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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

EVALUATION OF PLANT PROTEINS AS PARTIAL REPLACEMENT FOR ANIMAL PROTEINS IN DIETS FOR *PENAEUS INDICUS* AND *P. MERGUIENSIS* JUVENILES

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(Received 18.3.02, Accepted 4.6.02)

Key words: animal protein substitues, feeds, *Penaeus indicus, Penaeus merguiensis*, plant proteins

Abstract

The growth rate and survival of two white shrimps, *Penaeus indicus* and *P. merguiensis,* fed diets in which fishmeal was partially replaced with plant protein sources were investigated in three trials. In trial 1 with *P. indicus,* soybean, yeast and leaf meals of kangkong, papaya and *Cassia tora* L. were screened as partial substitutes for fishmeal. The total biomass of shrimp fed 20% yeast (20yeast) was highest but not significantly different than that of shrimp fed 10yeast and 10papaya. Survival was highest with 20yeast, 10papaya and 10yeast . Shrimp fed *Cassia tora* L*.* had the highest weight gain and SGR but their survival was similar to those fed poor performing diets. In trial 2 with *P. merguiensis,* the ingredients were modified by decreasing fishmeal and increasing the yeast and soybean substitution. The biomass of the shrimp fed 10yeast was similar to that of the shrimp fed 20yeast and 26soybean, the weight gain and SGR were similar to shrimp fed 20yeast while survival was highest but not different from 20yeast and 26soybean. In trial 3 with *P. indicus*, weight gain and SGR were best with 20yeast and 34soybean. However, biomass and survival did not differ among replacement levels.

The performance of the white shrimp varied with different levels of yeast and soybean meal incorporation. The response of *P. indicus* was best with 20yeast (15% by weight) or 34soybean meal (34% by weight) while that of *P. merguiensis* was with 10yeast (7% by weight), 20yeast (15% by weight) or 26soybean meal (26% by weight). Partial replacement of fishmeal with yeast or soybean meal would result in lower feed costs but the use of these feeds needs further refinement since survival was low in all treatments. Rearing techniques, such as increasing the feeding frequency, simulating deep pond conditions or using adequate substrates, should be refined.

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Introduction

White shrimps, *Penaeus indicus* and *P. merguiensis,* are usually secondary crops on extensive *P. monodon* and milkfish farms. However, there is a growing demand for them in local markets and the USA and Europe are prospective markets if the shrimps can be grown to the size of American shrimp (Apud, 1990). The period required to culture white shrimp is shorter than that of *P. monodon,* but Boonyaratpalin (1998) pointed out that relatively low yields and survival hinder intensive culture of these shrimps. Present culture practices use expensive commercial pellets for *P. monodon*. Fishmeal, a common source of protein, is also costly and sometimes of poor quality. Thus, there is a need for an alternate source of protein.

Generally, a combination of protein sources forms a better and more efficient diet than a single protein source. Soybean meal as a substitute protein source has been used in other penaeid feeds but the best level for white shrimp has not been tested. The use of single cell protein to partially replace the over-used fishmeal in diets for farmed animals is promising (Spinelli et al., 1979; Tacon, 1987). Alkane yeast was successfully incorporated in salmonid rations, replacing 25-50% with no loss in growth or feed efficiency (Tacon and Jackson, 1985). Although single cell protein is a poor source of dietary lipid and calcium, it is an excellent source of dietary vitamins (Tacon, 1987). Colvin (1976) incorporated food yeast at 6.5% by weight in *P. indicus* diets and obtained a weight gain of 500%.

P. indicus and *P. merguiensis* should have similar requirements since they are closely related species. The protein requirement of *P. merguiensis* is 34-50% (Aquacop, 1978; Sedgwick, 1979) with a 290-440 kcal/100 g diet. Colvin (1976) obtained good growth of *P. indicus* using 43% protein with a 3:2 fishmeal:shrimp meal combination. Various studies have been done on plant protein replacement in fish and some penaeid diets but, so far, none has been done on white shrimp diets. Hence, this study was undertaken to examine growth and survival of the white shrimps, *P. indicus* and *P. merguiensis*, fed diets in which fishmeal was partially replaced by different plant protein sources.

