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ORIGINAL ARTICLE

Growth performance of juvenile tiger shrimp Penaeus monodon Fabricius fed diets with fermented soybean meal as fishmeal substitute

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ABSTRACT

A 50d experiment was conducted to evaluate the efficacy of five diets (diets FI to FV) formulated by substituting fishmeal with fermented soybean meal (FSBM) at selected levels (20, 40, 60, 80 and 100%) on growth performance and digestibility of juveniles of the tiger shrimp *Penaeus monodon*. A diet (350-370 g kg^{-1} crude protein) containing 250g kg^{-1} fish meal (FM) without FSBM served as control (CF). The control and the five test diets were fed to triplicate groups (n= 60) of juvenile *Penaeus monodon* (initial weight: 100 mg ± 0.06). Analysis of growth data showed significant (P<0.01) difference between treatments with 100% survival in the control as well as all the treatments. Shrimp fed the diet FII, containing 40% FM substitution with FSBM, grew (specific growth = 2.12 g day^{-1}) faster than those in other treatments and the control. A 100% substitution of FM from the diet with FSBM had a severe negative effect on the body weight gain. The apparent dry matter digestibility (ADMD), apparent protein digestibility (APD) and apparent fat digestibility (AFD) also were the maximum for diet F II. The protein efficiency ratio (PER) and the feed conversion ratio (FCR) were not significantly different among the treatments and the control. The results revealed that up to 60% of FM could be effectively replaced in the diets by FSBM for rearing *P. monodon* juveniles without affecting growth and feed efficiency.

INTRODUCTION

There are about 30 species of shrimp and more than 300 finfish species that are commercially cultivated worldwide for which fishmeal forms the basis of feeds (Hertrampf and Pascual, 2000). Compounded commercial shrimp grow-out feeds contain about 250 g kg^{-1} fishmeal (Tacon and Barg, 1998). Probiotics, prebiotics, organic acids (OA), and fermented feeds/ ingredients, have been used as in-feed antibiotic alternatives to modulate poultry feed in order to improve animal health status and growth performance (Nava et al., 2009). The beneficial effects of probiotics and organic acids on performance indices and general health of different domesticated avian species are well documented (Levy et al., 2015; Seifi et al., 2017), although these effects have not always been consistent (Lee et al., 2010; Nosrati et al., 2017). Inclusion of fermented products in animal feed has been shown to induce advantageous effects on performance and gastrointestinal health by acting and exhibiting probiotic effects and could therefore be considered as an alternative for antibiotics growth promoters (Missotten et al., 2015). Fermented feed has a low pH, high concentration of lactic acid (>150 mM), and a high number of lactic acid bacteria (LAB) (approximately 109 CFU/mL of feed) (Ashayerizadeh et al., 2017). Engberg et al. (2009) showed that feeding fermented feed in laying hens increases LAB in the crop and decreases coliforms in the ileum. Furthermore, the OA content of fermented feed has been reported to modulate gastrointestinal microbiota balance through increasing acidity and lowering the pH (Niba et al., 2009). Besides the desired adverse effect on pathogenic bacteria, fermented feeds have some nutritional implications (Engberg et al., 2009). Microbial fermentation has been reported as an effective technique to eliminate or reduce anti-nutritional factors and improve nutritional value in plant-based protein meals (Ashayerizadeh et al., 2017; Jazi et al., 2017). Soybean meal (SBM) is the most commonly used plant protein source in poultry feed (Chiang et al., 2010). However, its application in poultry diets could often be limited due to some anti-nutritional factors, such as trypsin inhibitor (TI), oligosaccharides, phytic acid, and allergenic proteins, which interfere with digestion, absorption, and utilization of nutrients, especially for young chicks (Li et al., 2014; Feng et al., 2007a). Previous reports have indicated the reduction of TI and other anti-nutritional factors in SBM following fermentation (Wang et al., 2014; Sharawy et al., 2016). The hypothesis tested in the current study was that the antimicrobial compounds present in fermented SBM (FSBM) may act in a similar way to probiotics and OA in improving growth performance of Japanese quails through affecting gut microbiota and morphology. Plant materials of high protein content are good alternatives since these are available abundantly and their cost is low. However, plant protein supplements contain relatively low levels of some essential amino acids, lacks fatty acids like EPA and DHA, and low levels of bio-available energy and minerals such as phosphorus, as compared to animal protein sources.

