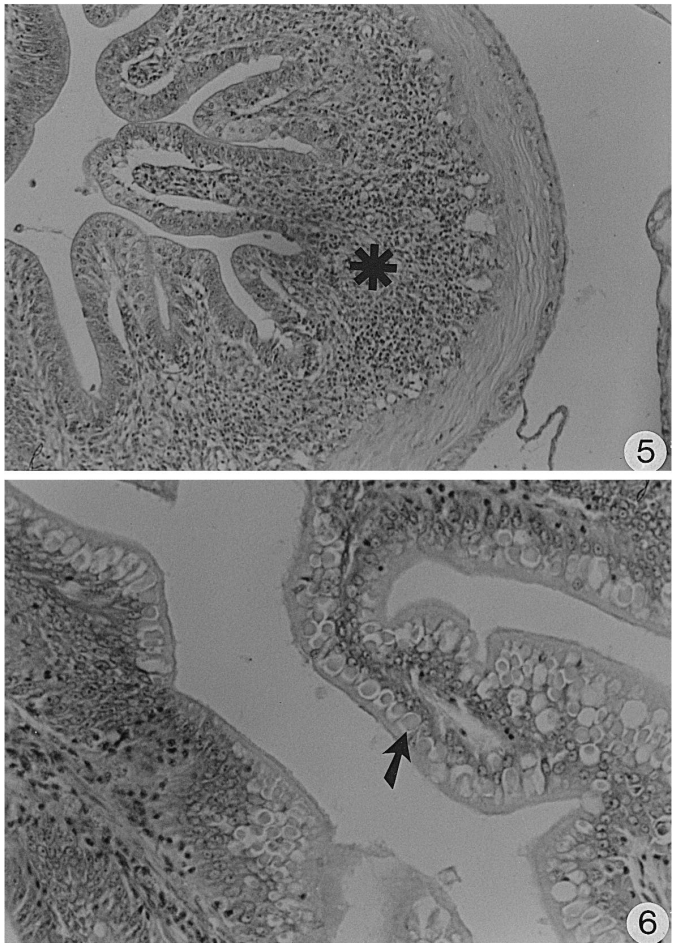


Fig. 5. The anterior intestine of the same fish in Fig. 4, shows hyperplasia of the lamina propia (asterisk). (H&E, 200×).

Fig. 6. Seabass fed the diet containing extruded SBM. The mucosal layer of the anterior intestine contains numerous vacuoles with eosinophilic inclusions (arrow). Microvilli are not clearly observed. (H&E, 390×).

large number of absorptive vacuoles were present in the cytoplasm. Goblet cells were regularly distributed among the mucosal epithelium (Fig. 3). Fish fed the diet containing the soaked raw full-fat SBM exhibited pronounced pathological changes of the intestinal epithelium. The mucosal epithelium had poorly developed enterocytes with decreased absorptive area (shortening of microvilli and decreasing absorptive vacuoles) (Fig. 4). Hyperplasia of the lamina propia was also observed (Fig. 5). The mucosal epithelial layers of the fish fed the extruded SBM diet had highly vacuolated enterocytes which contained eosinophilic inclusions in the vacuoles. A slight reduction of absorptive area (shortening of microvilli) was also observed (Fig. 6). Fish fed diets containing solvent-

extracted SBM and steamed full-fat SBM showed normal histology of anterior intestine but with a slight decrease in absorptive vacuoles and shortening of microvilli.

4. Discussion

Seabass juveniles fed diets in which solvent-extracted SBM replaced 37.5% of fish meal protein had identical survival and did not show significantly lower growth and feed performance than fish on the control fish meal diet. This is in agreement with results reported for rainbow trout and channel catfish. Growth differences were not significant when 9% fish meal in rainbow trout fingerling diet (as 25% replacement of fish meal) was replaced by 13% soybean meal (Dabrowski et al., 1989). The growth rate, however, was reduced significantly when 50% of the fish meal was replaced by soybean meal (Dabrowski et al., 1989; Alexis, 1990). Pongmaneerat and Watanabe (1993) succeeded in utilizing 30% SBM as a replacement of fish meal in an extruded high energy diet for small rainbow trout of 5 g initial weight. Liebowitz (1981) found that when energy and phosphorus requirements were met and when fish were fed to satiation, soybean meal could replace most of the fish meal in practical channel catfish diets. Robinson and Li (1994) also indicated that soybean meal could be used to completely replace fish meal in feeds for channel catfish reared in earthen ponds and fed daily to satiation.

