The Use of Concentrates and Other Soy Products in Shrimp Feeds

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ABSTRACT

Soy products have continued to be employed in a range aquaculture feeds, principally because they provide an acceptable source of amino acids at a very reasonable price. Advances in processing have led to the production of higher protein content soy products, namely soy protein concentrate (SPC) and protein isolate, which are manufactured by selective removal of carbohydrates. The protein level of these ingredients is similar to that of standard fish meal, although there are some important differences in amino acid profile. This paper discusses the use of soy products, especially SPC, in aquaculture feeds, and describes work conducted with SPC in diets for Pacific white shrimp *Litopenaeus vannamei*.

Two growth trials were conducted to ascertain the ability of SPC to replace fish meal in shrimp diets. One trial was conducted indoors with flow-through water for eight weeks. In this trial, SPC replaced 0, 25, 50, 75 and 100% of high quality fish meal with and without supplemental lysine (other indispensable amino acids were supplemented to all diets). In the second experiment, fish meal was replaced completely by either corn gluten meal or SPC. No amino acids were supplemented in any of these diets.

In the first trial, it was found that SPC could replace up to 75% of fish meal, although supplementation of lysine improved overall shrimp performance at 25 and 50% fish meal replacement. In the outdoor system, the final weight of shrimp fed the diet with complete replacement of fish meal by SPC was not significantly different than the control, even without indispensable amino acid supplementation. The result of this work indicates the

Forster, I. P., Dominy, W., Tacon, A. G., 2002. The use of concentrates and other soy products in shrimp feeds. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., Simoes, N. (Eds.). Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola. 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

high nutritional value of soy protein concentrate and highlights the importance of considering the culture system when conducting nutritional research.

Key words: soy products, soybean protein concentrate, shrimp, amino acids.

INTRODUCTION

For several years, there has been continuing interest in identifying and developing ingredients as alternatives to fish meal for use within aquafeeds (Hardy, 1995; Tacon, Dominy & Pruder, 1998). Originally, the impetus for this was the often-variable nature of the price and supply of fish meal of suitably high quality for the aquafeed industry. Recently, however, the concern raised about the negative impact of fish meal production on global fish stocks has heightened this interest (Naylor *et al*. 2000). Among the ingredients that are being investigated as alternatives to fish meal, products derived from soybeans are some of the most promising (Lim, Klesius & Dominy, 1998; Hardy 1999; Storebakken, Refstie & Ruyter 2000; Swick, 2002) because of the security of supply, price and protein/amino acid composition. According to FAO statistics, global soybean meal production has increased from about 15 million tons in 1961 to about 107 million tons in 2001, whereas fish meal production has remained fairly static at 5 – 7 million tons (Figure 1) and is expected to remain at these levels for the foreseeable future.

