Efficacy of fermented prawn shell waste as a feed ingredient for Indian white prawn, *Fenneropenaeus indicus*

B. AMAR[1], R. PHILIP[1] & I.S. BRIGHT SINGH[2]
Schools of [1] Marine Sciences and [2] Environmental Studies, Cochin University of Science and Technology, Cochin, Kerala, India

Abstract

Prawn shell waste collected from shrimp-processing plants in Cochin, India, was subjected to fermentation using 20 chitinoclastic and proteolytic/non-proteolytic bacterial strains. The products generated were analysed for protein, lipid, total sugars, *N*-acetyl glucosamine, free amino acids and ash. Shrimp diets were prepared using these 20 fermented products and a control diet using raw prawn shell waste. Feeding experiment was conducted with postlarvae (PL21) of Indian white prawn, *Fenneropenaeus indicus* for a period of 21 days. Biogrowth parameters such as mean weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio were estimated and the animals were challenged with white spot virus orally via diet. Enhanced growth could be observed in prawns fed F134 and F124, incorporated with the fermentation products generated using *Bacillus* spp., C134 and C124 respectively. The percentage survival of prawns after 7 days of challenge was found to be highest for groups fed diet F111 incorporated with fermentation product generated using *Bacillus* sp. These products of bacterial fermentation hold promise as growth enhancers and immunostimulants in aquaculture.

KEY WORDS: biogrowth parameters, feed ingredient, *Fenneropenaeus indicus*, fermentation, prawn shell waste, survival

Received 8 July 2005, accepted 19 June 2006

Correspondence: Dr Rosamma Philip, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682 016, Kerala, India. E-mail: rose@cusat.ac.in

Introduction

The shellfish-processing industry in India generates about 8.5 million tonnes of waste per year. Although a part of this is used for chitin/chitosan preparation, feed manufacture and as manure, a major portion still remains unused. This waste contains 200–350 g kg^{-1} crude protein, 150 g kg^{-1} chitin and 50 g kg^{-1} fat. A method for the complete utilization of this waste is required as a means of recovering and recycling nutrients, as opposed to disposal and subsequent environmental consequences. Prawn shell waste protein is rich in essential amino acids (Forster 1975; Penaflorida 1989) and the oil extracted from shrimp head contains polyunsaturated fatty acids (PUFA) essential for shrimps (Joseph & Meyers 1975; Joseph & Williams 1975). Dietary glucosamine was also found to be a growth-promoting factor in shrimp (Kitabayashi *et al*. 1971). Shrimp waste seems to act as an attractant in shrimp diets (Pascual & Destajo 1978) and the shell (chitin) in shrimp waste is found to have a growth-promoting effect in *Penaeus indicus* (Vaitheswaran & Ahamad Ali 1986; Clarke *et al*. 1993).

The utilization of shrimp waste in shrimp diets has been studied by various workers (Venkitaramaiah *et al*. 1978; Ahamad Ali 1982; Ahamad Ali & Mohamed 1985; Akiyama *et al*. 1989; Penaflorida 1989; Lim & Dominy 1990; Nwanna 2003). Differences in performance were attributed to differences in quality and shrimp waste was considered a potential feed ingredient having high biological value. The effect of dietary chitin on the growth and survival of juvenile *P. monodon* was studied by various workers (Fox 1993; Lan & Pan 1993; Das *et al*. 1995; Sudaryono *et al*. 1996). Fermentation is a process that enhances the nutritive quality of the substrate. The lactic acid fermented fish silage has been successfully used as aquafeed ingredient (Fagbenro *et al*. 1994; Hall & De Silva 1994; Fagbenro & Jauncey 1995; Fagbenro *et al*. 1997). The enhanced nutritional value and digestibility of fermented shrimp head waste by fish was reported by Plascencia-Jatomea *et al*. (2002) and Nwanna (2003).

The process of fermentation is therefore a feasible alternative that envisages the bioconversion of prawn shell waste into a partially hydrolysed product enriched with microbial

biomass. The present work evaluates the use of chitinoclastic and proteolytic bacterial strains for fermentation of prawn shell waste as a means of value addition by transforming it into a more nutritious feed ingredient.

Materials and methods

Microorganisms used

Twenty bacteria (chitinoclastic and proteolytic/non-proteolytic) belonging to *Bacillus*, Coryneforms, *Vibrio*, *Pseudomonas*, *Streptococcus* and Enterobacteriaceae maintained in the Microbiology Laboratory of the School of Marine Sciences were used for the study (Table 1). These strains were isolated from shellfish waste as well as from coastal sediments off Cochin, India. Characterization and identification of the cultures were carried out as per Buchanan & Gibbons (1974). The chitinoclastic and proteolytic properties of the selected strains were assessed by clearing zone formation on chitin agar plates and prawn flesh agar plates respectively (Amar & Philip 2004).

Prawn shell fermentation product

Shell waste collected from prawn-processing plants in Cochin were dried in an oven at 80 °C overnight, powdered, sieved through a 0.5-mm sieve and used as the raw material for fermentation. Chitinoclastic bacteria (20 strains) isolated from shell waste, and maintained in the laboratory were used for the study (Table 1). All the strains used were indexed as strain number with prefix C for chitinolytic property exhibited. One hundred grams of powdered shell waste was inoculated with the selected bacterial strains for fermentation. Solid state (1 : 1.5 w/v shell powder and 15 g L^{-1} sea water) and submerged state (1 : 4 w/v shell powder and 15 g L^{-1} sea water) fermentations were carried out as per the preference of the selected strains based on previous study (Amar & Philip 2004). The flasks were incubated at 28 ± 2 °C for 14 days during which regular mixing was performed. Then the fermentation product was dried at 60 °C for 18 h and used as an ingredient for feed preparation. All the fermented products were successively named as FP-strain number to signify the fermented product corresponding to the strain used.