Materials and Methods

Diet formulation and preparation. Various plant protein sources (Table 1) were screened to assess their effect on growth and survival of white shrimps. These plant sources were incorporated at 4.5% protein or 10% of the calculated crude protein in the diet. Two sets of diets were formulated (Table 2), containing roughly 50% and 40% protein, respectively (Table 3). The major protein source was fishmeal. Prawn head meal contributed around 8% of the dietary protein and was constant in all the diets to provide the quality protein needed by the shrimps. Gelatinized bread flour varied from diet to diet to compensate for the differences in weights of the plant protein sources. Carrageenan was used as a binder in addition to the flour.

Based on total biomass and survival from trial 1, soybean and a combination of yeast and soybean were chosen to replace some of the fishmeal in succeeding trials and the protein level was reduced to 40%. The same diets were used in trial 2 with *P. merguiensis* and trial 3 with *P. indicus*.

The ingredients were thoroughly mixed in a Kitchen Aid mixer. An adequate amount of water was added to produce dough that could pass through the 2 mm diameter die of the grinder. Resulting pellets were steamed for about three minutes and dried at 60°C in a convection oven for about six hours. Pellets of about 2 x 3 mm were stored at 4°C in sealed plastic containers, ready for the feeding trials.

Feeding experiment. White shrimp juveniles of *P. indicus* (trials 1 and 3) and *P. merguiensis* (trial 2) with average initial weights of 0.92, 0.21, and 0.57 g, respectively, were obtained from nursery tanks at SEAFDEC, Tigbauan, Iloilo, Philippines. The shrimps were acclimatized with commercial diets for two weeks prior to stocking in tanks. The animals were randomly distributed at ten ani-

Table 1. Plant protein sources used to formulate the diets.

Common or local name	*Scientific name*	*Description; source*
Prawn head meal		By-product in *P. monodon* processing; from fishing port complex
Fishmeal		Poultry grade; Peru
Soybean meal	*Glycine max*	Defatted or full, poultry grade
Yeast	*Candida utilis*	By-product of coconut research; Biotechnology Department, Univ. of the Philippines, Los Baños, Laguna
Swamp cabbage	*Ipomea reptans*	Leaves and tops, dried and ground
or kangkong	or *Ipomea aquatica*	
Papaya	*Carica papaya*	Leaves, dried and ground
Kamantolan	*Cassia tora* L.	Leaves and tops, dried and ground; local native
Bread flour		Food grade
Rice bran		First class; from rice mills
Cod liver oil		Poultry grade
Soybean oil		Food grade
Carrageenan		Crude; Shemberg Corp., Cebu City, Philippines

Table 2. Composition of experimental diets.

	Ingredients (g/100 g diet)					
Diets[1]	*Fishmeal*	*Plant source*	*Bread flour*	*Rice bran*	*Basal mix*[2]	*Animal:plant proteinratio*
Trial 1						
10Soybean, defatted	44.00	11.00	15.00	3.00	27.00	8:1
10Soybean, full	44.00	11.00	15.00	3.00	27.00	8:1
10Yeast	44.00	9.40	14.60	5.00	27.00	8:1
20Yeast	38.00	18.80	14.20	2.00	27.00	4:1
10Kangkong	44.00	11.80	15.20	2.00	27.00	9:1
10Papaya	44.00	20.00	9.00	—-	27.00	9:1
10*Cassia tora L.*	44.00	23.70	5.30	—-	27.00	9:1
Diets[1]	*Fishmeal*	*Soybean*	*Yeast*	*Bread flour*	*Basal mix*[3]	*Animal:plant protein ratio*
Trials 2 and 3						
17Soybean	34.00	17.00	—-	14.50	34.50	4:1
26Soybean	27.80	26.00	—-	11.70	34.50	2.5:1
34Soybean	21.60	34.00	—-	9.90	34.50	1.5:1
10Yeast	27.80	17.00	7.60	13.10	34.50	2.5:1
20Yeast	21.60	17.00	15.20	11.70	34.50	1.5:1

1 The number in the diet name corresponds to the percent of dietary protein contributed by the plant in the diet name.
2 Basal mix for trial 1 (g/100 g diet): prawn head meal 15; soybean oil 2; cod-liver oil 2; vitamin mix 1.5; mineral mix 1.5; carrageenan 5.
3 Basal mix for trials 2 and 3 (g/100 g diet): prawn head meal 17; rice bran 9.45; soybean oil 2; cod liver oil 2; vitamin-22 (vitamin-mineral mix for poultry) 1; vitamin C 0.05; carrageenan 3.