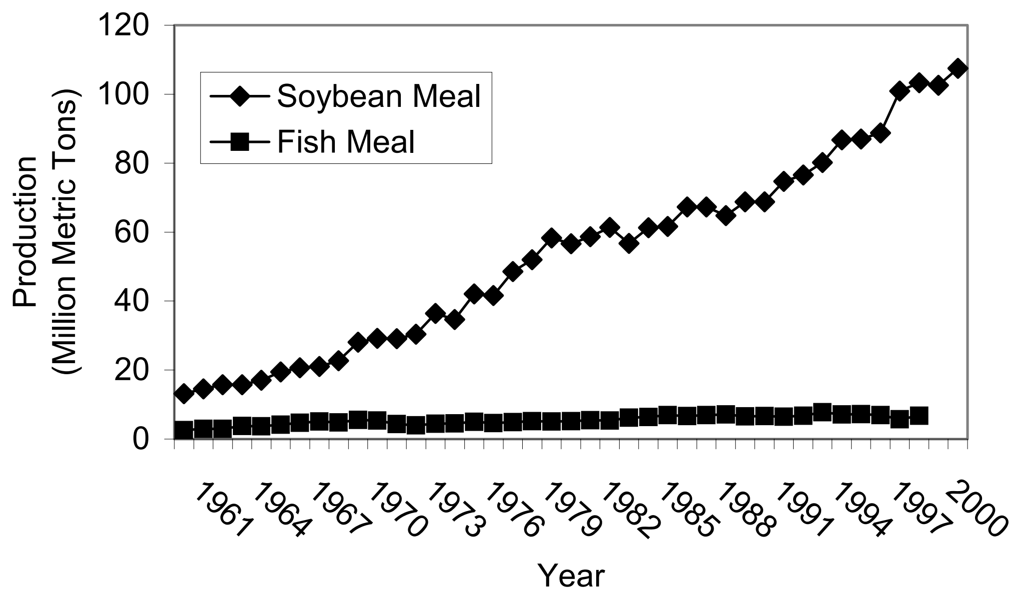

Figure 1. Global production of soybean meal and fish meal (source: Food and Agriculture Organization of the United Nations, 2001)

Soy products relevant to aquaculture feeds can be divided into three categories: protein products, oil, and lecithin. In the scientific literature, most attention has been paid to soy protein, although soy lecithin is used in commercial aquaculture feeds (e.g., for shrimp).

This paper provides a very cursory summary of soy products in aquaculture feeds and describes research conducted at the Oceanic Institute on the use of soy protein concentrate as replacement of fish meal in diets for Pacific white shrimp *Litopenaeus vannamei* (Boone). For more information on the production and uses of soy products the reader is referred to Lim et al., 1998; Hardy, 1999; Storebakken et al. 2000; Swick, 2002. A great deal of information can be found at several websites, including: http://www.unitedsoybean.org/, http://www.centralsoya.com/, and http://www.soygrowers.com/.

A number of processes go into the manufacture of protein products from soybeans. In general, the hulls are removed, and the beans are rolled into flakes, which are de-oiled by a solvent. After toasting to remove trypsin inhibitor, this product is commonly referred to as soybean meal, a low oil high carbohydrate product with about 48% crude protein. This product can be blended with ground soybean hull to produce a meal with about 44% protein.

In addition to trypsin inhibitor, several antinutritional and/or allergenic compounds are associated with the carbohydrate fraction exist in soybeans, such as, glycinin, ß-conglycinin, oligosaccharides, lectins, and saponins (Liener, 1994). As well, some specific types of carbohydrates that exist in soybean meal can impart a “beany” taste, and may influence its palatability. Many of these anti-nutritional factors can be selectively removed by solvent (aqueous alcohol) extraction, or by isoelectric leaching, producing a range of products with elevated protein content (above 65%) called Soy Protein Concentrates. The duration and temperature of the process, as well as modification of the aqueous alcohol solvent used, combine to influence the characteristics of the final product, for example reduced antigen products. Texturized SPC is produced by extrusion of traditional SPC.

A number of studies have demonstrated the usefulness of soy protein products, especially soybean meal (SBM), in animal feeds, and this ingredient is routinely added to commercial formulations for many species, including shrimp. Lim & Dominy (1990; 1992) found that SBM (both solvent-extracted and full-fat extruded SBM) could effectively replace up to 42% of fish meal protein in practical feeds for *Litopenaeus vannamei*. Divakaran *et al*. (2000) showed that the apparent digestibility of protein in soybean meal is at least 89% in *L. vannamei*. Tacon & Akiyama (1997) provide an extensive list of published work on the use of soybean meal and other products in crustacean feeds.

Soy protein concentrate (SPC) shows good promise for aquatic feeds. Removal of specific carbohydrates may reduce the palatability issue that is sometimes encountered with plant products in feeds for some aquatic species. The protein content of SPC is approximately 65% (as fed basis), with a lipid level of less than 1% and ash content of about 6% (low phosphorus). The level and balance of amino acids is considered to be among the most appropriate among plant proteins, although it is low in methionine (Storebakken *et al*., 2000).

