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Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*

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Abstract

The use of a co-extruded soybean poultry by-product meal (CEPM) and flashed dried poultry by-product meal (FD-PBM) was evaluated as replacements for fish meal in a practical diet formulated to contain 32% crude protein and 8% lipid. Each meal was substituted for menhaden fish meal on an iso-nitrogenous basis and offered to juvenile *Litopenaeus vannamei* (mean initial wt. \pm S.D., 0.37 ± 0.015 g) over a 6-week period. Inclusion levels ranged from 0 (30 g fish meal/100 g diet) to 80% replacement (6 g fish meal/100 g diet). Replacement of fish meal with CEPM resulted in equivalent values for final weight, percent weight gain and feed efficiency (FE) and a significant increase in protein conversion efficiency (PCE). Similarly, replacement of 40%, 60%, and 80% of the fish-meal protein in the basal diet with FD-PBM resulted in a significant increase in weight gain and FE. Although not significant, there was also a general increase in PCE when FD-PBM was included in the diet. Under the reported conditions, survival, FE, and PCE values were either improved or were not significantly influenced by the replacement of menhaden fish meal with either CEPM or FD-PBM. Hence, these products can be used to reduce the fish-meal content of practical diets from 30 to 6 g/100 g dry wt. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Litopenaeus vannamei*; Poultry meal; Co-extrusion; Soybean meal

1. Introduction

The quantity and quality of dietary protein are primary factors influencing shrimp growth, nitrogen loading of the culture system and feed costs. Consequently, considerable research has been conducted to evaluate protein requirements (Guillaume, 1997)

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and the acceptability of various feedstuffs as protein sources (Tacon and Akiyama, 1997). Feedstuffs containing at least 20% crude protein are considered to be protein supplements and include fish meal, marine by-products, meat and bone meal, poultry by-products, cottonseed meal, peanut meal, and soybean meal. In commercially manufactured feeds for shrimp culture, marine protein sources (fish, shrimp and squid meals) are one of the primary protein sources being included in the feed at approximately 25%, by weight (Tacon and Barg, 1998). Marine protein sources are often utilized in aquatic feeds because they are an excellent source of indispensable amino acids, essential fatty acids, vitamins, minerals, and generally enhance palatability. Marine by-products such as scallop waste, lobster waste, squid viscera and shrimp head meal have been evaluated as alternative marine protein sources (Carver et al., 1989; Sudaryono et al., 1995). However, fish meals and marine by-products are commodities for which supplies are limited and demand is expected to continue to increase. Hence, maintenance of the economical viability of commercial aquaculture will require the replacement of expensive marine proteins, with lower cost ingredients for which production is not limited.

Protein sources that can be utilized to replace marine protein sources, either partially or completely, include both terrestrial plant and animal sources that are either locally available or traded on the world market. Replacement of marine proteins in shrimp feeds has met with different degrees of success. Considerable attention has been devoted to the evaluation of plant proteins such as: soybean meal (Lim and Dominy, 1990; Piedad-Pascual et al., 1990; Tidwell et al., 1993; Sudaryono et al., 1995), solvent-extracted cottonseed meal (Lim, 1996), lupin meals (Sudaryono et al., 1999), various legumes (cowpea, green mungbean, rice bean) and leaf meals (Eusebio, 1991; Eusebio and Coloso, 1998), and papaya or camote leaf meal (Penaflores, 1995). Because of their low price and consistent quality, plant proteins are often an economically and nutritionally viable source of protein. However, due to potential problems with deficient levels of indispensable amino acids (e.g., lysine and methionine), anti-nutrients and poor palatability, their use is often limited.

Sources of terrestrial animal protein are primarily rendered by-products such as meat and bone meal and poultry by-product meal, which generally contain 45–65% crude protein and are often good sources of indispensable amino acids. The quality of these meals depends on both the quality of raw ingredients and the type of processing. Animal by-product meals can be very high in lipid, ash and indigestible fiber (feathers, tendons, etc.), resulting in reduced digestibility (Robinson and Li, 1996). However, if suitable raw ingredients are chosen and then properly processed, a high-quality protein source can be produced. One of the more promising alternative ingredients is poultry by-product meal (Dong et al. 1993), which is a waste material of the poultry industry. Currently, few studies have evaluated the use of terrestrial-animal by-product meals in practical shrimp feeds. Hence, the objective of this study was to evaluate the use of two poultry by-product meals as replacements for fish meal in practical shrimp diets.