Table 3. Proximate composition of feedstuffs and experimental diets (g/100 g DM).

	Moisture	*Crude protein*	*Crude fat*	*Crude fiber*	*NFE*	*Ash*	*ME**
Feedstuffs							
Prawn head meal	8.58	53.04	4.86	14.88	4.24	22.98	
Fishmeal	1.86	73.33	13.59	1.59	0.00	11.49	
Soybean meal, full	5.38	41.84	18.28	4.65	29.45	5.78	
Soybean meal, defatted	8.39	42.78	0.71	5.16	43.99	7.36	
Yeast	10.25	51.30	1.01	1.70	37.73	8.26	
Kangkong	3.86	38.21	4.86	14.00	34.01	8.92	
Papaya	3.98	22.84	10.05	11.56	42.27	13.28	
Cassia tora L	5.03	19.28	3.78	12.83	53.86	10.25	
Trial 1							
10Soybean, defatted	3.87	49.24	9.60	6.22	22.10	12.84	371
10Soybean, full	3.33	50.66	10.91	4.99	20.09	13.35	381
10Yeast	4.44	51.18	10.58	4.70	19.67	13.87	380
20Yeast	7.86	49.57	9.44	4.25	23.34	13.40	376
10Kangkong	4.03	47.90	9.87	6.42	23.86	11.95	373
10Papaya	3.59	48.64	10.14	5.99	23.19	12.04	376
10*Cassia tora L*.	5.23	46.90	10.57	5.67	24.39	12.47	376
Trials 2 and 3							
17Soybean	4.51	42.50	7.24	4.87	28.11	17.28	342
26Soybean	3.85	42.14	8.16	4.92	29.44	15.34	352
34Soybean	3.86	42.36	7.19	6.86	28.96	14.63	344
10Yeast	4.38	41.83	7.56	5.03	30.63	14.95	350
20Yeast	3.72	41.88	8.72	5.67	29.11	14.62	354

* Metabolizable energy (kcal/100 g) is based on standard physiological values of 4.5, 3.3 and 8 kcal/g for protein, carbohydrate and fat, respectively (Brett and Groves, 1979).

mals per 50 l experimental fiberglass tank. Each treatment was replicated thrice in a completely randomized design. A flow-through water system with aeration was used. To prevent molting shrimp from being cannibalized, a net was suspended across the oval tank to serve as a substrate in addition to the standard four pieces of 2” PVC pipe placed in each tank. Black net covered the tanks to prevent escape of the shrimp. Initially, shrimp were fed 10% of their body weight, half at 09:00 and half at 16:00. Every two days, the feed amount was adjusted according to excess feed, which was siphoned out of the tank before feeding. Mortality was inspected every day. Aquaria were scrubbed every two weeks. The daily cleaning and flow-through system were designed to minimize accumulation of metabolites and growth of natural food organisms in the culture system. The animals were starved for 18 h prior to stocking. Each group of shrimps was weighed at the start, every two weeks and at the end of the 37 (trial 3) or 42-day period (trials 1 and 2).

Chemical analysis. Proximate analyses (AOAC, 1990) of the formulated feeds are shown in Table 3. Energy was calculated using 4.5, 3.3 and 8 kcal/g for protein, carbohydrate and fat, respectively.

Data analysis. Total biomass, percent weight gain, arcsine transformation of survival, and specific growth rate were analyzed using the general linear model procedure of SAS software (SAS Inst. Inc., Cary, NC). Treatment differences were compared using one-way analysis of variance and Duncan’s multiple range tests ($p<0.05$).

Results

Trial 1 - P. indicus. After 42 days, shrimp fed the diet with 20% yeast yielded the highest total biomass, although it was not significantly different from that of shrimp fed 10% yeast or 10% papaya (Table 4). Survival was significantly better amongst shrimp fed 10% papaya, 10% yeast or 20% yeast. Shrimp fed *Cassia tora* L. had the highest weight gain and SGR but their survival was low and similar to poor performing diets.

