Indian J. Fish., 66(3): 60-68, 2019
DOI: 10.21077/ijf.2019.66.3.88889-08

Feeding diets with graded levels of fermented soybean meal to Pacific whiteleg shrimp *Penaeus vannamei* (Boone, 1931): Effect on digestive enzymes, immune responses and carcass composition

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ABSTRACT

A 45-days indoor trial was performed to assess the effect of fermented soybean meal (FSBM) on digestive enzyme activity, immune responses and carcass composition of amino acids, fatty acids, and minerals in *Penaeus vannamei* (Boone, 1931). Five iso-nitrogenous diets were formulated by replacing fishmeal (w/w) with FSBM (200, 250, 300. 350 and 400 g kg^{-1}). A total of 300 juveniles (3.08±0.07 g) were randomly distributed to the experimental tanks at the rate of twenty shrimp per tank with three replications for each treatment. Protease activity ($p<0.05$) decreased with increase in the inclusion level of fermented ingredients. Shrimp fed with FSBM300 diet had a significantly ($p<0.05$) higher amylase activity than others. whereas, the lipase activity was not affected significantly due to the dietary change. Total haemocyte count varied from 11.24 to18.54x10^6 cells ml^{-1} in FSBM diet fed shrimps. Control group showed highest activity of phenoloxidase (2.85 dopochrome ml^{-1}) but it did not significantly differ from other treatments. Shrimp fed fermented ingredients had no significant difference in carcass amino acids. Eicosapentaenoic (C20:5) and docosahexaenoic acids (C22:6) were significantly ($p<0.05$) affected in shrimp reared with FSBM350 and FSBM400 diets. Calcium had a significant difference between the treatments, while other elements were not influenced. The results concluded that fishmeal could be partially substituted with fermented ingredients in the diet of *P. vannamei* without having any negative effect on immune responses and carcass micro-nutrients.

Keywords: Carcass composition, Digestive enzyme, Fermented ingredient, Immune response, *Penaeus vannamei,* Soybean meal

Introduction

Pacific whiteleg shrimp *Penaeus vannamei* (Boone, 1931) though a native species of Eastern Pacific Ocean, it is widely cultured in many of the countries worldwide due to its tolerance to wide range of salinity, temperature and the availability of genetically improved specific pathogen free post-larvae. Shrimp feed exceedingly relies upon fishmeal, given its wholesome attributes like excellent amino acids (methionine, lysine and tryptophan), essential fatty acids, in particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), highly available phosphorus and other essential nutrients along with the higher palatability and digestibility. Therefore, more than 70% of fishmeal in global production is utilised by the aqua-feed sector, wherein the largest consumer is shrimp (29%) (FAO, 2015). The scenario of diminished availability and escalating price of fishmeal in recent years prompted the researchers to substitute its considerable quantity by using various alternatives. One among them is fermented oilseed meals/cakes (Shiu *et al.,* 2015; Sun *et al.,* 2015; Sharawy *et al.,* 2016; Jannathulla *et al.,* 2017, 2018a,b). Though fermented plant proteins are being used as a potential substitute of fishmeal in shrimp feed, its relationship on digestive enzymes and their distribution pattern in *P. vannamei* is scarce. Study on digestive enzymes in aquatic species is vital, since they are associated with the innate feeding behaviour and diet composition and their assessment would be helpful in selecting the suitable feed ingredients for feed formulations. Similarly, dietary changes in general, regularly cause no perceptible signs, however had a severe impact on the health status of organisms that would not emerge from the nutritional parameters. Rumsey *et al.* (1994) reported that when rainbow trout fed diets containing graded levels of soybean meal by replacing fishmeal had observed changes in serological parameters

and non-specific defence mechanisms. The inclusion of cottonseed meal and supplementation of iron significantly affected the immune parameters in channel catfish (Barros *et al.,* 2002). However, published reports on the effect of inclusion of fermented ingredients in shrimp on immune parameters are very limited.