Kaushik *et al*. (1995) were able to replace 100 % of fish meal with SPC in diets for rainbow trout *Oncorhynchus mykiss* (Walbaum) with no reduction in growth or nutrient utilization. Refstie et al. (2001) found that Atlantic salmon *Salmo salar* (L.) fed diets in which SPC replaced 30% of high quality fish meal (LT) protein grew significantly better than those fed the fish meal control diet. At this level of inclusion, the addition of DL-methionine had no effect. Recent work by Paripatananont *et al*. (2001) examined the nutritional quality of SPC in relation to fish meal in diets for *Penaeus monodon* Fabricius. These researchers fed a series of diets with SPC replacing 0, 25, 50, 75, and 100% of fish meal for eight weeks, with no supplementation of amino acids. They found that SPC could replace up to 50% of the fish meal with no adverse effect on growth, feed intake, FCR or mortality.

To date, there is no information on the use of soy protein concentrate in diets for *L. vannamei*. A trial, conducted indoors in flow-through conditions, was undertaken to examine the ability of SPC to replace high quality fish meal in diets for Pacific white shrimp in the presence of methionine, and to examine if supplemental lysine effects the utilization of this protein source. A second trial was conducted to examine the ability of SPC to replace fish meal under outdoor in static water (with ample endogenous feed sources) conditions.

MATERIALS AND METHODS

Indoor trial

Animals and feeding

Shrimp were obtained from the Oceanic Institute hatchery (Kona, Hawaii) and transferred to our testing facility at Makapu'u. Prior to the commencement of the trial, the shrimp were maintained on a commercial feed (Rangen 35 crude protein, 2.5 squid). Animals were stocked into 52 rectangular glass aquaria (76 x 31 x 31-cm dimensions and 52-L water volume) at an initial density of 18 shrimp aquarium^{-1} (corresponding to 75-shrimp m^{-2}). Individual weight at stocking was 1.73 g (sd = 0.12). Water (26.1-26.4 °C) was supplied to each aquarium at the rate of 1 L min^{-1}. Analysis of variance procedures revealed no significant variation in stocking weight among dietary treatments. A 72-h stress replacement period after stocking was observed to avoid bias in the treatments.

Each diet was fed to four randomly selected aquaria for 8 weeks. Shrimp were fed a fixed ration 8 times day^{-1} (0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 H), by an automatic feed dispenser. The daily ration was calculated according to shrimp size and temperature-dependent feeding rates (Tacon *et al*., 2002) and used as an estimate of the feeding requirement. Feeding rates were then adjusted daily following observations of the quantity of feed residue present in each tank to determine whether rations were excessive, sufficient, or insufficient. Each tank was siphoned before the first feeding of the day to remove diet residues and fecal matter.

Diets

High quality fish meal (LT-94; SSF Norway) and soy protein concentrate (SPC; Profine VF, Central Soya Co., Inc. Fort Wayne, IN, USA) were obtained for this trial. A commercial laboratory (CN Labs, Courtland, MN, USA) determined the amino acid level of each protein source used in this trial, as well as of the shrimp whole body (Table 1).

Table 1. Essential amino acid (plus cystine and tyrosine) levels of shrimp whole body, fish meal and soy protein concentrate.

Amino Acid	Whole Shrimp (% freeze-dried)	Fish Meal LT-94 (% as fed)	Soy Protein Concentrate (% as fed)
Tryptophan	0.688	0.731	0.771
Lysine	4.843	5.752	4.278
Histidine	1.621	1.820	1.790
Arginine	6.101	4.763	5.004
Threonine	2.519	3.036	2.742
Cystine	0.751	0.593	1.030
Valine	3.096	3.795	3.353
Methionine	1.917	2.268	1.554
Isoleucine	2.651	3.062	3.145
Leucine	4.692	5.400	5.378
Tyrosine	2.577	2.283	2.472
Phenylalanine	2.813	2.705	3.392
Taurine	0.562	0.812	0.000
EAA SUM	**34.828**	**37.017**	**34.908**