Trial 2 - P. merguiensis. The animal to plant protein ratio ranged 2-4:1 while the metabolizable energy ranged 342-354 kcal/100 g. The shrimp responded positively to 10% and 20% yeast replacement in all growth parameters after 42 days. Diets with 26% soybean performed similarly except for a lower weight gain. Increasing the soybean content to 34% resulted in poorer shrimp performance.

Trial 3 - P. indicus. Using the same diets as in trial 2, *P. indicus* biomass and survival did not significantly differ whether yeast or a greater proportion of soybean meal was incorporated*.* Weight gain and SGR, however, were highest with the 20% yeast and 34% soybean diets.

Discussion

Cannibalism was very prominent during the trials in spite of the shelters that were provided. In earlier feeding experiments with *P. monodon,* appendages were missing from dead animals. In this experiment, none or only a very small portion of the shrimp was left at all. There are undocumented reports of the disappearance of white shrimps, probably due to cannibalism. Survival was poor in all trials compared to earlier *P. monodon* trials (Peñaflorida, 1995). Therefore, the current trials were conducted only 37-42 days. Survival could possibly be improved if the feeding frequency were increased since food would be available to the shrimp at all times, preventing their need to attack one another. In a pond experiment, Apud (1990) obtained lower survival at a depth of 40-70 cm (38%) than at 70-100 cm (70%). Hence, tanks should simulate deep pond conditions by covering the area with black plastic sheets.

In trial 1, fishmeal was replaced with plants at a 10% level, except for yeast which was also tried at a 20% replacement level. The *P. indicus* responded positively to soybean meal and yeast. Studies have shown that combinations of animal and plant proteins yield better results than a single source of protein. However, the ratio between animal and plant proteins in earlier studies did not vary. Shrimp fed the diet with 20% yeast, the

Table 4. Biomass, weight gain, survival and SGR of white shrimp fed different diets.

Diets	*Initial weight (g)*	*Final weight (g)*	*Total biomass (g)*	*Weight gain (%)*	*Survival[1] (%)*	*SGR[2]* (%/day)
Trial 1 - P. indicus, 42 days						
10Soybean, defatted	0.91±0.02[a]	1.65±0.13[bc]	5.57±0.95[b]	80.19±9.68[b]	35±2[b]	1.39±0.13[b]
10Soybean, full	0.99±0.07[a]	1.91±0.06[ab]	5.61±0.91[b]	93.10±8.28[b]	33±4[b]	1.56±0.10[b]
10Yeast	0.87±0.07[a]	1.65±0.10[bc]	8.85±1.07[ab]	89.5±10.24[b]	47±2[a]	1.52±0.06[b]
20Yeast	0.97±0.10[a]	1.81±0.21[ab]	12.28±2.47[a]	86.91±2.91[b]	55±4[a]	1.43±0.03[b]
10Kangkong	1.01±0.01[a]	1.81±0.11[ab]	4.71±0.92[b]	79.31±0.67[b]	31±4[b]	1.38±0.14[b]
10Papaya	0.76±0.08[a]	1.34±0.13[c]	8.46±0.80[ab]	75.52±4.75[b]	53±2[a]	1.33±0.06[b]
10*Cassia tora* L.	0.90±0.06[a]	2.23±0.18[a]	8.07±0.17[b]	148.31±5.12[a]	37±2[b]	2.15±0.14[a]
Trial 2 - P. merguiensis, 42 days						
17Soybean	0.52±0.03[a]	1.02±0.04[b]	5.49±0.56[c]	98.70±6.91[bc]	47±2[c]	1.63±0.08[bc]
26Soybean	0.61±0.03[a]	1.33±0.07[a]	9.71±0.17[a]	117.00±1.49[b]	59±2[ab]	1.84±0.01[ab]
34Soybean	0.58±0.02[a]	1.08±0.04[b]	7.63±0.92[b]	88.54±9.05[c]	57±4[b]	1.50±0.11[c]
10Yeast	0.58±0.02[a]	1.37±0.07[a]	11.37±0.25[a]	137.13±7.92[a]	66±3[a]	2.05±0.08[a]
20Yeast	0.56±0.02[a]	1.32±0.05[a]	10.14±0.44[a]	138.20±0.95[a]	61±2[ab]	2.07±0.00[a]
Trial 3 – P. indicus, 37 days						
17Soybean	0.22±0.02[a]	0.44±0.04[a]	1.85±0.16[a]	98.56±2.58[b]	41±4[a]	1.85±0.03[b]
26Soybean	0.22±0.01[a]	0.49±0.04[a]	1.25±0.21[a]	124.81±18.13[b]	31±4[a]	2.17±0.21[b]
34Soybean	0.19±0.00[a]	0.50±0.01[a]	1.52±0.32[a]	169.49±7.00[a]	33±3[a]	2.68±0.07[a]
10Yeast	0.23±0.02[a]	0.48±0.03[a]	1.63±0.27[a]	114.69±4.2[b]	35±2[a]	2.06±0.05[b]
20Yeast	0.22±0.02[a]	0.60±0.04[a]	1.79±0.13[a]	172.64±3.87[a]	33±0[a]	2.71±0.03[a]