Reduced weight gains of fish fed diets 3 and 4 appeared to be related to palatability, since feed efficiency ratio and apparent protein digestibility of these diets were similar to those of diets 1 and 2. This suggests that the extruded full-fat SBM and steamed full-fat SBM might contain substances that cause poor palatability. It has also been reported that soybean products are less palatable to some species of fish. Fowler (1980) reported that chinook salmon refused to eat a diet containing 80% full-fat SBM. In contrast, full-fat SBM has been shown to be a good protein and energy source for rainbow trout (Smith, 1977; Reinitz et al., 1978; Tacon et al., 1983) and channel catfish (Saad, 1979). Another factor which might have affected feed intake in our experiment was the dietary level of fish oil, even though the diets had the same level of oil and that the $n-3$ highly unsaturated fatty acid level was within the requirement range of 1.2 to 2.2%, it has been observed in our laboratory that seabass consumed more feed if it contains a higher level of fish oil.

Soaked raw full-fat SBM was poorly utilized by seabass as shown by a marked reduction in feed intake, feed efficiency, protein efficiency ratio, growth performance, survival and apparent protein digestibility. Similar effects on growth, feed efficiency and survival were found in rainbow trout (Smith, 1977), common carp (Abel et al., 1984), channel catfish (Wilson and Poe, 1985a), *Clarias gariepinus* (Balogun and Ologhobo, 1989) and Nile tilapia (Wee and Shu, 1989) fed raw soybean meal. A high level of trypsin inhibitor activity was likely responsible for decreased growth and protein digestibility of the soaked raw full-fat SBM diet. The results of the estimated trypsin inhibitor activities which was indirectly estimated from urease activity using the regression equation (Herkelman et al., 1991) were 0.77, 0.92, 0.28 and 14.64 mg/g for solvent-extracted SBM, extruded full-fat SBM, steamed full-fat SBM and soaked raw full-fat SBM, respectively. Sandholm et al. (1976) also indicated that protein digestibil-

ity was negatively correlated with trypsin inhibitor activity. Similar observations have been reported for rainbow trout (Krogdahl and Holm, 1983) and Atlantic salmon (Olli et al., 1989). Rainbow trout fed diets containing purified Kunitz soybean trypsin inhibitor had diminished growth, decreased intestinal trypsin activity, and an increase in fecal excretion of protein and lipids (Berg-Lea et al., 1989).

Properly heated soybean products (solvent-extracted SBM, extruded full-fat SBM and steamed full-fat SBM) were well utilized by seabass. Protein digestibility values of solvent-extracted SBM, extruded full-fat SBM and steamed full-fat SBM were not significantly different ($P > 0.05$) from that of fish meal. Similar results were obtained for channel catfish (Lovell, 1977; Wilson and Poe, 1985b) common carp and rainbow trout (Pongmaneerat, 1992), and Nile tilapia (Popma, 1982).

Rackis (1965) observed pancreatic hypertrophy in rats fed unheated soybean meal and pancreatic atrophy with prolonged feeding. He indicated that trypsin inhibitors were responsible for nearly all of the pancreatic hypertrophy in rats. Thus, pancreatic atrophy found in seabass fed the soaked raw full-fat SBM diet was likely attributed to trypsin inhibitor activity. The decrease in absorptive area of the anterior intestine of the seabass fed the soaked raw full-fat SBM diet was similar to the changes in the distal intestine of salmon fed a full-fat SBM diet (Van den Ingh et al., 1991). This change would likely have an effect on protein and fat absorption due to its function in protein and fat absorption. Van den Ingh et al. (1991) indicated that morphological changes in the distal intestine of salmon fed the full-fat SBM might be due to antinutritional factors other than protease inhibitors, such as lectin. Whether the morphological changes in the anterior intestine of seabass was due to lectin or other anti-nutritional factors is still unknown. The mild histopathological changes in the anterior intestine of seabass fed solvent-extracted SBM, extruded full-fat SBM, and steamed full-fat SBM may be due to other anti-nutritional factors. However, no conclusion could be drawn.

According to Wright (1981) and Hardy (1996), a 0.079 and 0.061 pH unit rise in urease activity for extruded full-fat SBM and solvent-extracted SBM, respectively, indicates proper processing of these soybean meals. As no increase in pH was detected for the urease test, the steamed full-fat SBM may have been overheated. Urease activity of the soaked raw full-fat SBM was 1.8, which is slightly lower than the reported value of 2.25 or greater for unheated SBM (Hardy, 1996). The potential use of steamed full-fat soybean as a protein source for making on-farm feed may be of practical benefit to small-scale farmers who can grow the crop and prepare this material.

5. Conclusion

Results of this study indicate that fish meal is better utilized by juvenile seabass than the soybean products tested. Solvent-extracted SBM and extruded and steamed full-fat SBM have comparable nutritional value. The growth reduction of fish fed diets containing these soybean meal products was probably due to reduced feed intake since FER and protein digestibility of these diets were comparable to those of the all fish meal diet. Thus, if palatability of the diets can be improved, at least 37.5% of protein from fish meal or 15% fish meal can be replaced by solvent-extracted SBM, extruded, or steamed full-fat SBM.

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