2. Materials and methods

A 6-week feeding trial was conducted to determine the nutritional value of two commercial protein sources relative to growth and survival of *Litopenaeus vannamei*

juveniles. The protein sources tested were a co-extruded soybean/poultry by-product meal (CEPM) consisting of a mixture of ground dry clean parts of the chickens from USDA-inspected poultry processing plants and soybean meal (Profound™, American Dehydrated Foods, Verona, MO, USA) and a flashed dried poultry by-product meal (FD-PBM, Griffin Industries, Cold Spring, KY, USA). Proximate analyses and amino acid profiles of these ingredients were determined by independent laboratories and are presented in Table 1. Pepsin digestibility values were determined by Woodson-Tenent Laboratories (Little Rock, AR, USA) and were found to be 94.09% for fish meal, 89.4% for FD-PBM and 91.5% for the CEPM. A practical basal diet containing menhaden fish meal and soybean meal as the primary protein sources was formulated to contain 32% protein and 8% lipid (Table 2). The test diets were then developed based on the replacement of fish meal on an equal protein basis. Lipid levels of the diets were also adjusted, albeit slightly, using menhaden fish oil resulting in seven test diets that contained equivalent levels of protein and lipid. Additionally, a phosphorus source was added to ensure that dietary levels were replete. Prior to use, the fish and soybean meals were ground with a laboratory type hammer mill using a #40 screen (1.02 mm diameter hole) and analyzed for protein and moisture content. After processing, the dry ingredients and oil were mixed in a food mixer (Hobart, Troy, OH, USA) for 15 min. Hot water was then blended into the mixture to attain a consistency appropriate for pelleting. Each diet was pressure pelleted using a meat grinder and a 2-mm die. After pelleting, the diets

Table 1
Profile of selected nutrients for the two test ingredients (g/100 g dry wt.)

	Flash dried poultry by-product meal	Co-extruded soybean/poultry by-product meal
Moisture ^a	8.0	6.5
Crude protein	72.2	53.1
Crude fat	12.0	8.5
Ash	12.3	10.7
Calcium	3.4	2.1
Phosphorus	2.1	1.6
Arginine	5.60	3.72
Glycine	8.12	3.09
Histidine	1.36	1.29
Isoleucine	2.84	2.32
Lysine	4.53	3.20
Methionine	0.91	0.89
Cystine	0.66	0.79
Phenylalanine	2.99	2.61
Tyrosine	2.53	1.80
Serine	3.20	2.60
Threonine	3.27	2.15
Tryptophan	0.57	0.68
Valine	3.53	2.58

^aExpressed as an as is basis.

Table 2
Ingredient composition of the test diets (g/100 g dry wt.)

	CEPM ^a					FD-PM ^b		
	0	20	40	60	80	40	60	80
Menhaden fish meal ^c	30.0	24.0	18.0	12.0	6.0	18.0	12.0	6.0
CEPM	0.0	7.9	15.7	23.6	31.4	0.0	0.0	0.0
FD-PM	0.0	0.0	0.0	0.0	0.0	10.8	16.2	21.6
Soybean meal ^d	17.7	17.7	17.7	17.7	17.7	17.7	17.7	17.7
Menhaden fish oil ^e	4.1	4.1	4.1	4.1	4.1	4.1	4.0	4.0
Wheat gluten ^f	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Wheat starch ^f	35.9	33.7	31.5	29.4	27.2	36.4	36.7	37.0
Nutribinder ^g	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Trace mineral premix ^h	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix ⁱ	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin C ^j	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calcium phosphate ^k	0.2	0.5	0.8	1.2	1.5	0.9	1.2	1.6
Soy lecithin ^l	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

^aProfound™, Co-extruded soybean and poultry by-product meal. American Dehydrated Foods, Verona, MO, USA.

^bFlashed dried poultry by-product meal, Griffin Industries, Cold Spring, KY, USA.

^cSpecial Select™, Zapata Protein USA, Randeville, LA, USA.

^dSolvent extracted, Producers Coop, Bryan, TX, USA.

^eOmega Protein, Reedville, VA, USA.

^fUnited States Biochemical, Cleveland, OH, USA.

^gIndustrial Grain Products, Lubbock, TX, USA.

^hg/100 g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.