Experimental feed formulation

Ingredients as given in Table 2 were mixed well into a dough with water, steamed for 10 min in an autoclave under atmospheric pressure and pelletized using a laboratory model hand pelletizer having a 1-mm-diameter die. Pellets were dried in an oven at 50 °C for 24 h. Twenty different feeds

Table 1 Characteristics of the bacterial strains selected for the study

	Morphological and biochemical tests						Physiological grouping		
Culture no.	Gram's reaction	Morphology	MOF	Oxidase	Motility	Spore	Chitinase	Protease	Genera
C14	+	Rods	F	+	+	–	+++	++	Coryneforms
C15	+	Rods	F	+	–	+	+++	+++	*Bacillus*
C18	+	Rods	F	+	+	–	++++	++	Coryneforms
C29	–	Rods	F	+	+	–	++	–	*Vibrio*
C33	–	Rods	F	+	+	–	++	–	*Pseudomonas*
C48	+	Cocci	Falk	+	–	–	++	++	*Streptococci*
C52	–	Rods	Falk	+	–	–	++	+	*Vibrio*
C84	–	Rods	F	+	+	–	+	+	*Pseudomonas*
C111	+	Rods	F	+	–	+	+++	+	*Bacillus*
C123	+	Rods	F	+	+	–	++	++	Coryneforms
C124	+	Rods	Falk	+	–	+	+++	++	*Bacillus*
C134	+	Rods	F	–	–	+	++	++	*Bacillus*
C149	–	Cocci	F	–	–	–	+++	++	Enterobacteriaceae
C153	+	Rods	Falk	+	–	+	+++	++	*Bacillus*
C154	+	Rods	F	+	–	+	+++	++++	*Bacillus*
C157	–	Rods	F	+	+	–	++	–	*Vibrio*
C163	–	Cocci	F	–	–	–	++	++	Enterobacteriaceae
C218	–	Rods	F	+	+	–	++	–	*Vibrio*
C219	–	Rods	F	+	+	–	+++	–	*Vibrio*
C220	+	Rods	F	–	–	+	+++	–	*Bacillus*

++, +++, ++++ represent increasing grades of halo size; MOF = Marine Oxidation Fermentation Test.

Table 2 Composition of experimental diets

Ingredients	Control diet (FC) (g kg^{-1})	Experimental diet (g kg^{-1})
Prawn shell powder	250	–
Fermented prawn shell[1]	–	250
Fish meal	280	280
Groundnut oil cake[2]	80	80
Soybean meal[3]	150	150
Maida[4]	100	100
Rice bran[5]	100	100
Vitamin and mineral mix[6]	20	20
Agar	20	20
Water (mL)	1000	1000

[1] Twenty different fermented products were prepared which were incorporated in the 20 experimental diets prepared.
[2] Prepared by grinding the cake remaining after oil extraction from ground nuts.
[3] Prepared by grinding the flakes remaining after oil extraction from soybean.
[4] Refined wheat flour.
[5] Finely ground rice bran.
[6] Vitamin and mineral mix (mg g^{-1} vitamin and mineral mix): thiamine, 0.61 mg; riboflavin, 0.48 mg; pantothenic acid, 2.42 mg; pyridoxine, 0.72 mg; cyanocobalamin, 0.02 mg; biotin, 0.02 mg; retinol, 0.13 mg; menaptone, 0.24 mg; folic acid, 0.13 mg; niacin, 2.42 mg; α-tocopherol, 2.42 mg; banox, 0.30 mg; cholecalciferol, 0.06 mg; ascorbic acid, 6.05 mg; K_2HPO_4, 4.68 mg; $Ca_3(PO_4)_2$, 6.36 mg; $MgSO_4{\cdot}7H_2O$, 7.12 mg; $NaH_2PO_4{\cdot}2H_2O$, 1.84 mg.

were prepared incorporating the 20 fermented products plus the control diet with raw prawn shell powder. Water stability of the feed was checked by immersing pellets in sea water for 18 h and examining the structural stability by visual observation. Feeds were stored in polythene bags in a freezer (−20 °C). All the feeds prepared were named F-strain number to indicate the respective strain and successive fermented product derived by it.

Proximate composition of the fermented products and the experimental diets

Biochemical composition of the shell fermentation products and the experimental diets were estimated: protein in the fermented product (Lowry *et al.* 1951), lipid by chloroform-methanol extraction (Folch *et al.* 1957), total sugars (Roe 1955), *N*-acetyl glucosamine (NAG) (Reissig *et al.* 1955) and free amino acids (FAAs) (Yemm & Cocking 1955). Micro-Kjeldahl distillation method (Barnes 1959) was employed for the estimation of protein ($N \times 6.25$) in the diet. Crude fibre in experimental feed was estimated as per AOAC (1990) and lipid by chloroform-methanol extraction (Folch *et al.* 1957). Moisture content was assessed by drying in an oven at 80 °C to constant weight and ash by incineration at 600 °C in a muffle furnace for 5 h. The nitrogen-free extract (NFE) was computed by difference (Crompton & Harris 1969).

Feeding experiment

Postlarvae (PL21) of Indian white prawn (30–50 mg, *Fenneropenaeus indicus*) were brought to the laboratory from a commercial prawn hatchery in Kannamali, Cochin. They were acclimatized to experimental tanks and maintained on prepared control diet for a period of 1 week. Larvae were then stocked into 30-L rectangular fibreglass tanks, 25 individuals per tank, and reared on the experimental diets for 21 days. Feeding trials were conducted using triplicate tanks for each treatment. The initial body weight of all the postlarvae in a tank was taken and the average weight was calculated. Fifty per cent water exchange was made with fresh sea water on alternate days. Aeration was provided from a 1 hp compressor through air stones. Physico-chemical parameters of the rearing water were monitored daily and adjusted to the desired range by water exchange (Table 3). Salinity, NH_3-N, NO_2-N, NO_3-N and dissolved O_2 were estimated as per APHA (1995).

Twenty-one different feeds were given to the prawns including one control diet. Prawns were fed twice daily morning and evening at 10 a.m. and 4 p.m. respectively at 15–20% of the body weight per day. Faecal matter was siphoned out twice daily, before the supply of fresh diet and water exchange was performed in the morning after removal of faeces. Uneaten feed was also collected twice daily (before the supply of fresh diet), placed on preweighed filter paper and washed with fresh water to remove salt, dried in an oven

Table 3 Rearing conditions and water quality parameters of the experimental system

Parameter	Mean ± SD
Initial body weight (average)	12.4 ± 0.8 mg
Stocking density	25 (per tank)
Rearing and feeding conditions	
Tank capacity	30 L
Feeding level	10–15% body weight
Feeding frequency	Twice daily
Feeding period	21 days
Temperature	28 ± 2 °C
pH	7 ± 0.5
Salinity	26 ± 2 g L^{-1}
Dissolved oxygen (mg O_2 L^{-1})	7 ± 1
Ammonia (mg NH_4^+ L^{-1})	0.01 ± 0.005
Nitrate (mg NO_3^- L^{-1})	n.d.
Nitrite (mg NO_2^- L^{-1})	<0.01

n.d. = not detectable.