Soybean (*Glycine max* (L)) is valued because of its high protein content, relatively well balanced amino acids profile, reasonable price and reliability of supply (Chou *et al.*, 2000). Soybean meal is the most widely used plant protein and is considered to be the most effective alternative for quality fishmeal found in feeds for many aquatic organisms (Watanabe, 2002). However, extensive use of soybean meal in aquafeeds is undesirable, because of phytic acid an anti-nutritional factor that commonly occur in soybean meals, which can be removed only by the enzyme phytase. Therefore, the development of techniques like bacterial fermentation that reduce the harmful anti- nutritional properties of soybean meal would make possible a high quality and cost- effective protein with valuable functional benefits for cultured organisms. Application of solid-state fermentation (SSF), an age-old practice for improving nutritional value of food, appears promising due to natural potential and advantages it offer (Pandey *et al.*, 1999). Using GRAS (generally regarded as safe) microbial strains, SSF has been found to be applicable in production of enzymes and other biomolecules, and animal feed (Ramachandran *et al.*, 2004), and in nutritional enrichment of raw materials (De-Lena *et al*., 1997; Imelda-Joseph *et al.*, 2004, 2005).

The objective of this study was to evaluate the efficacy of partial or total replacement of fishmeal in the diets with fermented soybean meal (FSBM) on the growth performance of juvenile *Penaeus monodon* maintained under laboratory conditions. The effect of incorporation of FSBM in diets on apparent digestibility of dry matter, protein and lipid was also investigated.

MATERIALS AND METHODS

MICROORGANISM

Bacillus coagulans, a gram-positive, spore forming, bacteria obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, was used for SSF. The culture was maintained in Zobell Marine Agar (Himedia, Mumbai) with fortnightly subculture.

Solid state fermentation (SSF)

Soybean meal (SBM) was procured from the local market. Two kilogram SBM with 60 % moisture was autoclaved for 20 min at 121°C and after cooling to room temperature (30±2 °C) one percent inoculum (10^7- 10^8 cells ml^{-1}) of *B. coagulans* was added for fermentation. After 48h of fermentation, the fermented soybean meal (FSBM) was dried at 50-60°C to a final moisture content of about 10 %. Dried FSBM was ground in a laboratory pulverizer and used as ingredient for shrimp diets.

DIETS

Dried and milled anchovy (*Stolephorus indicus*) was used as fishmeal (FM). Dry whole shrimp without shell was ground and used as shrimp meal (SM). Groundnut and sesame oil cake and cassava flour were purchased from local market. Five experimental diets were formulated to contain 350- 370g kg^{-1} crude protein by replacing FM with 20, 40, 60, 80 and 100 % FSBM (Diets FI to FV). The control feed (CF) contained 250 g kg^{-1} fishmeal and no FSBM. All the diets contained 10 g kg^{-1} chromic oxide as an inert marker for apparent digestibility measurements. For diet preparation, dry ingredients were mixed with gelatinized cassava flour and 50g kg^{-1} vitamin- mineral premix (Supplevit, animal feed grade) was added prior to pelletization. Diets were pelletized (2 mm diameter) with a hand pelletizer and were stored at 4°C in sealed containers in a domestic refrigerator until feeding.