Ten diets (Table 2) were formulated to contain 40% crude protein and 9% crude lipid (as fed basis). All but one of the diets were supplemented with crystalline indispensable amino acids (IAA) (arginine, methionine and phenylalanine; lysine was added to some diets) to ensure that the diets contained all IAA at least at the level of shrimp whole body (Tables 2 and 3). One diet contained no SPC and no IAA (only glutamic acid). Five diets contained SPC in replacement of 0, 25, 50, 75 and 100% of the fish meal protein, with no supplemental lysine. In the four remaining diets, SPC replaced fish meal at 25, 50, 75 and 100% and crystalline lysine was added to bring the dietary level to that of shrimp whole body. All IAA additions were made at the expense of glutamic acid.

Table 2. Formulation and analysis of diets used to assess efficacy of Soy Protein Concentrate in diets for shrimp. SPC replaced fish meal at 0, 25, 50, 75, 100% of protein, with and without lysine (Lys) supplementation. All formulation values are g/100 g diet as fed.

Ingredient	No IAA	0	25	50	75	100
Fish Meal[1[1]]	300.0	300.0	225.0	150.0	75.0	0.0
Soy Protein Concentrate		0.0	90.8	181.5	272.3	363.0
Wheat	463.4	460.4	436.6	413.9	390.1	367.9
Fish Oil	25.0	25.0	33.0	40.0	48.0	54.5
Lys	0.0	0.0	0.0	0.0	0.0	0.0
Glu	41.0	20.0	20.0	20.0	20.0	20.0
DL-Met	0.0	5.0	5.0	5.0	5.0	5.0
Arg	0.0	16.0	16.0	16.0	16.0	16.0
Phenylalanine	0.0	3.0	3.0	3.0	3.0	3.0
Agar	10.0	10.0	10.0	10.0	10.0	10.0
Squid liver powder	25.0	25.0	25.0	25.0	25.0	25.0
Brewer's Yeast	50.0	50.0	50.0	50.0	50.0	50.0
Vital Wheat Gluten	40.0	40.0	40.0	40.0	40.0	40.0
Soy Lecithin	20.0	20.0	20.0	20.0	20.0	20.0
Cholesterol	2.3	2.3	2.3	2.3	2.3	2.3
Min Px	0.6	0.6	0.6	0.6	0.6	0.6
Vit Px	4.0	4.0	4.0	4.0	4.0	4.0
Choline Chloride	1.2	1.2	1.2	1.2	1.2	1.2
Stay C	0.7	0.7	0.7	0.7	0.7	0.7
Potassium phos	5.6	5.6	5.6	5.6	5.6	5.6
Sodium phos	5.6	5.6	5.6	5.6	5.6	5.6
Magnesium phos	5.6	5.6	5.6	5.6	5.6	5.6
Total		1000.0	1000.0	1000.0	1000.0	1000.0
Analysis (dry weight basis)						
Crude Protein (%)		44.4	44.3	44.4	44.1	43.9
Crude Lipid (%)		9.8	10.3	9.6	10.8	10.0
Ash (%)		5.8	5.5	5.2	5.2	5.1
Gross Energy (kJ/g)		20.76	20.66	20.61	20.71	20.77

[1[1]] LT-94. SSF Sildolje-og Sildemelindustriens Forskningsinstitut, Norway .

Table 2 continued. Formulation and analysis of diets used to assess efficacy of Soy Protein Concentrate in diets for shrimp. SPC replaced fish meal at 0, 25, 50, 75, 100% of protein, with and without lysine (Lys) supplementation. All formulation values are g/100 g diet as fed.