Table 4 Proximate composition of the various fermented products (on dry matter basis)

Feed	Protein (g kg^{-1})	Lipid (g kg^{-1})	NAG (g kg^{-1})	Total sugars (g kg^{-1})	Free amino acids (g kg^{-1})
Control	325	51	0.43	25	15
FP14	239	96	0.30	22	7
FP15	449	50	0.45	28	9
FP18	355	70	0.30	38	7
FP29	462	36	0.29	27	14
FP33	258	32	0.29	45	17
FP48	278	72	0.26	46	5
FP52	238	75	0.29	28	14
FP84	459	28	0.25	25	13
FP111	378	107	0.24	42	22
FP123	448	48	0.25	22	8
FP124	472	82	0.12	30	10
FP134	290	106	0.27	43	16
FP149	339	52	0.24	41	13
FP153	262	49	0.26	23	16
FP154	390	52	0.37	26	10
FP157	271	87	0.14	27	8
FP163	448	81	0.39	44	14
FP218	322	73	0.44	37	10
FP219	306	35	0.23	23	18
FP220	365	73	0.32	43	26

NAG, *N*-acetyl glucosamine.

at 80 °C for 24 h and the weight was recorded. After 21 days of feeding, final wet body weight of all the prawns were taken and the average weight was calculated. Parameters including mean weight gain of prawns, food conversion ratio (FCR), specific growth rate (SGR) and protein efficiency ratio (PER) were determined based on the data collected during the experimental period. Survival for the various treatment groups and control was recorded.

Challenge experiment

After termination of the feeding experiment (21 days) all treatment groups including the control, were maintained under the same rearing conditions. A challenge with white spot virus (WSV) was performed through oral administration. For this, prawns were fed with WSV-infected prawn flesh (*F. indicus* adult) in the morning (after a starvation period of 12 h) and evening *ad libitum* for 1 day ensuring availability of infected meat to all the prawns in the tank and then maintained on the corresponding experimental diets for the following days. All the rearing conditions were maintained as in Table 3.

Survival rate was recorded every day for a period of 7 days by which time almost complete mortality was recorded in 90% of the treatment groups. Mortality was noted at the end of the experiment and expressed as percentage survival. Statistical analysis of the results was performed using ANOVA and differences between mean values examined by Duncan's multiple range test with SPSS software package (SPSS Inc. 2003).

Results

Proximate composition of the fermented products

The proximate composition (on dry weight basis) of the fermented products are given in Table 4. Protein was maximum in FP124 (472 g kg^{-1}) followed by FP29 (462 g kg^{-1}) and FP84 (459 g kg^{-1}). Lipid content varied from 32 to 107 g kg^{-1}. Lipid was highest in FP111 and FP134 (107 and 106 g kg^{-1} respectively). In the case of NAG except for FP15 (0.45 g kg^{-1}) and FP218 (0.44 g kg^{-1}), all values recorded were lower than that in the control (raw prawn shell powder). For total sugars the highest value noted was for FP48 (46 g kg^{-1}) followed by FP33 (45 g kg^{-1}). Considerable variation could be noted in the FAA content of various fermented products ranging from 5 to 26 g kg^{-1} with the highest value for FP220.

Proximate composition of experimental feeds

The proximate composition of the experimental diets utilized for screening the efficacy of the fermentation products as potential feed ingredient are presented in Table 5. The protein content of the feeds ranged from 416 to 572 g kg^{-1}, the highest value in feed F124 (572 g kg^{-1}) was followed by F29 (566 g kg^{-1}) and F163 (556 g kg^{-1}). Lipid content in the feed was highest for feed F111 (139 g kg^{-1}) and F134 (136 g kg^{-1}). Fibre content was maximum in F163 (68 g kg^{-1}) followed by F18 (54 g kg^{-1}). Ash content did not vary significantly (121–133 g kg^{-1}) among the feeds with the highest value recorded for F153. Moisture content varied greatly among the different experimental feeds ranging from 29 to 66 g kg^{-1} and NFE ranged from 93 to 293 g kg^{-1}.

Biogrowth parameters

Growth performance was assessed by measuring the mean weight gain, the SGR (percentage weight gain per day), the FCR and PER at the end of the experiment (Table 6). Significant weight gain was noted with *F. indicus* maintained on most of the experimental feeds. The best mean weight gain was recorded for feed F134 (53.02 mg) followed by F124 (33.81 mg). FCR showed significant variation ranging from 1.7 to 6.47, the best FCR obtained

Table 5 Proximate composition of the experimental feeds (on dry matter basis)

Feeds	Crude protein (CP) (g kg^{-1})	Lipid (L) (g kg^{-1})	Crude fibre (CF) (g kg^{-1})	Crude ash (CA) (g kg^{-1})	Moisture (g kg^{-1})	NFE (g kg^{-1})
Control	475	83	42	102	35	298
F14	417	106	34	127	29	316
F15	557	56	38	121	49	228
F18	495	84	54	130	39	237
F29	566	51	48	129	37	206
F33	429	48	40	127	66	356
F48	443	94	43	127	58	293
F52	416	106	28	125	60	325
F84	564	41	41	106	30	248
F111	510	139	48	128	50	175
F123	423	67	42	121	54	347
F124	572	108	51	123	53	146
F134	451	136	45	128	55	240
F149	484	70	46	132	59	268
F153	432	69	41	133	45	325
F154	518	75	33	123	52	251
F157	439	119	48	131	43	263
F163	556	105	68	124	54	147
F218	472	91	41	124	30	272
F219	462	42	48	126	40	322
F220	440	101	30	126	41	303

NFE = nitrogen-free extracts including crude fibre = 1000 − (CP + L + CF + CA).
Values are not given with ± SD.

Table 6 Biogrowth parameters of *Fenneropenaeus indicus* postlarvae fed various experimental diets

