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Efficacy of bacterial fermented oilcake mix as fishmeal substitute in the diet of tiger shrimp, *Penaeus monodon* (Fabricius) post larvae

IMELDA JOSEPH AND R. PAUL RAJ

Central Marine Fisheries Research Institute, Post Box No. 1603, Ernakulam North P. O., Kochi-18, Kerala, India

ABSTRACT

Diets incorporated with varying levels (5, 15, 25 and 35%) of bacterial fermented oil cake mix, derived by solid-state fermentation (SSF) as fishmeal replacement, were evaluated in *Penaeus monodon* post larvae for growth, digestibility and body composition through a 42 days laboratory experiment. A diet containing 35% fishmeal and no fermented ingredient mix was used as the control. Pure culture of *Bacillus coagulans* MTCC-2449 was used for SSF and the 36 h fermented product was incorporated in the diets. Analysis of response data revealed that the shrimp fed diet containing 35% fermented ingredient mix (100% fishmeal substitution) had significantly higher ($P<0.05$) mean weight gain (0.63±0.03 g), better feed conversion ratio (1.58), apparent protein utilization (25.65) and protein efficiency ratio (1.71) than all other diets as well as the control. The apparent protein digestibility (86.56%) and apparent fat digestibility (94.55%) were also found to be the highest for this diet. The survival rate was 100% in all the treatments and the control.

Introduction

Feed cost and quality are the major concern of shrimp farmers as growth, production and profitability largely depends on feeds. Feed should contain all the essential nutrients in adequate levels and balanced proportions, properly digestible and available for assimilation and growth. Fishmeal, with a well-balanced mixture of essential amino acids and other nutrients, which are readily digested, is the major protein source in aquafeeds (Dong *et al*., 2000). Since the world supply of fishmeal is affected due to over-exploitation besides other reasons (Kikuchi, 1999), replacement of fishmeal with cheaper ingredients in aquafeed is necessary to reduce the cost of production (Kaushik, 1990; Higgs *et al.*, 1995). Plant protein sources are the only ingredients for which expanded production in future is possible (Crowder, 1990). Certain oilseeds and oilcakes are good protein sources and can be used as main protein sources in shrimp and fish feed when properly supplemented with essential amino acids (Paulraj, 1993; Davis *et al*., 1995, Stickney *et al.*, 1996). But, plant derived ingredients may be deficient in some essential amino acids and contain anti-nutritional factors that impair the efficiency of digestion (Paulraj, 1993; Lemos *et al*., 2000). Digestibility values of feedstuffs are important parameters to be considered in diet formulation. The apparent digestibility of feedstuffs and nutrients in compounded diets (protein, carbohydrate, lipid, minerals, amino acid and sterols) has been estimated for a few species of shrimp (Deshimaru, 1972; Colvin, 1976; Fenucci *et al*., 1982; Lee *et al.*, 1984; Smith *et al*., 1985; Akiyama *et al*., 1989).

Solid-state fermentation (SSF) is a cheaper and relatively simple technique holding

tremendous potential to be utilized to improve the nutritional quality of plant ingredients. The fermented ingredients can be used directly as enzyme source, which in turn will increase the digestibility of nutrients (Tengerdy, 1998 Pandey *et al.*, 1999). The present investigation was conducted to elucidate the suitability of bacterial (*Bacillus coagulans*) fermented oil cake mix, as a substitute for fishmeal in the diet of tiger shrimp *Penaeus monodon* post larvae.

Materials and methods

Solid State Fermentation (SSF)

Bacillus coagulans MTCC-2449 for SSF was procured from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The raw materials (soybean flour, wheat flour, groundnut oil cake and sesame oil cake) were ground in a laboratory model pulverizer and sieved using 400 µm screen and analysed for proximate composition. The sieved materials were weighed and mixed in the ratio of 4:3:2:1, (soybean flour: wheat flour: groundnut oil cake: sesame oil cake respectively) to obtain protein content of about 39%. For SSF, 500 g of the mix with 60% moisture was autoclaved at 121°C for 15 min. It was then inoculated with 100 ml of *B. coagulans* culture containing 10^7 – 10^9 cells ml^{-1} (absorbance at 530 nm 0.7) and incubated at 30°C and pH 7.0 in a static condition with occasional shaking. Since the protein content of the product obtained after 36 h fermentation was significantly higher ($p<0.05$) than that obtained after 12 h, 24 h and 48 h, it was selected for evaluation in four experimental diets for *P. monodon* post larvae as fishmeal substitute.