Notwithstanding, the nutritional composition of shrimp mainly depends on feed. Hence, formulating feeds with suitable ingredients having adequate nutrients is an indispensable prerequisite. Nowadays, the awareness of consumers is increasing with regard to the nutritional quality of foods. In most of the countries, nutritional labelling is obligatory in all the marketed processed foods, which helps the consumers to maintain a healthy food regime. The Act of Nutrition Labelling and Education (NLEA) of 1990 in the USA has already formulated the labels having information on the nutrient contents of processed foods. In addition, the Association of Official Analytical Chemists (AOAC) has also formed a task force to determine the methods for nutrient labelling analysis. Therefore, producing healthy shrimps with good nutritional constituents is important for humans. Nonetheless, the greater part of earlier reports on fermentation have been restricted in assessing only macronutrients (proximate composition) in shrimp, yet till date, there is no information related to fermented ingredients on micronutrients (amino acids, fatty acids and minerals). Hence, the present study aimed to investigate the effect of inclusion of fermented soybean meal on digestive enzymes activity, immune responses and carcass composition of amino acids, fatty acids and minerals in *P. vannamei.* The results from this study would help to explore the usage and limitation of fermented ingredients in producing good quality shrimp.

Materials and methods

Fermentation methodology

The fungus, *Aapergillus niger* listed under GRAS (Generally Recognized As Safe) notifications by UFDA (GRAS Notice No. 35, 2010) was used for fermentation. *A. niger* culture (ATCC 6275) acquired from Himedia Laboratories (Mumbai, India) was grown on potato dextrose agar (PDA) for five days at 35±1°C in an incubator and the suspension was adjusted to approximately 10^7 spores ml^{-1}. Meanwhile, commercial solvent extracted soybean meals (SBM) was purchased at six different places in and around Chennai, India and were ground to a particle size of <500 µm. The ground samples were hydrated by adding water to adjust the moisture content between 60 and 65% and then subsequently, sterilised by autoclaving at 121 °C (105 kPa) for 15 min. The cooled autoclaved samples were inoculated with 5% *A. niger* suspension (10^7 spores ml^{-1}) and allowed to ferment for three days at 35±1°C in an incubator (Jannathulla *et al.,* 2017). Post-fermentation, all the samples were dried at 50°C for 48 h to bring down the moisture content below 10%.

Experimental diets

A control diet was formulated with locally available ingredients based on the nutritional requirements of *P. vannamei* (Jannathulla *et al.,* 2018a), which contained 250g kg^{-1} of fishmeal as a major protein source. In test diets, fermented soybean meal (FSBM) was included at the rate of 250, 300, 350 and 400 g kg^{-1} by replacing fishmeal (Table 1). The experimental diets were prepared according to Dayal *et al.* (2011). As FSBM had diverse protein and lipid levels compared to fishmeal, it was replaced with test ingredients on a w/w basis to assess the specific effect of fermentation in shrimp. However, the experimental diets were formulated to iso-nitrogenous and iso-lipidic by adjusting the level of corn gluten meal and palm oil, respectively. The nutritional composition of experimental diets is given in Table 2.

Experimental conditions

A 45 days indoor laboratory trial was performed with the juveniles of *P. vannamei* procured from the local farm. Prior to the experiment, juveniles were acclimatised to the indoor laboratory conditions and fed with a control diet having 374.46 g kg^{-1} of crude protein for 15 days. Post-acclimatisation, a total of 300 healthy shrimps with an average weight of 3.08±0.07 g were randomly distributed to 15 nos. of 500 l (1.31x0.64x0.73 m^3) oval-shaped fiberglass reinforced plastics (FRP) tanks at the rate of 20 shrimps per tank with three replicates for each treatment. All the tanks were equipped with aquaculture flow-through system (1.5 ml min^{-1}) and covered with a fiber mat to prevent the escape of shrimps. Shrimps were fed respective diets (6% of the biomass) three times at 07.00 hrs, 12.00 hrs and 17.30 hrs and the feed given was adjusted according to body weight, survival and consumption. The uneaten feed particles (if any) were siphoned out from the experimental tanks after an hour of feeding in a clean Falcon tube and dried at 60°C overnight in a hot air oven to measure the feed intake on a daily basis. During the experimental period, ultraviolet treated water was used after filtering through a 5 µm cartridge filter. The water quality parameters *viz.,* salinity (20g l^{-1}), temperature (26.5 to 28.5°C), dissolved oxygen (5.8 to 7.8 mg l^{-1}), pH (8 to 8.5) and total ammonia-nitrogen (<0.1 mg l^{-1}) were recorded and found to be within the normal range.