Diets	Weight gain (g)[1]	FCR[2]	SGR[3] (% day^{-1})	PER[4]
Control	12.5^{b} ± 0.8	3.4^{e} ± 0.2	3.7^{a} ± 1.4	2.6^{ef} ± 0.17
F14	13.7^{b} ± 0.8	4.0^{fg} ± 0.2	6.8^{bc} ± 0.9	2.7^{fg} ± 0.12
F15	28.2^{h} ± 0.9	2.9^{d} ± 0.0	6.3^{bc} ± 1.1	2.3^{cd} ± 0.08
F18	15.9^{c} ± 0.6	4.0^{g} ± 0.2	7.5^{cd} ± 0.3	2.1^{c} ± 0.08
F29	9.4^{a} ± 0.8	5.5^{i} ± 0.5	4.3^{b} ± 0.9	1.4^{a} ± 0.12
F33	17.8^{cd} ± 0.9	3.7^{fg} ± 0.2	5.9^{bc} ± 1.2	2.4^{de} ± 0.12
F48	15.2^{bc} ± 0.9	4.1^{g} ± 0.3	5.0^{bc} ± 0.9	2.3^{cd} ± 0.14
F52	8.6^{a} ± 0.7	4.7^{h} ± 0.3	8.7^{d} ± 3.0	2.8^{gh} ± 0.15
F84	22.5^{ef} ± 0.7	2.8^{cd} ± 0.1	5.2^{bc} ± 0.1	2.5^{ef} ± 0.07
F111	22.8^{fg} ± 1.1	3.8^{fg} ± 0.2	6.6^{bc} ± 0.8	2.1^{c} ± 0.10
F123	19.8^{de} ± 0.6	3.1^{de} ± 0.1	6.1^{bc} ± 1.4	3.4^{kl} ± 0.10
F124	33.8^{i} ± 1.0	2.2^{bc} ± 0.01	8.1^{cd} ± 0.7	3.2^{jkl} ± 0.09
F134	53.0^{j} ± 1.2	1.7^{a} ± 0.03	9.8^{d} ± 3.1	5.2^{n} ± 0.11
F149	27.7^{h} ± 1.0	2.9^{d} ± 0.1	7.6^{cd} ± 0.4	3.0^{hi} ± 0.11
F153	25.6^{g} ± 1.0	2.9^{d} ± 0.1	12.9^{d} ± 0.6	3.6^{m} ± 0.14
F154	19.0^{de} ± 0.8	4.2^{g} ± 0.2	4.9^{bc} ± 0.1	1.9^{b} ± 0.08
F157	23.6^{fg} ± 1.1	2.5^{bc} ± 0.1	6.1^{bc} ± 0.3	3.4^{l} ± 0.16
F163	27.9^{h} ± 0.5	6.4^{j} ± 0.1	7.6^{cd} ± 1.7	2.5^{def} ± 0.09
F218	15.8^{c} ± 0.8	2.9^{d} + 0.1	7.4^{bcd} ± 2.5	3.1^{ijk} ± 0.13
F219	14.9^{bc} ± 1.3	2.6^{cd} + 0.2	9.2^{d} ± 1.5	3.0^{hij} ± 0.18
F220	16.4^{c} ± 0.7	3.0^{d} + 0.01	8.9^{d} ± 3.3	3.3^{kl} ± 0.10

Values are given as mean ± SD, $n = 3$.
[1] Weight gain = $(w_2 - w_1)$ (g).
[2] FCR = Food consumed (g) × live weight gain (g)$^{-1}$.
[3] SGR = (ln w_2 − ln w_1) × t^{-1} × 100, $t = 21$ days.
[4] PER = $(w_2 - w_1)$ × [protein consumed (g)$^{-1}$ d.m.].
Values with the same superscript does not vary significantly.

being with *F. indicus* fed F134 (1.7) followed by F124 (2.21). SGR was maximum with feed F153 (12.9% day^{-1}) followed by F134 (9.9% day^{-1}). PER varied from 1.4 to 5.2 for the various feeds. The highest PER for feed F134 (5.2) was found to be significantly different from the next highest value of 3.6 by feed F153.

Duncan's multiple range test showed that the performance of the feeds in terms of biogrowth parameters differed significantly and it is presented in Table 6. Feeds F134 and F124 gave the best performance.

The percentage survival of the postlarvae after 21 days of feeding on experimental diets is shown in Fig. 1. The shrimp fed control feed showed 80% survival. The percentage survival for treatment diets varied from 41% to 100%. The survival was best with feed F218 (100%) followed by F157, F15, F33, F48 and F52, all showing above 90% survival. The percentage survival of the postlarvae fed on experimental diets after challenge with WSV is given in Fig. 2. Postchallenge survival after 7 days was best with F111 (75%) followed by F48 (66.7%). Very low survival was recorded for shrimps fed diet F163 (i.e. below 20%).

The correlation between the biogrowth parameters and proximate composition of the various fermented products incorporated in the feeds is given in Table 7. Correlation of biogrowth parameters with that of the proximate composition of the fermented products did not show a significant positive correlation with any of the factors except for the free fatty acids, which showed a positive correlation ($P < 0.1$) with SGR. Postchallenge survival had a positive correlation ($P < 0.1$) with the lipid content in the fermented products.

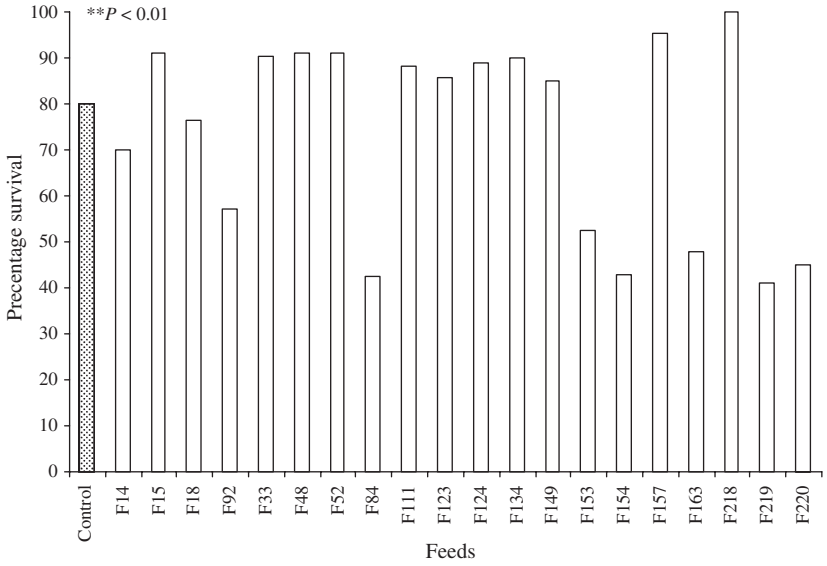

Figure 1 Survival of the postlarvae after 21 days of feeding experiment with various experimental diets.

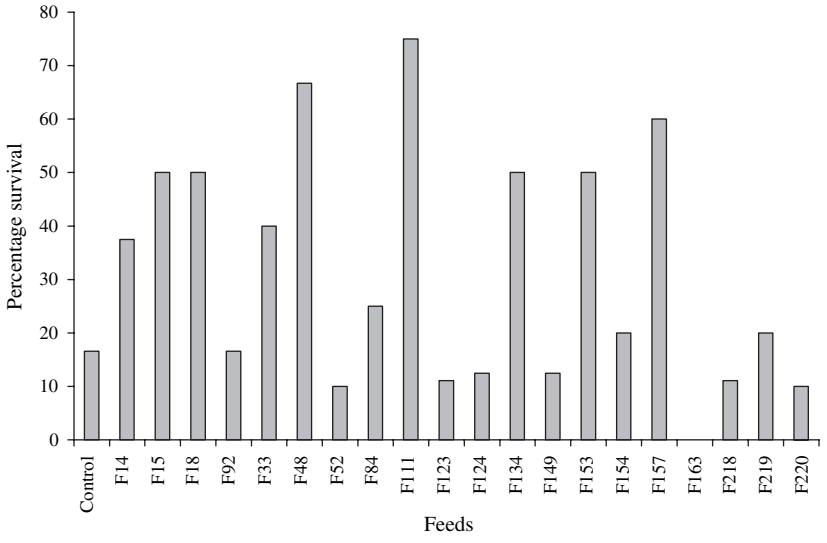

Figure 2 Percentage survival of postlarvae fed on experimental feed for 21 days and then challenged with white spot virus (day 7).