Diets

The 36 h bacterial fermented ingredient mix (BFI) was dried to constant moisture level, ground, sieved (<1mm) and incorporated into the test diets. Four experimental diets (@38.5% crude protein) were formulated by incorporating BFI at 5, 15, 25 and 35%, designated as F1, F2, F3 and F4 respectively by replacing fishmeal in the same proportion. A diet with 35% fishmeal devoid of BFI served as the control (CF). The ingredient and proximate compositions of the diets are presented in Table 1. For each diet, the dry

TABLE 1: *Composition of experimental diets F1 to F4 and CF* (*% dry matter basis*)

Ingredient	F1	F2	F3	F4	CF
BFI[a]	5	15	25	35	-
Fish Meal	30	20	10	0	35
Shrimp meal	16	16	18	22	15
Wheat flour	27.5	22	17	16	26.5
Peanut cake	17.5	15.0	8	5	19.3
Sesame oil cake	0.5	7.3	15.5	20	2.0
Fish oil	2.0	2.5	3.0	3.5	2.0
Mineral mix[b]	0.5	1.2	2.5	2.0	1.0
Vitamin Mix[b]	1.0	1.0	1.0	1.0	1.0
Proximate composition (% dry matter basis)					
Crude protein	38.77	38.17	38.78	38.73	38.46
Crude fat	6.77	6.80	6.76	6.54	7.18
Crude Ash	11.77	12.18	12.15	12.29	13.21
Acid insoluble ash	1.81	1.64	1.27	0.89	1.93
Crude Fibre	1.98	2.33	2.58	2.83	1.85
NFE[c]	40.71	40.52	39.73	39.61	39.30
Calcium	2.37	2.31	2.15	2.21	2.45
Phosphorus	1.30	1.23	1.21	1.15	1.40

[a]*Bacillus* fermented Ingredient
[b]Commercial grade (supplevit)
[c]Nitrogen-free extract (calculated by difference)

ingredients and cod liver oil along with 1% chromic oxide were mixed manually into gelatinized wheat flour, and blended to attain a consistency appropriate for pelleting. The diets were pelleted using 2 mm die in a hand pelletizer. After pelleting, the feeds were dried to 8-10% moisture and stored in airtight plastic containers in a domestic freezer until use. The proximate analysis of the diets was determined after processing (AOAC, 1990). The amino acid profiles of the diets (Table 2) were determined by HPLC (Waters, India) after hydrolyzing the sample with 6N HCl. Tryptophan was measured spectrophotometrically after hydrolyzing with NaOH (AOAC, 1990).

Kerala, India. The shrimp were held in a 500 L fiberglass tank containing 15ppt water, and acclimated to laboratory conditions for three days prior to the commencement of the experiment. The feeding trials were conducted in 50 L circular perspex tanks containing 35 L of 15ppt water. Each treatment consisted of three replicate groups of shrimp (10 shrimp per tank; mean weight: 30 ± 0.09mg) along with the control groups, with continuous aeration. Dissolved oxygen concentration was monitored weekly and maintained at 4.11- 5.83 mg L^{-1} throughout the experimental period. Water temperature ranged from 25 to 29°C and pH from 7.5 to 7.8. The shrimp were fed at the rate

TABLE 2: *Amino acid profile of diets incorporated with BFI at different levels* (*g* $100g^{-1}$ *feed*)

Amino Acids	Diets				
	F1	F2	F3	F4	CF
Aspartic acid	4.42	3.82	3.66	4.03	3.27
Glutamic acid	6.67	8.97	8.63	6.47	7.27
Serine	2.25	1.90	1.86	1.99	1.65
Glycine	2.35	1.75	1.80	2.54	1.83
Histidine	1.45	0.80	0.77	1.67	0.69
Arginine	3.61	3.37	2.86	3.52	2.63
Threonine	1.88	2.78	1.27	2.27	1.23
Alanine	2.52	0.86	1.88	1.47	1.94
Proline	2.46	1.74	1.85	1.86	1.76
Tyrosine	1.86	1.47	1.52	2.12	1.63
Valine	1.67	1.61	1.27	1.93	1.24
Methionine	1.03	0.68	0.85	1.11	0.99
Cystine	0.08	0.09	0.09	0.12	0.11
Isoleusine	1.17	1.16	0.86	1.30	0.84
Leucine	0.34	2.66	2.68	3.84	2.63
Phenyl alanine	2.47	2.08	1.98	2.90	2.03
Lysine	3.30	2.30	2.31	2.80	2.64
Tryptophan	2.09	1.21	1.51	1.88	1.86

The water stability of the diets was evaluated by estimating the dry matter retention of the pellets at 1h, 2h, 3h and 4h intervals in 15ppt water (Obaldo *et al*., 2002).