Digestive enzymes

At the end of the experiment, a total of 15 shrimps in a treatment (5 shrimps per replication) were collected and washed with deionized water to remove the adhering

contaminations. Shrimps were dissected to remove the hepatopancreas and was rinsed again with de-ionized water. Hepatopancreas was homogenised with ice-cold de-ionized water (1:10) at 4°C and the extract was centrifuged at 5000 *g* for 10 min. The collected supernatant was kept at -80°C and used as crude enzyme. The total soluble protein was determined as per Lowry *et al.* (1951) using bovine serum albumin (Sigma-Aldrich, USA) as a standard in a UV-Visible spectrophotometer (UV-1800, Shimadzu). All the digestive enzymes, including protease, lipase and amylase were determined by the method of Moreno-Arias *et al.* (2017) and their activity was expressed as U mg^{-1} of protein.

Immune responses

Haemolymph samples were acquired from five shrimps in each replication of a treatment (15 shrimps per treatment) through the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on a one ml syringe containing an equal volume of fixative solution (10% formalin in 0.45 M sodium chloride). Total haemocyte (THC) was determined following Sritunyalucksana *et al.* (2005) using haemocytometer (Neubauer, Marienfeld, Germany) in five out of 25 squares (0.2 x 0.2 x 0.1 mm^3) and the activity of phenoloxidase was assayed as per Cheng and Chen (2001).

Biochemical composition

Proximate composition of experimental diets was analysed as per the method of AOAC (1997). Shrimp, not used for haemolymph collection and immune response analysis, were pooled treatment-wise and analysed for carcass nutrient composition. Amino acid profiles were analysed using a pre-column derivatisation HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6 N hydrochloric acid (Finlayson, 1964). Tryptophan, being labile to acid hydrolysis, was measured after alkali hydrolysis by spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). Lipid was extracted following the method by Folch *et al*. (1957) and the respective fatty acid methyl esters (FAMEs) were prepared as per Metcalfe *et al.* (1966). Routine analysis of methyl esters was performed by a gas chromatograph (GC-2014 Shimadzu) on a RTX wax capillary column (100 m length x 0.25 mm I.D x 0.2 µm film thickness). The sample (1 g) was digested using microwave digestion method (Anton Par microwave system) for mineral analysis with 6 ml of nitric acid and 2 ml of hydrogen peroxide in inert polymeric microwave vessels. The minerals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent 5100 SUV) using the 5.2 software (Jannathulla *et al.,* 2017).

Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA) and the multiple comparisons of

Table 1. Ingredient composition of experimental diets containing graded levels of fermented soybean meal (g kg^{-1} as fed basis)

Particulars	Control (CNT)	Diets with fermented soybean meal (FSBM)			
		FSBM 250	FSBM 300	FSBM 350	FSBM 400
Fishmeal1	250	200	150	100	50
FSBM2	-	250	300	350	400
Acetes3	80	80	80	80	80
Squid meal	15	15	15	15	15
Soybean meal	200	-	-	-	-
Corn gluten	20	24	28	32	36
Sesame cake	50	50	50	50	50
Wheat flour	324	315	306	297	288
Fish oil1	20	20	20	20	20
Palm oil	-	5	10	15	20
Lecithin	10	10	10	10	10
Pre-mix4	20	20	20	20	20
Binder5	10	10	10	10	10
BHT6	1	1	1	1	1