Ingredient	25Lys	50Lys	75Lys	100Lys
Fish Meal	225.0	150.0	75.0	0.0
Soy Protein Conc.	90.8	181.5	272.3	363.0
Wheat	428.6	404.9	379.1	355.9
Fish Oil	33.0	40.0	48.0	54.5
Lys	13.0	15.0	18.0	20.0
Glu	15.0	14.0	13.0	12.0
DL-Met	5.0	5.0	5.0	5.0
Arg	16.0	16.0	16.0	16.0
Phenylalanine	3.0	3.0	3.0	3.0
Agar	10.0	10.0	10.0	10.0
Squid liver powder	25.0	25.0	25.0	25.0
Brewer's Yeast	50.0	50.0	50.0	50.0
Vital Wheat Gluten	40.0	40.0	40.0	40.0
Soy Lecithin	20.0	20.0	20.0	20.0
Cholesterol	2.3	2.3	2.3	2.3
Min Px	0.6	0.6	0.6	0.6
Vit Px	4.0	4.0	4.0	4.0
Choline Chloride	1.2	1.2	1.2	1.2
Stay C	0.7	0.7	0.7	0.7
Potassium phos	5.6	5.6	5.6	5.6
Sodium phos	5.6	5.6	5.6	5.6
Magnesium phos	5.6	5.6	5.6	5.6
Total	1000.0	1000.0	1000.0	1000.0
Analysis (dry weight basis)				
Crude Protein	44.2	45.2	45.2	45.3
Crude Lipid	10.1	9.6	9.9	9.2
Ash	5.5	5.4	5.1	4.5
Gross Energy	20.73	20.89	20.86	20.64

Table 3. Relative amino acid composition of shrimp whole body, fish meal and soy protein concentrate. The values represent the ratio (x 100%) of each amino acid in fish meal (FM), soy protein concentrate (SPC) and shrimp whole body.

Amino acid	FM/Shrimp (%)	SPC/Shrimp (%)	SPC/FM (%)
Tryptophan	106	112	105
Lysine	119	88	74
Histidine	112	110	98
Arginine	78	82	105
Threonine	121	109	90
Valine	123	108	88
Methionine + Cystine	107	97	90
Isoleucine	115	119	103
Leucine	115	115	100
Phenylalanine + Tyrosine	93	109	118
Taurine	145	0	0

The experimental diets were manufactured at the facilities of the Oceanic Institute as follows: All the major dry feed ingredients were mixed for 15 min in a Hobart food mixer (Model D-300, Hobart Manufacturing Corporation, Troy, Ohio, USA). A warm (approx. 60 °C) aqueous solution of sodium phosphate, potassium phosphate, choline chloride, and trace element premix, was then added to the dry ingredient mix, to bring the moisture content of the resulting mash to approximately 34-35%. The amino acids were dissolved in hot water (approximately 90 °C) in which the agar had been dissolved, and added to the mash. The mash was then blended for a further 15 min. Half the supplemental oil and lecithin and all the cholesterol were blended in a KitchenAid mixer (Model K5SS, KitchenAid, St. Joseph, Michigan, USA), added to the mash and mixed for a further 15 min. The resulting mash was then passed through a California Pellet Mill (Model CL5, San Francisco, California, USA) fitted with a 2.5 mm diameter die. No steam was used and the pellet temperature at the die was below 70 °C. The resulting moist pellets were then dried overnight in a drying cabinet using an air blower at approx. 38 °C until the moisture level was below 10%. The vitamin premix and vitamin C source (Table 2) were then emulsified with the remaining oil and lecithin in a KitchenAid mixer and this mixture was added to the dry cooled pellets by top coating using a Hobart D300 food mixer with a whisk beater. The finished pellets were then stored in plastic bins at 19 – 20 °C until used.

Outdoor trial

A trial to assess the efficacy of soy protein concentrate in diets for shrimp in pond water conditions was conducted in an outdoor mesocosm laboratory at the Oceanic institute This work was part of a larger trial that examined several aspects of shrimp nutrition.