Table 7 Correlation (r value) between the biogrowth parameters and biochemical components of the various fermented products

Biogrowth parameters	Proteins	Carbohydrates	Lipids	NAG	Total sugars	Free fatty acids
Weight gain	0.01	−0.107	0.248	−0.298	0.153	0.122
FCR	0.1156	0.201	−0.091	0.204	0.005	−0.093
SGR	−0.375	0.074	0.109	−0.288	−0.027	0.364*
Survival (prechallenge)	−0.300	0.012	0.421**	−0.072	0.265	−0.268
Survival (postchallenge)	−0.183	0.026	0.385**	−0.237	0.188	−0.094

*$P < 0.1$, **$P < 0.01$.

Discussion

Considerable increase in nutrient content was noted in terms of protein, lipid and total sugars in most of the fermented products. Proliferation of bacteria in the chitinous substrate might have contributed to the enrichment of protein because of biomass buildup. The chitinoclastic and proteolytic strains performed better in terms of product enrichment as evidenced by the biochemical analysis of the fermented product. Almost all high protein enrichment values were observed for products by chitinoclastic and proteolytic forms like FP124 (472 g kg^{-1}), FP48 (459 g kg^{-1}), FP15 (449 g kg^{-1}) and FP163 (448 g kg^{-1}), except for FP29 (462 g kg^{-1}) which is a product by a chitinoclastic non-proteolytic strain. Hydrolysis of chitin in prawn shell, and to a certain extent the protein hydrolysis, would have enhanced the nutritional quality in addition to improvement in texture and odour of the product. Microbial protein is believed to contribute significantly to the protein content of the fermentation product. Protein enrichment of prawn shell waste have been reported by Rhishipal & Philip (1998). A similar enrichment of protein and lipid was noted in fermented sesame seed meal by Mukhopadhyay & Ray (1999).

Increase in total sugars can be attributed to the hydrolysis of the complex substrate (chitin) leading to increased levels of soluble oligosaccharides thereby enhancing digestibility. Very low value of NAG obtained by fermentation may indicate the rapid uptake of monomeric NAG by bacteria as soon as it is released during hydrolysis of the substrate. FAA enrichment of the fermented product helps in assimilability of the product. Increase in FAAs (10-fold) and vitamin content of rye fodder by fermentation has been reported by Penaloza *et al*. (1985) and Klappach *et al*. (1991). In this study, only very few fermented products showed an increase in FAAs which may be due to the utilization of FAA by the microorganisms themselves.

Dietary protein has been reported as the most essential nutrient for the growth of prawns (Andrews *et al*. 1972; Balazs *et al*. 1973; Forster & Beard 1973; Venkitaramiah *et al*. 1975; Alava & Lim 1983). Penaeid shrimps require 350–400 g kg^{-1} protein, 80–100 g kg^{-1} fat rich in PUFA and 350 g kg^{-1} carbohydrate in their diet. The protein content of the feeds ranged from 416 (F52) to 574 g kg^{-1} (F124). This range was found acceptable for optimum growth in penaeid prawns as shown by various earlier feeding experiments (Lee 1971; Hanson & Goodwin 1977; Lin *et al*. 1982; Alava & Lim 1983; Bautista 1986; Millamena *et al*. 1986; Shiau & Chou 1991; Shiau *et al*. 1991; Fox *et al*. 1994; Bautista & Subosa 1997). The varying content of protein obtained in the different feeds can be attributed to bacterial biomass generated during the fermentation process.

The protein quality of a feed ingredient depends on several factors like digestibility and content of essential amino acids, which are also crucial to the biological value of the protein. The presence of protein rich in lysine and methionine together with n-3 fatty acids (Sick & Andrews 1973; Sandifer & Joseph 1976; Kanazawa *et al*. 1979a,b; Menesveta *et al*. 1983) in prawn shell waste was found to enhance growth in shrimps. The quality of shrimp meal protein in terms of amino acid content was found to be close to that of prawn muscle (Deshimaru *et al*. 1985; Penaflorida 1989). This quality of the protein could play a very important role in the current growth enhancement reported in this study.

As the strains selected were highly proteolytic in nature, luxuriant bacterial growth was observed during the fermentation of the substrate. The nutritional value of the microorganisms used in aquaculture depends on their digestibility and assimilation characteristics and the target animal. The essential amino acid index of almost all bacteria was noted to be in the range 91–94. Being above the value 90, it is of good quality for use as aquaculture feed ingredient (Teshima *et al*. 1986; Penaflorida 1989). Bacteria like *Pseudomonas* and *Methylophilus* spp. have been investigated for use as single cell protein in aqua feeds. They have approximately 730 g kg^{-1} crude protein and 57 g kg^{-1} lipid and 27 g kg^{-1} NFE by weight (Kant 1996). Yeast was examined as a replacement for fish meal in rainbow trout diets by Dabrowski *et al*. (1980) and Aarseth *et al*. (2005). Yeasts and

bacteria have been evaluated as food for bivalve aquaculture. Protein is the major constituent of both yeast and bacteria (250–490 g kg^{-1} d.m.) (Kant 1996). In this study, bacteria belonging to the genera *Bacillus*, *Vibrio*, *Pseudomonas*, *Serratia* and Coryneforms were used for the fermentation of prawn shell waste. Products generated with *Bacillus* sp. gave the best performance in terms of various growth parameters (F134 and F124).

Recommended lipid levels for commercial shrimp feeds range from 60 to 75 g kg^{-1} and a maximum level of 10 g kg^{-1} was suggested by Akiyama & Dominy (1989). In the present study, lipid was found to be highest (139 g kg^{-1}) in feed F111 followed by F134 (136 g kg^{-1}). Lipid enhancement could be by bacterial production. Yongmanitchai & Ward (1989) have reported marine bacteria that produced EPA (elcosa pentanoic acid). Among the lipid compounds in the diets of shrimps, PUFA (poly unsaturated fatty acids), phospholipids and sterols have received the most attention in crustacean lipid nutrition. Microorganisms contain a diverse range of fatty acid composition and are rich sources of useful PUFA (Brown *et al.* 1996) and 20:5n-3 (Yazawa *et al.* 1988).