[1]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India
[2]Fermented soybean meal
[3]Mantis shrimp used as a protein source
[4]Pre-mix (g kg^{-1}): Thiamine hydrochloride (25.5g), riboflavin (25 g), prydoxine hydrochloride (50. g), cyanocobalamin (0.1g), menadione (5.00 g), all-trans tocopherol acetate (99g), retinyl acetate (10g), vitamin D (50 g), nicotinic acid (101g), D-Ca-pantothenate (61.g), biotin (25g), folic acid (6.25 g), inositol (153.06 g), ferric citrate (13.7g), ZnSO4.7H2O (28.28 g), MgSO4.7H2O (0.12 g), MnSO4.H2O (12 .43 g), CuSO4.5 H2O (19.84 g), CoC12.6H2O (4.07 g), KIO4 (0.03 g), KCl (15.33 g), Na2SeO3 (0.02 g)
[5]Pegabind, Bentoli Agri Nutrition Asia Pvt Ltd, Singapore
[6]Butylated hydroxytoluene: Sigma Aldrich (Cat. No: PHR1117)

Table 2. Nutritional composition of experimental diets containing graded levels of fermented soybean meal (g kg^{-1} as fed basis)

Particulars	Control (CNT)	Diets with fermented soybean meal (FSBM)			
		FSBM 250	FSBM 300	FSBM 350	FSBM 400
Proximate composition					
Moisture	87.6	88.2	87	87.1	87.3
Crude protein	374.4	372.6	371.4	369.8	376.5
Ether extract	67.6	69.8	71.1	71.5	72.2
Crude fiber	29.8	29.8	32.6	36.2	37.6
NFE1	297.1	305.4	315.1	319.6	315.5
Total ash	143.5	134.2	122.8	115.8	110.9
Essential amino acids					
Arg	23.1	24.2	26.4	23.9	25.3
His	8.8	9.1	9.4	9.3	10.1
Ile	15.3	15.6	15.8	16.9	16.3
Leu	26.4	26.7	27.3	27.2	27.5
Lys	21.4	21.2	22.2	20.8	21.4
Met	8.4	8	7.8	7.4	7.2
Phe	17.3	17.6	18.7	18.1	19.1
Thr	14.3	14.1	13.9	14.7	13.5
Trp	4.2	4.2	4.8	4.4	4.7
Val	17.1	16.7	16.8	15.7	15.3
Major fatty acids					
C16:0	14.7	15	15.4	15.7	15.9
C18:0	3.2	3.1	3.0	2.7	2.7
C16:1	4.9	4.3	3.9	3.3	2.7
C18:1	9.1	10.7	11.9	13.3	15.1
C18:2	11.2	11.5	12.7	12.7	13.4
C18:3	1.2	1.3	1.2	1.4	1.2
C20:4	0.7	0.6	0.6	0.4	0.4
C20:5	3.6	3.4	3.2	2.7	2.5
C22:6	2.0	2.0	1.9	1.7	1.4
Macro minerals					
C	29.7	27.2	23.7	21.9	17.7
P	14.8	14.1	12.6	11.1	9.6
Na	2.0	2.4	1.6	1.5	0.9
K	5.6	7.8	8.1	8.9	9.6
Mg	3.3	3.7	3.5	3.3	3.7

[1]Nitrogen free extract was calculated by a difference

treatments were done using Tukey's test to find significant differences between the treatments. Regression analysis was performed to assess the relationship between the inclusion level and THC. Prior to statistical evaluation, data were checked for ascertaining a normal distribution and then determining the homogeneity of variance. The entire data were analysed using SPSS version 16.0 and statistical tests were evaluated at 5% significance ($p<0.05$).