Experimental design and feeding trial

A feeding experiment was conducted for 42 days using the test and the control diets.

P. monodon post larvae were procured from a local private shrimp hatchery at Kochi,

of 15% of the body weight throughout the experimental period. This daily ration was divided into two meals of 40 and 60% and fed at 0930h and 1600h respectively. The treatments were randomly assigned and shrimp were weighed on days 0, 10, 20, 30, and 40 and the daily ration was adjusted accordingly.

Faecal matter was collected daily, separated from the left over feed from each of the treatments and the control, before water

exchange into a collection sieve (40µ), and washed with distilled water without any disturbance to remove adhering salts, pooled, dried (60±1°C) and stored in desiccator for further analyses. Apparent digestibility of protein and fat were determined for the diets based on relative composition of chromic oxide percentage in the feed and the faeces.

Chemical analyses of feed ingredients, feeds and shrimp were done following AOAC (1990) procedures. Phosphorus and calcium content in the diets were determined by titrimetric method (AOAC, 1990). Chromic oxide in samples of feeds and faecal matter was estimated by the method described by Furukawa and Tsukahara (1966).

Diet performance

Diet performance was evaluated by determining survival, weight gain, feed conversion ratio (FCR), mean growth rate (GR), protein efficiency ratio (PER), apparent protein utilization, apparent nutrient (protein and fat) digestibility and body composition of the experimental animals as highlighted below:

Feed conversion ratio (FCR) = Weight of feed offered/Weight gain

Weight gain = Final weight – Initial weight

Mean growth rate (GR) = (Final weight – Initial weight) /Time (day) x 100

Protein efficiency ratio (PER) = Weight gain/ Protein intake

Apparent protein utilization = Protein gain/ Protein intake x 100

Apparent nutrient digestibility =
100-100 (% Cr_2O_3 in feed/% Cr_2O_3 in faeces) x

(% nutrient in faeces/% nutrient in feed)

At the start of the experiment, 200 numbers of post larval shrimp were weighed and dried at 55°C to a constant weight and the dry matter percentage was calculated. The dried samples were used for initial body composition analyses. On day 42, the final weights of the shrimp in different treatments and the control were recorded, and shrimp from each treatment were dried and ground, and the body composition was determined (AOAC, 1990).

Data Analyses

The growth performance of the shrimp were analyzed by one-way ANOVA and new Duncan multiple range tests to determine if significant ($p<0.05$) differences existed among the dietary treatments. The digestibility and body composition data were statistically analyzed by two-way ANOVA without replication. All the statistical analyses were conducted using SPSS for Windows (Statistical Package for Social Sciences, Windows Version, Chicago, IL, USA).

Results

In the present study, an attempt has been made for partial or complete replacement of fishmeal with BFI in *P. monodon* post larval diets. The raw materials for the present study were selected based on their nutritional profile (New, 1976). The BFI obtained after 36 h had crude protein content of 37.07, crude fat 2.30, crude fibre 3.49, crude ash 8.91 and nitrogen free extract of 48.22.

Water stability tests of the diets have shown that the dry matter loss was the least for diet F1 (19.62 %) and the highest for F4 (25.47) after 4 h (Table 3). After 42 days feeding trial, the survival rate was cent percent in all the treatment groups as well as in the control.

The shrimp groups fed diet F4, with 100% fishmeal substitution using BFI, exhibited the maximum weight gain (0.63± 0.03 g), which

TABLE 3: *Dry matter weight loss (%) of control and experimental diets in 15 ppt water*

Diet	1h	2h	3h	4h
CF	17.82	20.39	22.18	22.87
F1	12.78	12.83	13.84	19.62
F2	13.73	14.94	17.63	19.75
F3	16.71	17.52	18.68	19.56
F4	16.45	16.76	17.25	25.47

was significantly higher (P<0.05) than those fed other diets as well as the control (Table 4). The mean growth rates (GR) of shrimp fed different diets are presented in Table 4. GR was significantly (p<0.05) higher for diet F4 (12.12) and the lowest GR (2.87) was for the control diet.

The feed conversion ratio (FCR) ranged from 3.04 to 1.58 for diets with varying levels of BFI (Table 4). The diet F4 presented the best FCR (1.58) (P<0.05) among the treatments and the control. The PER was also significantly (p<0.05) the best (1.71) with the diet F4. The control diet with 35% fishmeal recorded significantly poor (P<0.05) FCR (3.30) and protein efficiency ratio (PER) (Table 4).