A positive correlation even though not significant could be obtained between the percentage survival and the lipid concentration in the various diet. Prawns fed shrimp head oil-augmented diet were found to grow significantly larger (Sandifer & Joseph 1976). Millamena *et al.* (1988) noted greater survival and growth in *P. monodon* larvae that were fed lipid-enriched *Artemia* nauplii. Sheen & Chen (1993) found that growth of *P. monodon* fed iso-nitrogenous diets supplemented with 80, 100 and 120 g kg^{-1} lipid were significantly higher than those with lower lipid content. Various studies with *P. japonicus* have demonstrated that dietary phospholipids enhance growth and survival of larvae (Teshima *et al.* 1982; Kanazawa *et al.* 1985; Camara *et al.* 1997) and postlarvae (Levine & Sulkin 1984). A detailed analysis of the fatty acid profile might reveal the presence and quantum of specific fatty acid components responsible for this better survival.

Growth performance coupled with high survival (after viral challenge) could not be obtained for any of the strains. Feeds which showed best growth performance (F134 and F124) exhibited an average postchallenge survival. F111 on the other hand exhibited an average performance for all other biogrowth parameters while exhibiting a significant postchallenge survival. Growth performance of some of the experimental feeds was superior when compared with earlier studies with raw prawn shell waste feed (Balazs & Ross 1976; Menesveta *et al.* 1983; Fox *et al.* 1994; Nwanna 2003; Nwanna *et al.* 2003). An FCR of 2.8 was recorded for shrimp meal diet by Jayalaksmy & Natarajan (1994). In the present study, better FCR could be obtained with F134 (1.7) and F124 (2.21). According to Forster (1970) and Nwanna (2003) only an FCR of 2–3 could be anticipated in prawns because of loss incurred during moulting, though the order of acceptance of the feeds varies in different species (Goswami & Goswami 1982).

Correlation of biogrowth parameters with that of the proximate composition of the fermented product did not show a significant positive correlation with any of the factors except for free fatty acids, which showed a positive correlation ($P < 0.1$) with SGR. The survival percentage after challenge with WSV had significant correlation with the lipid content. This observation indicate the importance of lipids as a component of the diet and necessitate a detailed analysis of the lipid profile of various fermented products to find out the specific fraction responsible for boosting growth as well as immune system leading to better survival.

The present study shows that the shell fermentation product can find good application as a potential feed ingredient in the aquaculture industry both in hatchery and grow out systems. The process would be cost-effective as the raw material is cheap and there being no additional energy input for its production. Moreover being an effective way of recycling of the waste through an upgradation technology, the process deserves much attention as far as the aquaculture and the processing industry is concerned.

Acknowledgements

Authors are grateful to the Director, School of Marine Sciences, for the facilities provided. The first author is thankful to the Cochin University of Science and Technology, Cochin, and the Council for Science and Industrial Research, New Delhi, for research fellowship granted.

References

Aarseth, K.A., Sørensen, M. & Storebakken, T. (2005) Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength: Weibull analysis. *Anim. Feed Sci. Technol.*, **126,** 75–91.

Ahamad Ali, S. (1982) Relative efficiencies of pelletized feeds compounded with different animal proteins and the effect of protein level on the growth of *Penaeus indicus*. *Proc. Symp. Coast. Aquacult. Mar. Biol. Assoc. India*, **1,** 321–328.

Ahamad Ali, S. & Mohamed, K.H. (1985) Utilization of prawn waste and mantis shrimp for compounding feeds for the culture of penaeid prawns. In: *Proceedings of the Symposium on the Harvest and Post Harvest Technology of Fish* (Ravindran, R.,

Nair, N.U., Perigreen, P.A. et al), pp. 615–618, 24–27 November 1982. Society of Fisheries Technologists, Cochin, India.

Akiyama, D.M. & Dominy, W.G. (1989) Penaeid shrimp nutrition for the commercial industry. In: *Proceedings of the People's Republic of China Aquaculture and Feed Workshop* (Akiyama, D.M. ed.), pp. 143–163, Singapore, September 17–30. American Soybean Association, Singapore.

Akiyama, D.M., Coelho, S.R., Lawrence, A.L. & Robinson, E.H. (1989) Apparent digestibility of feedstuffs by marine shrimp *Penaeus vannamei*. *Bull. Jpn. Soc. Sci. Fish.*, **55,** 91–98.

Alava, V.R. & Lim, C. (1983) The quantitative dietary protein requirement of *Penaeus monodon* juveniles in controlled environment. *Aquaculture*, **30,** 53–61.

Amar, B. & Philip, R. (2004) Nutritional enrichment of prawn shell waste by microbial transformation. In: *Marine Science into the New Millennium: New Perspectives and Challenges* (Moi, P.S., Ching, C.V., Chye, S.N. et al, eds), pp. 411–418. University of Malaya Maritime Research Centre, Malaysia.

Andrews, J.W., Sick, L.V. & Baptist, G.J. (1972) The influence dietary protein and energy levels on growth and survival of penaeid shrimp. *Aquaculture*, **1,** 341–347.

AOAC (1990) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th edn. Association of Official Analytical Chemists, Arlington, VA.

APHA (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th edn. American Public Health Association, Washington, DC.

Balazs, G.H. & Ross, E. (1976) Effect of protein source and level on growth and performance of captive freshwater prawn *Macrobrachium rosenbergii*. *Proc. World Maricult. Soc.*, **5,** 1–14.

Balazs, G.H., Ross, E. & Brooks, C.C. (1973) Preliminary studies on the preparation and feeding of crustacean diets. *Aquaculture*, **7,** 299–313.

Barnes, H. (1959) *Apparatus and Methods in Oceanography*. George Allen Unwin, New York. 125 pp.

Bautista, M.N. (1986) The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. *Aquaculture*, **53,** 229–242.

Bautista, M.N. & Subosa, P.F. (1997) Changes in shrimp feed quality and effects on growth and survival of *Penaeus monodon* juveniles. *Aquaculture*, **151,** 121–129.

Brown, M.R., Barrett, S.M., Volkman, J.K., Nearhos, S.P., Nell, J.A. & Allan, G.L. (1996) Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. *Aquaculture*, **143,** 341–360.

Buchanan, R.E. & Gibbons, N.E. (1974) *Bergey's Manual of Determinate Bacteriology*, 8th edn. Williams and Wilkins Co., Baltimore, MD.

Camara, M.R., Coutteau, P. & Sorgeloos, P. (1997) Dietary phosphatidylcholine requirements in larval and postlarval *Penaeus japonicus*. *Bate. Aquacult. Nutr.*, **3,** 39–47.