Juvenile shrimp (of the same strain and size as above) were stocked within outdoor free-standing 1500 L cylindrical black-coated fiberglass microcosm tanks (1.52 m diameter with a conical bottom) at an initial stocking density of 100 shrimp/tank (equivalent to a shrimp density of 51 individuals/m^2 cone surface area, 55 individuals/m^2 flat bottom surface area or 71/m^3 water volume), with three tanks allotted per dietary treatment. Water within the microcosms was continuously mixed and aerated and a zero-water-exchange `green water' management system operated within the tanks for the duration of the 70-day culture trial (for tank configuration and operation see Freeman & Duerr 1991). Air was continuously supplied to all experimental tanks with an EG&G Rotron 5 HP regenerative blower (Saugerties, New York, USA). Freshwater was used as required to replace evaporative losses. Diurnal water temperature, dissolved oxygen, pH and salinity measurements throughout the study were recorded.

The shrimp were fed either a control feed with fish meal, or a diet with either corn gluten meal or soy protein concentrate in complete replacement of the fish meals (Table 4). The diets were manufactured and ration size was determined as described above. Diets were introduced to the culture tanks continuously (24 hours) by automatic belt feeders. The animals were weighed initially and every two weeks. The growth and survival data after eight weeks was used to assess the nutritional quality of the protein sources relative to fish meal.

Table 4. Formulation of feeds in the outdoor trial.

Ingredient	Control %	CGM %	SPC %
Fishmeal	110.0	0.0	0.0
Corn gluten meal		150.0	
Soy protein concentrate			140.0
Wheat, whole	550.0	498.2	507.9
Vital wheat gluten	40.0	40.0	40.0
Brewers yeast	50.0	50.0	50.0
Squid liver powder	25.0	25.0	25.0
Soybean meal	150.0	150.0	150.0
Soy lecithin - CSM	20.0	20.0	20.0
Fish oil	35.9	47.7	48.0
Corn oil	0.0	0.0	0.0
Cholesterol-FG	2.3	2.3	2.3
Potassium phosphate	5.6	5.6	5.6
Sodium phosphate	5.6	5.6	5.6
Magnesium phosphate	5.6	5.6	5.6
Total	1000	1000	1000
CP (%)	30.83	30.75	30.79
CL (%)	8.73	8.77	8.79

Statistical analyses

The final weight, specific growth rate (ln (final weight/initial weight) * 100%/56 days) and survival data were subjected to analysis of variance procedures. Survival data was transformed using the arcsin square root (Zar, 1999) prior to analysis. One tank of animals in the outdoor trial that was fed the corn gluten diet crashed, with essentially complete loss of animals and the data was excluded from statistical considerations. Significance of the differences in treatment means in comparison with the control was determined using Dunnet's test, which examines the significance of differences between the control (Diet 2 in the indoor experiment – 0% SPC, with supplemental IAA) and test treatments. All calculations were made with a commercial computer software program (SigmaStat v. 2.03, SPSS Inc., Chicago, IL). Differences were considered significant at the 5% level of probability.

RESULTS

Indoor trial

Both the fish meal and SPC sources examined have similar amino indispensable amino acid content that of shrimp whole body (Table 3), although fish meal was lower in arginine and aromatic amino acids (phenylalanine and tyrosine), while SPC was lower in lysine, arginine and sulfur amino acids (methionine and cystine). It should be noted that on a protein basis, SPC exceeds the recommended levels for all indispensable amino acids of Akiyama, Dominy & Lawrence (1991) for shrimp.

Replacement of fish meal by SPC did not significantly affect the final body weight of the shrimp at levels up to 75% in the dietary series with no supplemental lysine (Figure 2), although there was a slight decrease in final weight at fish meal replacement above 25%. The diets containing supplemental lysine promoted better growth in shrimp fed diets with 25 and 50% replacement of fish meal by SPC than in the control. At 100% replacement of fish meal, the growth was significantly reduced from the control, and was similar to the shrimp fed diets not supplemented with lysine. At the highest levels of SPC inclusion, there was a reduction in feed intake, as evidenced by the failure to consume the allotted ration of these diets. A similar pattern was found for the specific growth rate (Table 5).