CHEMICAL ANALYSIS

Chemical analysis of the ingredients as well as the diets included moisture content, crude protein (CP), crude ash, crude fat, nitrogen free extract (NFE), calcium and phosphorus (AOAC, 1990). Amino acid analysis was performed by reverse-phase high-performance liquid chromatography after acid hydrolysis (6N HCl) and pre-column derivatization by phenyl isothiocyanate (PITC). HPLC was performed using a Waters 1525 Binary HPLC pump and Waters 2487 Dual Absorbance Detector (HPLC, Waters India Ltd). Data were processed and analyzed using Waters Breeze software. Operating conditions were: column temperature 38°C, column, pico-tag (Waters, pico tag system); absorbance, 254µm; pump pressure, 1500- 1700 psi. Tryptophan content in the samples was determined after alkaline hydrolysis (5 % NaOH) by spectrophotometry (AOAC, 1990). Total phosphorus of the diets was determined by titrimetric method and calcium using the residue from ash by titration method described by AOAC (1990).

FEEDING TRIAL

Penaeus monodon juveniles (100 mg ± 0.06) procured from a local shrimp hatchery (Aquaplaza Hatchery, Cochin) were transported in oxygen filled transportation bag containing 20 ppt water. The animals were acclimatized for seven days in a circular fibre glass tank containing approximately 500L water of 20 ppt salinity in the CMFRI Marine Research Hatchery, Kochi. The experimental set-up consisted of 18 numbers of 35 L perspex circular tanks each of them covered using black resin sheets to regulate light penetration and disturbance to the animals. Each treatment (five experimental diets and the control) consisted of three replicates, and were randomly distributed. Each of the tanks was stocked with 20 *P. monodon* juveniles with daily exchange of 50 % water; and aeration was provided through diffuser stones. The feeding trial was conducted for 50 days. Temperature and pH of the water were daily measured. While, dissolved oxygen (Winkler method), nitrite- nitrogen and ammonia (using kits from E- Merck Germany) levels were measured at weekly intervals to ensure optimal environmental conditions.

GROWTH PERFORMANCE AND FEED EFFICIENCY

The shrimp were fed daily at the rate of 12 g 100^{-1}g body weight throughout the trial and the daily ration divided into two dosages and fed at 0900 hrs (40%) and at 1600 hrs (60%). Faecal collections were made daily morning before water exchange and feeding. The faecal material from each of the tank was collected, pooled, dried at 50°C and the dry matter, total nitrogen, lipid and ash contents were determined (AOAC, 1990). Apparent digestibility was estimated by the indirect method, as described by Maynard and Loosli (1969) with chromic oxide as inert marker. The chromic oxide content was determined spectrophotometrically (Furukawa and Tsukahara, 1966). The performance of the diets was evaluated by determining weight gain, food conversion ratio (FCR), and specific growth rate (SGR) and protein efficiency ratio (PER) (Sudaryono *et al.*, 1999). Apparent net protein utilization (ANPU) defined as the percentage of ingested protein which is deposited as tissue protein was calculated by following Tacon (1987).

STATISTICAL ANALYSES

Mean results per tank were subjected to analysis of variance (ANOVA). Significant differences were indicated by Duncan's multiple range tests using SPSS statistical analysis system. The level of significance was P≤0.01, and all the results are presented as mean ± standard error of the mean (S.E.M.).

RESULTS AND DISCUSSION

The inclusion level of feed ingredients in the test diets and control and their proximate composition are given in Tables 1a and 1b. The proximate composition as well as the amino acids profile of the control and the test diets is shown in Tables 2a and 2b. The significant finding from this experiment is that substitution of FM up to 40% with FSBM in the diet for *P. monodon* (diet F II) had significantly (P<0.01) improved the growth performance of juvenile tiger shrimp and that FM replacement up to 60 % did not affect the shrimp growth or survival. However, FM replacement by FSBM at levels exceeding 60 % resulted in reduced performance.