ⁱg/kg premix: thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D₃ (400,000 IU/g) 0.002, dl-alpha-tocopheryl acetate (250 IU/g) 8.0, Alpha-cellulose 865.266.

^j250 mg/kg active C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins, Parsippany, NJ, USA.

^kCefkaphos® (primarily monobasic calcium phosphate) BASF, Mount Olive, NJ, USA.

^lAqualipid 95, Aqualipid 95, Central Soya Chemistry Division, Fort Wayne, IN, USA.

were dried to a moisture content of 8–10% and stored at 4°C. Moisture content and crude protein levels of the diets were determined after processing.

High health post-larvae were obtained from a commercial hatchery and held under quarantine conditions for approximately 1 month. During this holding period, the shrimp were maintained on a commercial shrimp feed (Rangen, Buhl, ID, USA) and evaluated for signs of viral and bacterial pathogens. The shrimp were then hand sorted to a uniform size and stocked in the research system. Each dietary treatment consisted of four replicate groups of shrimp (eight shrimp per tank; mean wt. \pm S.D., 0.37 ± 0.015 g) maintained in a common semi-closed re-circulating system consisting of a series of 32 rectangular tanks. Each tank contained 68 l of seawater, a circulation pump, rapid-rate sand filter, supplemental aeration, and biological filter. Dietary treatments were ran-

domly assigned and the shrimp were weighed at days 0 and 42. The shrimp were fed according to a fixed feeding rate, which was adjusted for expected growth and observed mortalities resulting in a total of 7.9 g dry wt. of feed per shrimp over the course of the growth trial. Shrimp were fed four times per day at approximately 0800, 1100, 1300 and 1600 h. Photo-period was set for 12:12 h light:dark cycle. Water quality was maintained by biological filtration, removal of settled solids, and replacement of the systems make-up water with pre-filtered ozone-treated seawater at a rate of 4 l/min. Water temperature, dissolved oxygen and salinity were maintained at 28.1 ± 1.4 C, 6.3 ± 0.4 mg/l and 28.3 ± 2.5 . Total ammonia-nitrogen, nitrite-nitrogen and pH were measured twice weekly following the methods of Spotte (1979) and were maintained at 0.005 ± 0.007 mg/l, 0.008 ± 0.008 mg/l and 7.9 ± 0.06 , respectively.

At the conclusion of the 6-week growth trial, mean percent weight gain and survival for each dietary treatment were determined. Based on feed inputs and comparative carcass analyses, feed efficiency (FE = weight gain \times 100/feed offered) and protein conversion efficiency (PCE = protein gain \times 100/protein offered) values were estimated. Samples of shrimp from the initial population, and four shrimp from each tank were collected at the termination of the feeding trial and frozen for subsequent analysis. Samples of shrimp were minced, oven dried (90°C) to a constant weight, and ground prior to biochemical analysis. All chemical analyses were conducted in duplicate. Whole body samples, feed ingredients and test diets were analyzed for crude protein content using the micro-Kjeldhal method (Ma and Zuazago, 1942).

Data were analyzed using an analysis of variance to determine if significant ($P < 0.05$) differences existed among treatment means. The Student–Neuman–Keuls multiple comparison test was used to determine where significant differences existed between treatment means (Steel and Torrie, 1980). All statistical analyses were conducted using the SAS Systems for Windows V7, (SAS Institute, Cary, NC, USA).

3. Results and discussion

At the conclusion of the 6-week growth trial, survival was good and the growth of the shrimp was typical for shrimp offered a high quality practical diet under research conditions. Additionally, there were no indications of the feed being rejected or that the highest levels of either test ingredient resulted in a decrease in palatability.

The use of co-extrusion technologies has a variety of advantages which include the destruction of potential pathogens and the reduction of the moisture content of wet by-products. It also inactivates and/or destroys endogenous heat liable anti-nutritional factors found in soybean meal and gelatinizes starch granules (Carver et al., 1989), thus producing a value-added product at a minimal cost. The replacement of fish meal with CEPM did not adversely affect the shrimp (Table 3) relative to final weight, percent weight gain, and FE values. The PCE values for the dietary treatments in which fish meal was reduced were significantly higher than that of the basal diet. Although digestibility coefficients were not determined, increases in PCE values may indicate that the protein in the CEPM has a higher availability than that of the fish meal. These