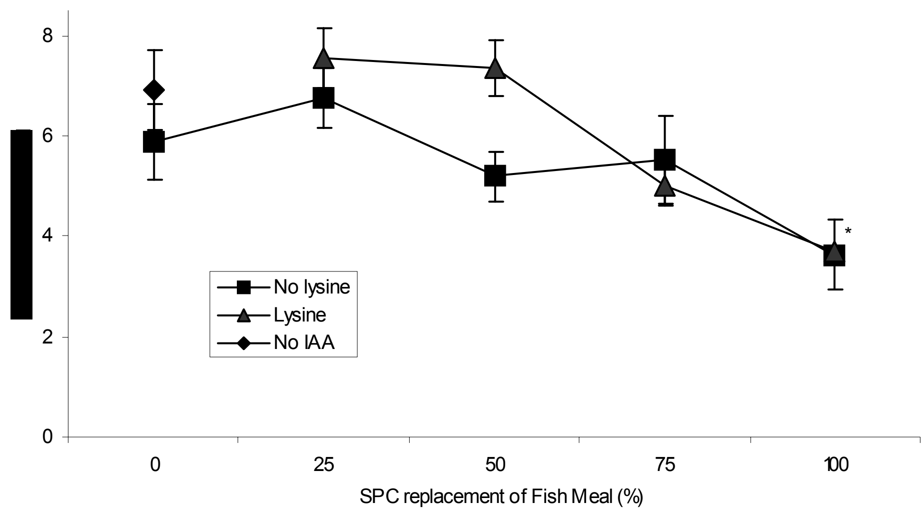

Figure 2. The final body weight of shrimp fed diets containing soybean protein concentrate in replacement of 0, 25, 50, 75 or 100% replacement of fish meal, with or without supplemental lysine.

Table 5. Specific growth rate (SGR) of shrimp fed diets containing soy protein concentrate at 0, 25, 50, 75, or 100% replacement of fish meal, with and without supplemental lysine (plus lys and no lys, respectively), or with no supplemental indispensable amino acids (no IAA). Values with and asterisk (*) are significantly less than the control (0, no lys) ($P<0.05$; Dunnet's test; n=4)

Dietary Treatment			
%SPC	Amino Acid	SGR	Std Dev
0	no IAA	2.41	0.10
0	no lys	2.21	0.18
25	no lys	2.42	0.15
50	no lys	1.95	0.15
75	no lys	2.04	0.30
100	no lys	1.28*	0.33
25	plus lys	2.63	0.12
50	plus lys	2.58	0.14
75	plus lys	1.89	0.18
100	plus lys	1.36*	0.20

Survival of the shrimp tended to be lower in animals fed diets containing 75 or 100% replacement of fish meal by SPC (Figure 3), especially in diets not supplemented with lysine.