Clarke, D.J., Lawrence, A.L. & Swakon, D.H.D. (1993) Apparent chitin digestibility in penaeid shrimp. *Aquaculture*, **109,** 51–57.

Crompton, E.W. & Harris, L.E. (1969) *Applied Animal Nutrition*, 2nd edn. Freeman and Co., San Francisco, CA.

Dabrowski, K., Hassard, S., Quinn, J.P., Pitcher, T.J. & Flinn, A.M. (1980) Effect of *Geotrichum candidum* protein substitution in pelleted fish feed on the growth of rainbow trout (*Salmo gairdneri* Rich.) and on utilization of the diet. *Aquaculture*, **21,** 213–232.

Das, M.M., Mukhopadhyay, P.K. & Nanda, S.K. (1995) Evaluation of four formulated diets in *Macrobrachium malcolmsonii* juveniles. *J. Aquat. Res.*, **87,** 53–63.

Deshimaru, O., Kuroki, K., Mazid, M.A. & Kitamura, S. (1985) Nutritional quality of compounded diets for prawn *Penaeus monodon*. *Bull. Jpn. Soc. Sci. Fish.*, **51,** 1037–1044.

Fagbenro, O.A. & Jauncey, K. (1995) Growth and protein utilization by juvenile catfish (*Clarias gariepinus*) fed dry diets containing co-dried lactic acid-fermented fish silage and protein feedstuffs. *Bioresour. Technol.*, **51,** 29–35.

Fagbenro, O.A., Jauncey, K. & Haylor, G. (1994) Nutritive value of diets containing dried lactic acid fermented fish silage and soyabean meal for juvenile *Oreochromis niloticus* and *Clarias gariepinus*. *Aquat. Living Resour.*, **7,** 79–85.

Fagbenro, O.A., Jauncey, K. & Krueger, R. (1997) Nutritive value of dried lactic acid fermented fish silage and soyabean meal in dry diets for juvenile catfish, *Clarias gariepinus* (Burchell, 1822). *Appl. Ichthyol.*, **13,** 27–30.

Folch, J., Lees, M. & Sloane, G.H. (1957) Simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226,** 497–509.

Forster, J.R.M. (1970) Studies in the development of a compounded diet for prawns with particular reference to *Palaemon serratus*. PhD Thesis, University College of North Wales, Marine Sciences Laboratories, Menai Bridge, Anglesey, 79 pp.

Forster, J.R.M. (1975) Studies on the development of compounded diets for prawns. In: *Proceedings of the First International Conference on Aquaculture Nutrition* (Price, K.S., Jr, Shaw, W.N. & Danberg, K.S. eds), pp. 229–248. Delaware Sea Grant College Program and US/Japan Aquaculture Panel, College of Marine Studies, University of Delaware, Newark, NJ, USA.

Forster, J.R.M. & Beard, T.A. (1973) Growth experiment with *Palaemon serratus* Pennant, fed with fresh and compounded foods. *Fish. Invest. Lond. II*, **27,** 16–20.

Fox, C.J. (1993) The effect of dietary chitin on the growth, survival and chitinase levels in the digestive gland of juvenile *Penaeus monodon* (Fab). *Aquaculture*, **109,** 39–49.

Fox, C.J., Blaw, P., Brown, J.H. & Watson, I. (1994) The effects of various processing methods on the physical and biochemical properties of shrimp head meals and their utilization by juvenile *Penaeus monodon* Fab. *Aquaculture*, **122,** 209–226.

Goswami, U. & Goswami, S.C. (1982) Formulation of cheap prawn diets and their biological evaluation of some penaeid prawns. In: *Proc. Symp. Coastal Aquaculture*, Part 1 (Silas, E.G., Rao, P.V., Nair, P.V.R. et al, eds), pp. 211–215. CMFRI, Cochin.

Hall, G.M. & De Silva, S. (1994) Shrimp waste ensilation. *Infofish. Int.*, **2,** 27–30.

Hanson, J.A. & Goodwin, H.L. (1977) *Shrimp and Prawn Farming in the Western Hemisphere*. Dowden, Hutchinson and Ross Inc., Stroudsburg, PA.

Jayalaksmy, B. & Natarajan, P. (1994) Growth, conversion efficiency and moulting frequency of *Macrobrachium idella* fed on different artificial feeds. *J. Aquacult. Trop.*, **9,** 193–200.

Joseph, J.D. & Meyers, S.P. (1975) Lipid fatty acid composition of shrimp meals and crustacean diets. *Feedstuffs*, **47,** 35–39.

Joseph, J.D. & Williams, J.E. (1975) Shrimp head oil: a potential feed additive for mariculture. *Proc. Symp. World Maricult. Soc.*, **6,** 147–152.

Kanazawa, A., Teshima, S., Tokiwa, H. & Ceccaldi, J. (1979a) Effects of dietary linoleic and linolenic acids on growth of prawn. *Oceanol. Acta*, **2,** 41–47.

Kanazawa, A., Teshima, S. & Endo, M. (1979b) Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comp. Biochem. Physiol.*, **63b,** 295–298.

Kanazawa, A., Teshima, S. & Sakamoto, M. (1985) Effects of dietary lipids, fatty acids and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. *Aquaculture*, **50,** 39–49.

Kant, S. (1996) Potentiality of single cell protein (SCP) for aquafeed. *Fish. Chimes*, **14,** 35–37.

Kitabayashi, K., Kurata, H., Shudo, K., Nakamura, K. & Ishikawa, S. (1971) Studies on formula feed for Kuruma prawn – III. On the growth promoting effects of both arginine and methionine. *Bull. Tokai Reg. Fish. Res. Lab.*, **65,** 119–127.

Klappach, G., Weichert, D., Zakordonets, D.A., Suprun, S.M. & Sholkevich, Y.G. (1991) Improvement of the nutritional value of rye products by solid-state fermentation with *Fusaria*. *Acta Biotechnol.*, **11,** 109–114.

Lan, C.C. & Pan, B.S. (1993) *In vitro* digestibility simulating the proteolysis of feed protein in the midgut gland of grass shrimp (*Penaeus monodon*). *Aquaculture*, **109,** 59–70.

Lee, D.L. (1971) Studies on protein utilization related to growth in *Penaeus monodon*. *Aquaculture*, **1,** 1–13.

Levine, D.M. & Sulkin, S.D. (1984) Nutritional significance of long chain polyunsaturated fatty acids to the zoeal development of the brachyuran crab, *Eurypanopeus depressus* (Smith). *J. Exp. Mar. Biol. Ecol.*, **81,** 211–223.

Lim, C. & Dominy, W. (1990) Evaluation of soybean meal, as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). *Aquaculture*, **87,** 53–63.