Means in a column followed by the same letter are not significantly different ($p<0.05$).
[1] Arcsine transformed
[2] $SGR = (\ln W_f - \ln W_i)/time \times 100$

only diet with a 4:1 animal:plant ratio, attained the highest biomass and survival. The rest of the shrimp, fed diets with a ratio of 8-9:1, did not perform as well, except for those fed 10% yeast or 10% papaya. These two diets produced shrimp with a survival rate similar to that of the shrimp fed the 20% yeast diet but, while their biomass was comparable with that of the shrimp fed 20% yeast, it did not differ from the rest of the diets. Papaya leaf meal was used to feed *P. monodon,* replacing 16% of the diet, and good growth was attained (Peñaflorida, 1995). The shrimp fed the leguminous *Cassia tora* L. had the highest weight gain and SGR but its total biomass and survival were low. It may be worthwhile to consider this plant as a potential food source since it grows wild locally and can easily be cultivated. Anti-nutritional factors in leaf meals (such as tannin) are not a problem. Peñaflorida (1995) showed that tannin was not a problem when leaf meal was included at 16% of the diet.

For the second and third trials, the protein level was reduced to 40% and the animal to plant ratio was reduced to 1.5-2.5:1. Calculated energy was also reduced to 342-354 kcal/100 g. The reductions of the animal:plant ratio and energy were minimal and did not seem to influence the performance of the diets, especially in trial 3. In trial 2, the combination of soybean and yeast (10yeast and 20yeast) resulted in better weight gain, survival and SGR than those attained with soybean alone, except for biomass in the 26soybean treatment. In trial 3, the combination of soybean and yeast (20yeast) performed equally well as 34soybean. This affirms the common observation that imbalances in protein sources can be corrected with a combination of feedstuffs. Since growth seems better with a combination of soybean and yeast, the use of yeast may constitute a considerable savings in feed costs. The yeast used in this study, *Candida utilis,* is a by-product of the coconut industry and could be an inexpensive ingredient if it can be used in its moist form (since drying requires added expense). The question, however, is the maximum tolerable amount for white shrimp; these feeding trials did not show the toxic level. Obtaining yeast may be problematic since it comes from experiments in the Biotechnology Department of the University of the Philippines in Los Baños. Yeast and *C. tora* L. are not commercially available but are intended for use in small-scale farm-made aquafeeds. The favorable results of these trials should encourage farmers to explore the use of exotic feed ingredients that are available locally.

The performance of the white shrimps varied with different levels of yeast and soybean incorporation. *P. indicus* responded best with 20% yeast protein (15% by weight) or 34% soybean protein (34% by weight) while *P. merguiensis* responded best with 10% yeast protein (7% by weight), 20% yeast protein (15% by weight) or 26% soybean protein (26% by weight). Partial replacement of fishmeal with yeast or soybean meal would result in lower feed costs but the use of these ingredients needs further refinement since the survival rate was low in all treatments. The feeding frequency should be increased so that feed is available to the shrimp at all times. Rearing techniques, such as simulating deep water conditions and using adequate substrates, should be refined.

Acknowledgments

The author is grateful to S. Magallanes and the CAL staff for technical assistance and M.T. Castaños for the review.

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