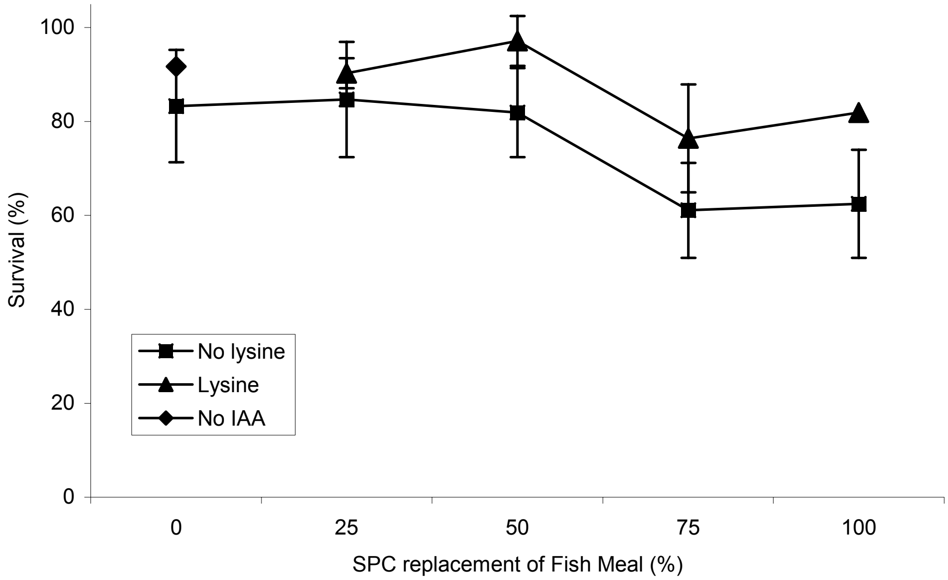

Figure 3. The survival of shrimp fed diets containing soybean protein concentrate in replacement of 0, 25, 50, 75 or 100% replacement of fish meal, with or without supplemental lysine.

Outdoor trial

Growth was better in the outdoor trial than in the indoor trial, as expected (Tacon *et al*., 2002). There were no significant differences in final weight or survival of the shrimp fed diets containing fish meal (14.58 (sd 0.49) g; 92.6 (sd 0.6) %) and soy protein concentrate (13.65 (sd 0.39) g; 83.0 (sd 6.1) %) in the outdoor trial. The final weight and survival of the shrimp fed corn gluten meal (12.08 (sd 0.65) g; 91.5 (sd 3.5) % n=2) in replacement of fish meal, were significantly less than those fed the fish meal control.

DISCUSSION

The results of the indoor trial indicate that, in the absence of endogenous source of nutrients, SPC may replace up to 75% of high quality fish meal in diets for Pacific white shrimp, when supplemented with sufficient arginine, methionine and phenylanine, with no reduction in growth and survival. Supplementing lysine improves the growth of animals up to 50% fish meal replacement level. These findings are in general agreement with those of Paripatananont *et al*. (2001) who found that they could replace 50% of fish meal in diets for tiger shrimp, although these researchers did not supplement their diets with any amino acids.

In the outdoor trial, the lack of a difference in final weight between shrimp fed the fish meal based diet or the diet in which SPC completely replaced fish meal, indicates the high nutritional value of SPC. Over the course of the trial in the outdoor system zero-water exchange system considerable levels of suspended particulate organic matter develops, which is a potential source of nutrition for the shrimp (Tacon *et al*., 2002). Research at our facilities and elsewhere has consistently demonstrated that shrimp grown in the presence of high levels of endogenous nutrients (e.g., from suspended particulate organic material) require less complete diets to perform well. It may be, that in these conditions, the requirement for marginally deficient indispensable amino acids is partially met from the endogenous material. It should also be borne in mind, that in the outdoor zero-exchange system, the diets contained lower protein (30% crude protein), and the actual level of SPC in the diet was only 14%, which is lower than that in the 50% replacement diet in the indoor trial (about 18%).

It is worth noting that SPC is generally priced at about $US880 – 1,100 per metric ton, although prices can be lower than $750, depending on supply (C. Russet, Central Soya, personal communication). This is higher than the current price of high-grade fish meal, but given the fluctuations and uncertainty in supply of fish meal, it is expected that SPC will be cost effective relative to fish meal as aquaculture continues to grow. It is important to further understand the potential for SPC in aquatic feeds in relation to soy bean meal, particularly in terms of palatability and antinutritional factors. SPC, which can be made to have less taste than soy bean meal, may indeed have greater flexibility in some species than soy bean meal.

ACKNOWLEDGEMENTS

The authors appreciate the technical assistance of the following staff members of the Aquatic Feeds and Nutrition program at the Oceanic Institute: Jesse Terpstra, Kekoa Nakachi, Eric Beyer, and Jeff Cody. This paper was prepared as part of the activities of the `Tropical Aquaculture Feeds and Culture Technology Development Project II: Development of Shrimp Feeds' awarded to the Oceanic Institute by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No. 59-5320-7-989. Mention of trade names or commercial products in this article is solely for providing specific information and does not imply recommendation or endorsement by the authors.

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