Tiger shrimp juveniles fed diets compounded by replacing fishmeal with FSBM at 20, 40, 60, 80 and 100 % exhibited varying responses for weight gain, SGR, FCR, and PER (Table 3). The shrimp fed diet (FII) with 40% replacement of FM resulted in significantly (P<0.01) the highest mean weight gain (1.04 g) and the lowest weight gain was for those fed the diet F V with no FM (0.36 g). While there are no previous studies to compare the efficacy of FSBM, a significant reduction in growth of Pacific white shrimp *Litopenaeus vannamei* at 100% replacement of fishmeal by soy protein concentrate (SPC) was reported by Forster *et al.* (2002), who found that replacement of fishmeal with SPC did not affect the final body weight of the shrimp at levels up to 75 %. FSBM substitution @40g $100g^{-1}$ fishmeal in the diet (FII) resulted in weight gain of 1.56 times to that of the control shrimp (0.68 g). Feed intake was also the highest for diet F II (2.49g $shrimp^{-1}$) and the lowest for the FM free diet FV (1.24 g $shrimp^{-1}$). Poor palatability and attractiveness could be the major reasons for the lower intake and poor growth performance of fishmeal free diet FV, since all the diets were formulated to be almost iso-nitrogenous. A similar observation of low feed intake and low weight gain was reported for hybrid tilapia (*Oreochromus niloticus* x *O. aureus*), while using FSBM (commercial grade) as fishmeal substitute by Guan-Shen *et al.* (2003).

In the present study the levels of arginine in CF and F II (Table 2b) were almost similar and in others it was above the recommended value for commercial shrimp feed (5.8 % of protein) (Akiyama et al., 1992). Lysine levels in the diets ranged from 74.5 to 109.6 g kg^{-1} protein and it is higher than the recommended value (5.3%) in all the diets. Methionine level in the diets ranged from 15.7 to 24.2 g kg^{-1} protein. Methionine level was the highest in diet FI, with only 20% FM replacement (2.42 g kg^{-1} protein). A gradual reduction in methionine was observed with increase in fish meal replacement (Table 2b). Considerable reduction in cystine was also observed with increased inclusion of FSBM in the diets. It is a well established fact that soybean is deficient in methionine and cystine (Amerio *et al.*, 1998). CF and FI had better levels of methionine: cystine and the poor performance of diets with FSBM above 60% may be attributed to the lower level of these sulphur amino acids than the recommended level (3.6 %). Inferior nutritive value for penaeid shrimp of formulated diets that have lower methionine (10 – 20 g kg^{-1} protein) and lysine (63- 69 g kg^{-1} protein) levels than those of clam meat and shrimp based diet (methionine 26- 28 g kg^{-1} protein; lysine 73- 89 g kg^{-1} protein) was reported by Deshimaru *et al.* (1985). Histidine (2.1%), threonine (3.6%) and phenylalanine-tyrosine (7.1%) levels were almost near the recommended levels (values in parenthesis) in the diets with FSBM and CF. Tryptophan was higher than the recommended level (0.8%) in all the diets. From the results it is evident that inclusion of FSBM at relatively lower levels results in better essential amino acids (EAA) balance in the diets probably due to the complementary effect of amino acids.

It is believed that palatability of a diet largely depends on the content of dietary attractants. The most widely recognized feed attractants are certain amino acids (taurine, glycine, arginine, glutamic acid and alanine), betaine, nucleotides and organic acids in combinations, and ingredients of marine origin are rich in these attractants and those of plant origin contain less of these substances (Lee and Meyers, 1997). In the present study, it was observed that alanine, valine, glycine, proline and leucine levels were relatively higher in the diet F II (Table 2b) which may be the reason for its better intake than other feeds. These amino acids may also be important sources of energy as observed by Polat *et al.* (1995) for African catfish.

Calcium and phosphorus levels were the lowest in the fishmeal free diet (FV) which might have resulted in reduced availability of these minerals to the shrimp.

The specific growth rate (SGR) expressed as percentage growth day^{-1} varied between 0.73 and 2.12 (Table 3) and significant difference was observed in SGR between the treatments and the control ($P<0.05$). The maximum SGR was obtained for the feed with 40% fishmeal replacement (FII).