(Duncan-Multiple Range, Student-Newman Keuls Multiple Comparison and Least Significant Difference tests) has shown that the control diet and diet F1 with 5% BFI were significantly (p<0.05) different from the other treatments. There was no significant difference (p>0.05) among the diets F2, F3 and F4 containing 15, 25 and 35% BFI respectively. The regression analysis (R^2= 1) revealed a linear relationship between growth and percentage incorporation of BFI in diets.

The results of body composition analysis of the test animals are shown in Table 5. The highest dry matter, crude protein and crude fat content were observed in shrimp fed diet F4, with no fishmeal. A significant (p<0.05)

TABLE 4: *Results of the 42 day feeding trial for* P. monodon *using BFI incorporated diets*

Index	CF	F1	F2	F3	F4
Mean wt. gain (g)	0.15	0.19	0.51	0.57	0.63
Mean growth rate (mg d^{-1} post $larva^{-1}$)	2.87	3.71	9.80	10.96	12.12
Feed conversion ratio	3.30	3.04	1.75	1.59	1.58
Protein efficiency ratio	0.78	0.91	1.55	1.68	1.71
Apparent protein digestibility	57.68	72.49	76.90	83.54	86.50
Apparent protein utilization	10.29	12.12	21.17	24.06	25.65
Apparent fat digestibility	79.31	85.62	90.23	91.08	94.55

The apparent protein and fat digestibilities were the highest for diet F4 (86.50% and 94.55% respectively). The lowest digestibilities for both the nutrients were recorded for the control diet (Table 4). The apparent protein utilization (APU) ranged from 10.29 for the control diet to 25.65% for diet F4, which was significantly (p<0.05) higher than other diets (Table 4). The statistical analysis of growth performance data by different methods

reduction in the ash content was observed in all the shrimp fed diets with varying levels of BFI, in comparison to the control animals (20.46%).

Discussion

In the present study, significantly (p<0.05) higher weight gain, mean growth rate (GR) and superior FCR and PER in the treatment fed diet F4 indicates the possibility of complete

TABLE 5: *Body composition of* P. monodon *from different treatments and control* (*% Dry matter basis*)

Initial & Treatments	Moisture	Dry matter	Crude Protein	Crude fat	Crude ash
Initial	82.08	17.92	59.27	2.29	21.96
CF	78.29	21.71	60.48	3.51	20.46
F1	78.08	21.92	60.74	3.67	19.03
F2	78.28	21.72	62.99	3.97	18.09
F3	77.54	22.46	63.77	3.98	18.14
F4	76.61	23.39	64.02	4.31	18.77

replacement of fishmeal in post larval shrimp diet with a mixture of ingredients enriched by the process of SSF. The better performance of diets incorporated with BFI suggests the significance of bacterial enrichment of nutrients to easily available forms by SSF possibly through the production of enzymes. Among the essential amino acids, histidine, threonine, valine, methionine, isoleusine, leucine and phenylalanine were observed to be relatively high in diet F4 compared to other experimental diets and the control (Table 2). This could be one of the reasons for the better performance of this diet. Millamena and colleagues studied the requirement of essential amino acids (EAA) for *P. monodon* ranging from 20 to 50 mg over an eight week period (Millamena *et al*., 1996 a, b; 1997; 1998 and 1999). Total sulphur amino acid requirement was estimated at 3.5% of dietary protein in a feed containing 0.41% cystine (Millamena *et al*., 1996). In the present study, the EAA levels were lower than the projected requirement by Millamena *et al*. (1996 a, b; 1997; 1998 and 1999). However, since their studies for amino acid requirements were based on incorporation of synthetic crystalline amino acids, the requirement would have been projected to be high. In the present study, no synthetic amino acids were incorporated in the diets and it is presumed that the dietary amino acids were better assimilated by the shrimp for their growth and metabolic utilization.

Proteins of marine origin such as fishmeal are likely to contain substances that are feeding effectors for shrimp, whereas proteins of terrestrial origin may not naturally contain these particular compounds (Smith *et al*., 2000). Even in the absence of fishmeal, the better acceptability of diet F4 was attributed to the incorporation of shrimp meal (22%) in the diet as in other test diets and control. It has been earlier reported that product based on shrimp processing waste was the most effective feeding effector for crustaceans (Saraç and Smith, 1998). Also a considerable increase in glycine content was observed in diet F4 (Table 2) which is an effective feeding stimulant. It was also reported that no single ingredient could completely replace fish meal in shrimp diet based on EAA study, indicating that any potential for replacement of fishmeal by one novel ingredient could be difficult, but rather possibly accomplished by a combination of ingredients (Fox *et al*., 2004).