Results and discussion

Protease activity was significantly ($p<0.05$) higher in the group fed a control diet (no fishmeal replacement) and was significantly pp<0.05) decreased with increasing the inclusion level of FSBM (Table 3). This result corroborates with the findings of Moreno-Arias *et al.* (2017) when replacing fishmeal with vegetable mix in the diet of *P. vannamei.* Amylase activity was significantly ($p<0.05$) higher in shrimp fed diets contained 300 g kg^{-1} of FSBM. However, the experimental groups fed FSBM containing diets, irrespective of the inclusion level, showed higher amylase activity than those fed a control diet. It has been suggested that the suitability of fermented ingredients usage was more in the diet of *P. vannamei* and is in agreement by Jannathulla *et al.* (2018a;). However, no specific trend was observed between the inclusion of FSBM and amylase activity in the present study. Becerra-Dorame *et al.* (2012) suggested that this would be due to the influence of certain extrinsic and intrinsic factors. The range of lipase activity was similar to those reported by Moreno-Arias *et al.* (2017) for the same species. However,

the activity of lipase was not significantly affected due to the dietary change in the present study as in the result of Pakravan *et al.* (2017).

The THC is an important indicator in assessing shrimp health as it is associated with cellular mechanisms. In our study, THC in control group was 11.24 x 10^6 cells ml^{-1} significantly ($p<0.05$) increased due to the inclusion of FSBM (Fig. 1) and ranged from 13.67-18.54 x 10^6 cells ml^{-1}. The values obtained in the present study was almost similar to those reported in *Macrobrachium nipponense* (Yang *et al.,* 2004), but slightly lower compared to *Penaeus monodon* (Lee and Shiau, 2002). The difference between the studies would probably be due to the variation in adopting capability of species to the culture environment (Le Moullac and Haffner, 2000). Though there is no information on THC in relation to dietary effect yet to date, the effect of various extrinsic factors, in particular temperature on THC have already been reported in different shrimp species earlier (Le Moullac and Haffner, 2000; Cheng and Chen, 2001). The regression analysis showed, $y = -0.9864x^2 + 7.0916x + 4.542$, $r = 0.8702$ for FSBM fed groups. Though THC gradually increased with increasing the inclusion level of FSBM, THC level dropped by >20% in shrimp fed a diet containing 400 g kg^{-1} of FSBM (FSBM400) compared to FSBM350 diet, which indicates that higher inclusion of fermented ingredients by substituting fishmeal had a negative impact on shrimp immune responses.

The important role of phenoloxidase on defence mechanism has largely been discussed earlier (Sritunyalucksana and Soderhäll, 2000). The activity of phenoloxidase is influenced by various factors majorly by certain cations, in particular calcium and magnesium. An enhanced phenoloxidase activity by calcium and magnesium was reported in *Penaeus paulensis* (Perazzolo and Barracco, 1997) and *P. monodon* (Sung and Sun, 2002). Fishmeal contained higher calcium content than FSBM and the dietary calcium decreased with increased FSBM inclusion (Table 2). However, the phenoloxidase activity was not significantly affected due to the dietary changes (Fig. 2), which indicates that the partial substitution of fishmeal using these fermented ingredients had no negative impact on the health status of *P. vannamei.*

Table 3. Digestive enzymes activity (U mg^{-1} of protein) of *P. vannamei* fed experimental diets having graded levels of fermented soybean meal

Particulars	Control(CNT)	Diets with fermented soybean meal (FSBM)				SEM	P-value
		FSBM250	FSBM300	FSBM350	FSBM400		
Protease	0.281^a	0.225^b	0.169^c	0.113^d	0.056^e	0.001	<0.001
Lipase	0.027^a	0.024^a	0.026^a	0.023^a	0.025^a	0.001	0.467
Amylase	0.494^d	0.591^c	0.862^a	0.741^b	0.487^d	0.001	<0.001

Means with the same superscript letters in the same row do not differ significantly ($p>0.05$)