Table 3

Response of juvenile *L. vannamei* (mean initial weight 0.37 ± 0.015 g) to practical diets containing increasing levels of co-extruded soybean/poultry by-product meal replacing fish meal on an equal protein basis^a

Percent replacement	Final weight (g)	Percent			
		Weight gain	Survival	FE ^b	PCE ^c
0	3.88	960	90.6	42.9	24.6 ^y
20	4.34	1030	100.0	48.2	27.3 ^{yz}
40	4.23	1090	93.8	47.3	27.3 ^{yz}
60	4.41	1125	100.0	49.4	28.6 ^{yz}
80	4.49	1119	93.8	50.3	29.1 ^z
PSE ^d	0.15	0.15	4.2	1.9	1.0
Pr > F	0.0953	0.0572	0.4380	0.0853	0.0485

^aMeans of four replicates. Numbers within the same column with different superscripts are significantly different ($P < 0.05$).

^bFE, feed efficiency = weight gain \times 100/feed offered.

^cPCE, protein conversion efficiency = protein gain \times 100/protein offered.

^dPooled standard error.

results indicate that this ingredient is a suitable partial substitute for fish meal in practical diets for *L. vannamei*.

The second ingredient tested was poultry by-product meal that had been flash dried. This drying technique is generally considered to minimize heat damage to the ingredient and produce high quality products. The replacement of 40–80% of the fish meal in the basal diet with FD-PBM resulted in a significant increase in weight gain and feed efficiency (see Table 4). There were no significant differences in PCE when FD-PBM was included in the diet. These results indicate that the FD-PBM used in this growth trial also serves as a suitable partial replacement for fish meal.

Table 4

Response of juvenile *L. vannamei* (mean initial weight 0.37 ± 0.015 g) to practical diets containing increasing levels of flash dried poultry by-product meal, replacing fish meal on an equal protein basis^a

Percent replacement	Final weight (g)	Percent			
		Weight gain	Survival	FE ^b	PCE ^c
0	3.88 ^y	960 ^y	90.6	42.9 ^y	24.6
40	4.26 ^z	1043 ^z	96.9	47.4 ^z	27.0
60	4.42 ^z	1087 ^z	93.8	49.4 ^z	27.4
80	4.43 ^z	1091 ^z	96.9	49.5 ^z	27.0
PSE ^d	0.12	0.12	4.8	1.5	1.0
Pr > F	0.0238	0.0169	0.7697	0.0211	0.2213

^aMeans of four replicates. Numbers within the same column with different superscripts are significantly different ($P < 0.05$).

^bFE, feed efficiency = weight gain \times 100/feed offered.

^cPCE, protein conversion efficiency = protein gain \times 100/protein offered.

^dPooled standard error.

Based on the observed results, either of the tested products can be used to reduce the fish-meal content from 30 to 6 g/100 g dry wt. Fish meal has been completely replaced in production diets for several species of fish (e.g., catfish, tilapia) and crustaceans (e.g., *Macrobrachium rosenbergii*); however, replacement of marine protein sources in practical diets for *L. vannamei* has been less successful. Utilizing a 32% crude protein practical diet, containing 45% marine protein mix (53% menhaden fish meal, 34% shrimp waste meal and 13% squid meal), Lim (1996) demonstrated that solvent-extracted cottonseed meal can be used to replace 40% of the marine protein mix. Higher levels of replacement with cottonseed meal resulted in reduced performance of the shrimp, presumably due to the gossypol content of cottonseed meal. Using the same marine protein mix, Lim and Dominy (1990) reported that 40% of the marine protein mix could be replaced by solvent-extracted soybean meal; however, higher levels of replacements resulted in reduced growth. In the current experiment, the fish-meal content of the diet was reduced to 6%, a level considerably lower than previous reports. The favorable response of the shrimp to these meals is probably due to the high quality of the meals in terms of both nutrient profile and possibly digestibility as well as a lack of apparent palatability problems.

In this study, growth, survival, FE, and PCE values were either improved or were not significantly influenced by the replacement of fish meal with either CEPM or FD-PBM. Although the tested products generally have a lower costs than high quality fish meal, the cost effectiveness of substituting these products for fish meal will vary depending on location and local cost of the ingredients. Because poultry by-product meals can vary considerably in quality, further studies to evaluate a range of products is recommended. Additionally, based on the positive results achieved in this study, further research evaluating the complete replacement of fish meal in *L. vannamei* diets is warranted.

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