The FCR was the lowest for the control with no FSBM (1.75) and the highest for diet FV (2.46) (Table 3). The PER observed for feeds ranged from 1.15- 1.63 with the highest for control and lowest for FV (Table 3). No significant ($P>0.05$) difference was observed in FCR and PER among control and test diets, though PER and FCR for fishmeal free diet was the poorest. One of the most significant findings from this experiment is the superior ANPU obtained for diet FII, a parameter which reveals the efficacy of utilization of the digested protein.

The digestibility data (Table 3) indicate that FSBM based diets are generally better digested by *P. monodon* juveniles. ADMD was relatively high for F II (57%) and APD was also significantly high ($P<0.05$) for this treatment (81.3%). ALD was also the highest for F II (94.90). ADMD of 60.26 % and APD of 75.38 % for soybean meal were reported for *Litopenaeus setiferus* by Brunson *et al.* (1997). Better digestibility of FSBM protein in the present study agrees with the earlier reports that protein from plant source was better digested by species like *Palaemon serratus* and *Pandalus platyceros* (Forster and Gabbot, 1971), *Macrobrachium rosenbergi* (Law *et al*., 1990) and *P. vannamei* (Akiyama *et al.*, 1989).

The results of the indoor trial indicate that, FSBM could replace up to 40- 60% of high quality FM in the diets of juvenile *P. monodon* without any significant adverse effect on performance. These findings are in general agreement with those of Paripatananont *et al*. (2001) who found that soy protein concentrate (SCP) could replace 50% of fish meal in diets for tiger shrimp. FSBM would be a cost effective substitute relative to fish meal and SCP as aquaculture continues to grow but needs further research to improve the potential use of FSBM in aquatic feeds, particularly in terms of improving palatability and reducing anti-nutritional factors.

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Table 1a Level of inclusion of different ingredients in control and experimental diets

Diet	g kg^{-1} inclusion of different ingredients						
	FSBM[a]	FM[b]	SM[c]	GNOC[d]	SOC[e]	CF[f]	V/M[g]
Control	0	250	100	200	150	250	50
[1]F I	50	200	100	150	200	250	50
[2]F II	100	150	100	150	200	250	50
[3]F III	150	100	100	150	200	250	50
[4]F IV	200	50	100	200	170	200	80
[5]F V	250	0	100	200	170	200	80

a-Fermented soybean meal; b-Fishmeal; c-Shrimp meal; d-Groundnut oilcake; e- Sesame oil cake;

f- Cassava flour; g- vitamin-Mineral mix;

1,2,3,4,5 - Diets with fishmeal replacement by FSBM 20, 40, 60, 80 and 100 g (100 g^{-1}fish meal)

Table 1b Nutrient composition of major ingredients used for diet preparation (Dry matter basis)

Raw material	Crude protein (%)	Crude Fat (%)	Crude fiber (%)	Total ash (%)	Nitrogen free extract (%)	Calcium	Phosphorus
Whole Fish meal	74.16±0.01	7.66±0.01	0.14±0.04	15.03±0.17	1.33±0.12	1.85	1.23
Shrimp meal	70.50±0.14	4.07±0.11	5.32±0.20	21.62±0.20	---	1.95	1.22
Soybean meal	55.64±0.21	0.63±0.02	2.64±0.08	7.42±0.01	29.37±0.08	1.69	1.01
Groundnut oil cake	33.73±0.12	6.40±0.21	11.27±0.12	8.32±0.17	31.14±0.89	1.64	1.1

Sesame oil cake	42.66±0.18	4.45±0.48	5.14±0.06	10.19±0.94	32.55±0.79	1.53	1.07
Fermented soybean meal	56.20±0.16	0.66±0.21	1.10±0.12	7.79±0.44	33.33±0.58	1.97	1.23
Cassava flour	1.16±0.19	0.33±0.17	0.95±0.07	1.96±0.55	95.57±0.03	0.03	0.61