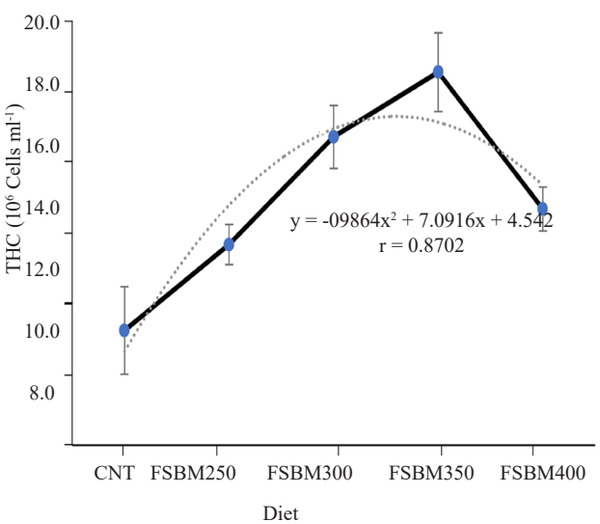

Fig. 1. Total haemocyte count (THC) of *P. vannamei* fed experimental diets with graded levels of fermented soybean meal

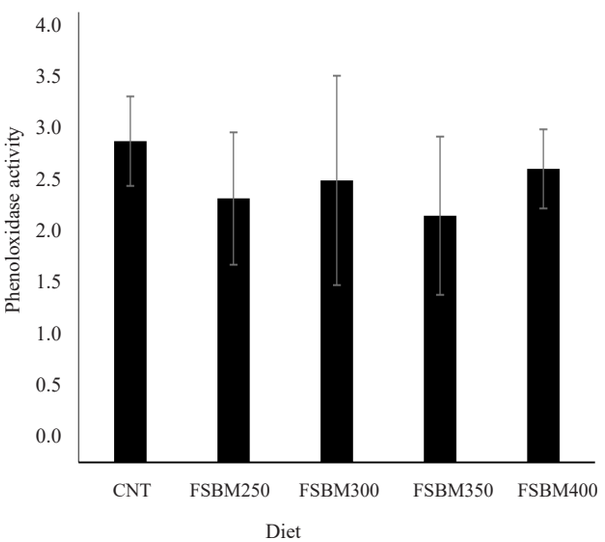

Fig. 2. Phenoloxidase activity of *P. vannamei* fed experimental diets with graded levels of fermented soybean meal

The amino acid composition of *P. vannamei* fed different experimental diets containing graded levels of FSBM is presented in Table 4. Of all the essential amino acids (EAA), arginine, leucine and lysine had >10 g kg^{-1} in shrimp carcass. However, the carcass amino acid compositions were not significantly influenced due to the substitution of fishmeal using both the fermented ingredients. This indicates that *A. niger* fermented ingredients could be used as a potential protein source which could also fulfill the EAA requirement of the shrimp as in fishmeal. Dayal *et al.* (2013) reported that the Protein Digestibility Corrected Amino Acids Score (PDCAAS) was 1 for shrimp, which obviously shows that shrimp has a superior quality of protein. In the present study, there was no significant difference in EAA of *P. vannamei* fed different experimental diets (Table 4). Similar results were reported in *P. monodon* while replacing fishmeal using a graded level of sunflower oil cake (Dayal *et al.,* 2011). The ratio of EAA/NAA was in the range of 1.01 to 1.03 regardless of shrimp fed different experimental diets in our study. A similar result (1.05) was reported in *Macrobrachium vollenhovenii* (Ehigiator and Oterai, 2012). However, Iwasaki and Harada (1985) reported that the average ratio of EAA/NAA was 0.70 in many fishes and 0.56 in crab and squid. In general, amino acids can be classified as nutritionally essential and nonessential. Moreover, fullness factor of shrimp was 3.3 even after replacing fishmeal using fermented ingredients on the scale of 0 to 5 (Dayal *et al.,* 2013) implying that fermented ingredients could be used as a potential protein source that fulfils essential nutrient requirements of the cultured species as in fishmeal.