Lin, C.S., Chang, B.G., Su, M.S. & Shitada, K. (1982) Requirement of white fish meal protein in diet of grass shrimp, *Penaeus monodon*. *China Fish. Mon.*, **337,** 13–15.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randell, R.J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275.

Menesveta, P., Panichayakul, S., Piyatiratitivorakul, P. & Piyatiratitivorakul, S. (1983) Effect of different diets on survival of giant prawn larvae (*Macrobrachium rosenbergii* de Man). *Proc. I. Int. Conf. Warm Water Crustacea*, 317–319.

Millamena, O.M., Primavera, J.H., Pudadera, R.A. & Caballero, R.V. (1986) In: Report of the FAO workshop on shrimp and fin fish feed development. *The Effect of Diet on the Reproductive Performance Pond-Reared Penaeus monodon* Fabricius Broodstock (MacLean, J.L., Dizon, L.B. & Hosillos, L.V. eds), pp. 593–596. The First Asian Fisheries Society, Manila, Philippines.

Millamena, O.M., Bombeo, R.F., Jumalon, N.A. & Simpson, K.L. (1988) Effects of various diets on the nutritional value of *Artemia* sp. as food for the prawn *Penaeus monodon*. *Mar. Biol.*, **98,** 217–221.

Mukhopadhyay, N. & Ray, A.K. (1999) Effect of fermentation on the nutritive value of sesame seed meal in the diets for rohu, *Labeo rohita* (Hamilton) fingerlings. *Aquacult. Nutr.*, **5,** 229–236.

Nwanna, L.C. (2003) Nutritional value and digestibility of fermented shrimp head waste meal by African catfish *Clarias gariepinus*. *Pak. J. Nutr.*, **2,** 339–345.

Nwanna, L.C., Balogun, A.M., Ajenifuja, Y. F. & Enujiugha, V.N. (2003) Replacement of fish meal with chemically preserved shrimp head meal in the diets of African catfish, *Clarias gariepinus*. *J. Food Agric. Environ.*, **2,** 1–6.

Pascual, F.P. & Destajo, W.H. (1978) Growth and survival of *Penaeus monodon* post larvae fed shrimp head meal and fish meal as primary animal source of protein. *Aquacult. Dep. Southeast Asian Fish Dev. Cent. Q. Res. Rep.*, **2,** 26–30.

Penaflorida, V.D. (1989) An evaluation of indigenous protein sources as potential component in the diet formulation for the tiger prawn *Penaeus monodon* using essential amino acid index. *Aquaculture*, **83,** 319–330.

Penaloza, W., Molina, M.R., Gomez, B.R. & Bressani, R. (1985) Solid-state fermentation: an alternative to improve the nutritive value of coffee pulp. *Appl. Environ. Microbiol.*, **49,** 388–393.

Plascencia-Jatomea, M., Olvera-Novoa, M.A., Arredondo-Figueroa, J.L., Hall, G.M. & Shirai, K. (2002) Feasibility of fishmeal replacement by shrimp head silage protein hydrolysate in Nile tilapia (*Oreochromis niloticus* L.) diets. *J. Sci. Food Agric.*, **82,** 753–759.

Reissig, J.L., Stromainger, J.L. & Leloir, L.F. (1955) A modified colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem.*, **217,** 959–966.

Rhishipal, R. & Philip, R. (1998) Selection of marine yeasts for the generation of single cell protein from prawn shell waste. *Bioresour. Technol.*, **65,** 255–256.

Roe, J.H. (1955) The determination of sugar in the blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, **212,** 335–343.

Sandifer, P.A. & Joseph, J.D. (1976) Growth responses and fatty acid composition of juvenile prawns (*Marcobrachium rosenbergii*) fed a prepared ration augmented with shrimp head oil. *Aquaculture*, **8,** 129–138.

Sheen, S.S. & Chen, J.C. (1993) The feasibility of extruded rice in shrimp feed to replace wheat flour for tiger prawn, *Penaeus monodon*. *J. Fish. Soc. Taiwan*, **20,** 65–72.

Shiau, S.Y. & Chou, B.S. (1991) Effects of dietary protein and energy on growth performance of tiger shrimp *Penaeus monodon* reared in seawater. *Nippon Suisan Gakkai Shi*, **57,** 2271–2276.

Shiau, SY., Kwok, C.C. & Chou, B.S. (1991) Optimal dietary protein level of *Penaeus monodon* juveniles reared in seawater and brackish water. *Nippon Suisan Gakkai Shi*, **57,** 711–716.

Sick, L.V. & Andrews, J.M. (1973) Effects of selected dietary lipids, carbohydrates and proteins on the growth, survival and body composition of *Penaeus duorarum*. *Proc. World Maricult. Soc.*, **4,** 263–276.

SPSS Inc. (2003) *Statistic Software Package*. SPSS Inc., Chicago, IL.

Sudaryono, A., Tsvetnenko, E. & Evans, L.H. (1996) Digestibility studies on fisheries by-products based diets *Penaeus monodon*. *Aquaculture*, **143,** 331–340.

Teshima, S., Kanazawa, A., Sasda, H. & Kawasaki, M. (1982) Requirements of larval prawn, *Penaeus japonicus*, for cholesterol and soyabean phospholipids. *Mem. Fac. Fish. Kagoshima Univ.*, **31,** 193–199.

Teshima, S., Kanazawa, A. & Yamashita, M. (1986) Dietary value of several proteins and supplemental amino acids for larvae of prawn *Penaeus japonicus*. *Aquaculture*, **51,** 225–235.

Vaitheswaran, S. & Ahamad Ali, S. (1986) Evaluation of certain substances as growth promoting agents for the prawn *Penaeus indicus*. *Indian J. Fish.*, **33,** 95–105.

Venkitaramaiah, A., Cook, D.W., Besiot, P. & Lakshmi, G.J. (1978) Nutritional value of high marsh grass and shrimp shell waste for commercial brown shrimp (*Penaeus aztecus* Ives.). *Proc. World Maricult. Soc.*, **9,** 217–224.

Venkitaramiah, A., Lakshmi, G.J. & Gunther, G. (1975) Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. *Aquaculture*, **6,** 115–125.

Yazawa, K., Akari, K., Okasaki, N., Watanabe, K., Ishikawa, C., Inoue, A., Numao, N. & Kondo, K. (1988) Production of eicosopentaenoic acid by marine bacteria. *J. Biochem.*, **103,** 5–7.

Yemm, E.W & Cocking, E.C. (1955) The determining of amino acids with ninhydrin. *Analyst*, **80,** 209–213.

Yongmanitchai, W. & Ward, O.P. (1989) Omega-3 fatty acids: alternative sources of production. *Process Biochem.*, **24,** 117–125.