Digestibility values were determined using chromic oxide as the inert marker. The loss of nutrients through leaching from the feed is often suggested as a problem in shrimp nutrition studies involving digestibility determinations using chromic oxide (Goldblatt *et al*., 1980). The problem of nutrient leaching from the diet prior to ingestion and from the faeces prior to collection during determination of apparent digestibility coefficients in crustaceans has been discussed by several authors (Fenucci *et al*., 1982; Taechanuruk and Stickney, 1982; Clark *et al.,* 1993). Excessive leaching of nutrients from diet or faeces can lead to an overestimation of digestibility coefficients. However, experiments conducted by Fenucci *et al*. (1982), Smith *et al*. (1985) and Law *et al*. (1990) showed evidence that there are no significant errors in the determination of diet digestibility coefficients for crustaceans due to nutrient and chromic oxide loss from the faeces, provided immersion in water is less than 6 h. In the present study, the maximum period that faecal material was left in water was <5 h. The results suggest that the indirect method using chromic oxide as a digestibility inert marker appeared to be reliable for *P. monodon***.** This is in agreement with Akiyama *et al.* (1989) and Wee *et al*. (1991) for penaeid shrimp and abalone, respectively. Results of digestibility and protein utilization reveal that the digestive system of shrimp was modulated by the components of the experimental diets, yielding a 15–30% improved protein digestibility, 6-15% fat digestibility and 2-15% improved protein utilization over the control (Table 4). Differences in protein digestibility might have also been attributed to differences in amino acid content of the diets. Lan and Pan (1993), in

in vitro studies using *P. monodon*, found that the protein digestibility of diets which contained proteins low in lysine, arginine and phenylalanine was lower than that of diets containing higher levels of these amino acids. Diet F4 had all these amino acids in higher level than other diets and the control (Table 2) suggesting its superior protein digestibility. The crude fiber content in diet F4 was the lowest. The lower fat and protein digestibility for diets other than F4 may also be attributed to the higher level of crude fiber, since *P. monodon* is known to be carnivorous and utilization of cellulose may be low (Sudaryono *et al*., 1996). Akiyama *et al*. (1989) also reported that high fibre content in diets decreases the protein digestibility in *P. vannamei.* Tacon (1987) also suggested that for carnivorous fish and shrimp species, the cellulose cell wall within plant protein sources may render the protein present within the cell inaccessible to digestive enzymes.

About 17% of the dry matter in the diet F4 was lost in the first 2 h and 25% was lost after 4 h (Table 3) suggesting relatively lower stability of diet F4. However, most of the feeds offered will be consumed by the shrimp within 2h and the post larvae have the tenacity to feed on broken feed particles, thereby lower water stability has not affected the diet performance. Whereas Fl, F2 and F3 lost only 12-19%, 15-20% and 17-19% of dry matter after 2 h and 4 h of immersion, respectively. The control feed showed a dry matter loss of 20-23 % after 2 and 4h of immersion. This may have been due to the fact that oil cake meals during processing do not gelatinize to the same efficiency as cereal grains such as wheat flour. Leaching of dry matter due to poorer water stability of the pellets may partly explain the lower APD, AFD and APU for the control diet.

Commercial shrimp feeds contain 30-50% crude protein, composed mostly of marine animal protein products such as fish, shrimp and squid meal. In the present study, the crude protein level in all the diets including control was about 39% and the animal protein composition in the diets varied between 22% (F4) and 50% (CF) and plant protein between 47.8% and 76 %. The control diet had animal protein: plant protein ratio of about 1:1 and diet F4 1:3. Lim (1997) has reported that about 12% peanut meal can be used as a replacement for 20% of animal protein mix in the diet of juvenile white shrimp *P. vannamei*. However, he has suggested that if the palatability of the diets can be improved, up to 35% peanut meal could be used to replace 60% of animal protein mix.

Overall, this study has shown that bacterial fermented oil cakes can be used as a partial substitute for marine protein sources in the diet for *P. monodon* with relatively high digestibility values and growth performance. The significant findings from this study emphasize the need for further research in elucidating the complete changes in nutritional profile and substrate modification by SSF of different plant ingredients, especially in terms of enzyme production and break down of anti-nutritional factors.

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