Table 2a Nutrient profile of the control and experimental diets

Diets	Moisture (%)	Crude Protein (%)	Crude fat (%)	Crude Fiber (%)	Total Ash (%)	Nitrogen Free extract (%)	Phosphorus (%)	Calcium (%)
Control	6.63±0.02	37.74±0.02	4.48±0.11	2.55±0.02	15.59±0.02	39.65±0.09	1.54	1.97
FI	8.24±0.02	39.22±0.13	4.17±0.16	3.09±0.01	15.05±0.13	39.25±0.14	1.66	1.85
FII	6.76±0.01	38.32±0.68	3.81±0.04	2.52±0.10	13.59±0.16	41.85±0.90	1.78	1.95
FIII	8.02±0.03	38.88±0.71	3.69±0.04	2.62±0.01	13.15±0.06	41.67±0.74	1.34	1.69
FIV	7.03±0.28	37.62±1.76	4.01±0.14	2.33±0.07	13.19±0.57	41.66±1.63	1.32	1.83
FV	8.77±0.09	38.24±0.29	3.88±0.03	2.49±0.13	12.33±0.01	43.07±0.14	1.22	1.64

Table 2b Amino acid profile of the control and test diets (g/ 100g protein)

Amino Acid	Control[1]	F I[2]	F II[3]	F III[4]	F IV[5]	F V[6]
Aspartic acid	9.55	7.54	8.30	7.64	9.67	8.97
Glutamic acid	14.63	13.42	14.09	13.03	14.24	14.36
Serine	6.09	5.36	5.76	5.49	5.95	6.09
Glycine	9.82	9.12	10.80	10.97	10.25	9.43
Histidine	1.91	1.67	1.55	2.43	2.27	2.27
Arginine	5.60	4.85	5.50	6.51	6.73	6.66
Threonine	3.16	3.18	2.87	3.80	3.93	3.87
Alanine	7.06	7.35	7.44	7.30	7.05	7.18
Proline	5.31	5.60	5.55	6.62	5.68	6.68

Tyrosine	2.66	2.71	3.02	2.98	2.82	2.75
Valine	5.00	5.03	5.41	5.05	4.74	4.27
Methionine	1.80	2.42	1.97	1.71	1.57	1.62
Cystine	3.54	3.60	0.55	0.60	0.59	0.48
Isoleusine	7.71	7.80	3.61	4.05	3.87	3.59
Leusine	4.22	4.20	8.12	7.82	7.91	7.69
Phenylalanine	2.01	2.1	4.74	4.43	4.26	4.22
Lysine	8.04	10.96	9.19	7.68	7.48	7.45
Tryptophan	1.65	2.50	2.07	2.516	2.45	2.34

Table 3 Growth response and feed utilization efficiency of shrimp fed diets containing various replacement levels of FM by FSBM for 50d

Parameters	Diets and Percentage Replacement of fishmeal					
	CF	FI	FII	FIII	FIV	FV
	0	20	40	60	80	100
Wt gain (g)	0.77±0.14	0.82±0.23	1.14±0.3	0.62±0.16	0.53±0.14	0.46±0.06
SGR (% day-1)	1.53	1.22	2.12	1.06	0.86	0.73
Feed intake (g shrimp-1)	1.35±0.04	1.46±0.15	2.26±0.12	1.4±0.16	1.23±0.09	1.13±0.01
FCR	1.75±0.06	1.58±0.36	1.55±0.31	1.89±0.44	2.42±0.39	2.46±0.22
Survival (%)	100	100	100	100	100	100
PER	1.63±0.05	1.43±0.29	1.44±0.29	1.27±0.22	1.25±0.21	1.15±0.11
ANPU (%)	10.43	11.23	17.43	10.77	9.48	8.66
ADMD (%)	49.47	53.76	57.00	56.8	57.95	55.29
APD (%)	76.48	81.13	81.3	79.2	79.26	74.35
ALD (%)	92.16	94.9	93.8	90.1	87.19	86.32

SGR- specific growth rate; FCR- Feed Conversion Ratio; PER- Protein Efficiency Ratio; ANPU- Apparent Net Protein Utilization; ADMD- Apparent dry matter digestibility