The dietary fishmeal replacement with fermented plant protein sources significantly ($p<0.05$) increased the lipid retention in shrimp carcass while replacing fishmeal using FSBM and fermented sunflower oil cake (Jannathulla *et al.,* 2018a;b) but a similar trend was not discernible in the fatty acid composition. Fatty acids like C16:0, C18:0, C16:1, C18:1, C18:2, C20:5 and C22:6 were high in experimental feeds (Table 2) and to a certain extent; the same were reflected in shrimp carcass compositions in the present study. The result is in agreement with earlier findings in *P. monodon* (Deering *et al.,* 1997) and in *Penaeus indicus* (Colvin, 1976). Among SFAs, C16:0 and C18:0 were significantly ($p<0.05$) reduced in diets with fishmeal substitution using FSBM (Table 3). Besides, the fatty acids that were low in quantity in the diets appeared to be actively synthesised or retained by shrimps (Gonzalez-Félix *et al.,* 2002). For example, C18:1 was relatively low in control diet than those formulated with fermented ingredients, but its level remained unchanged in shrimp carcass. This effect would probably be due to the result of preferential utilisation of fatty acids by shrimp. Sparing and retention of fatty acids has already been demonstrated in *Penaeus chinensis* (Xu *et al.,* 1994), *P. monodon* (Deering *et al.,* 1997; Khan *et al.,* 2018) and *P. vannamei* (Gonzalez-Félix *et al.,* 2002). However, the dietary change did not influence other major fatty acids in shrimp carcass.

The inclusion of FSBM upto 300 g kg^{-1} by replacing fishmeal did not significantly affect carcass EPA and DHA (Table 5) and beyond such levels of inclusion significantly ($p<0.05$) reduced the level of carcass EPA and DHA. Gonzalez-Felix *et al.* (2002) reported that a plant-based diet, in which 90% of fish oil was replaced by soy oil, had no adverse effects on shrimp growth performance, but dramatically reduced body PUFAs content. This indicates that 10% of fish oil could meet the essential fatty acid requirement of shrimp, but was not adequate to maintain PUFAs in the body. The higher replacement of fishmeal could be a reason for the lower values of PUFAs evidenced in shrimps fed diet FSBM350, whereas the same diet showed a similar growth with the control group (Jannathulla *et al.,* 2018a). This result is in agreement in *Dicentrarchus labrax* while replacing fish oil using various plant oils *viz*., rapeseed, linseed and palm oils (Montero *et al.,* 2005). Shrimp fed diet with FSBM had

Table 4. Essential amino acid composition of *P. vannamei* fed experimental diets having graded levels of fermented soybean meal (g kg^{-1} wet basis)

Particulars	Control (CNT)	Diets with fermented soybean meal (FSBM)				SEM	p-value
		FSBM 250	FSBM 300	FSBM 350	FSBM 400		
Arg	11.07[a]	10.59[a]	11.13[a]	11.28[a]	11.38[a]	0.192	0.541
His	14.02[a]	12.86[a]	14.22[a]	13.35[a]	13.46[a]	0.715	0.603
Ile	6.51[a]	6.45[a]	6.75[a]	6.91[a]	6.64[a]	0.084	0.613
Leu	11.47[a]	11.25a	11.80[a]	11.93[a]	11.91[a]	0.208	0.581
Lys	10.4 [a]	10.49[a]	11.57[a]	11.64[a]	11.03[a]	0.521	0.413
Met	3.84[a]	3.52[a]	3.82[a]	4.25[a]	3.94[a]	0.065	0.219
Phe	5.96[a]	6.01[a]	6.23[a]	6.34[a]	6.33[a]	0.083	0.642
Thr	4.25[a]	4.14[a]	4.41[a]	4.67[a]	5.33[a]	0.138	0.099
Trp	1.15[a]	1.22[a]	1.12a	1.16[a]	1.16[a]	0.006	0.821
Val	6.66a	6.60[a]	6.90[a]	7.08[a]	6.76[a]	0.079	0.544

Means with the same superscript letters in the row do not differ significantly ($p>0.05$)

a higher content of calcium (7.02 to 7.44 g kg^{-1}) than those fed a control diet (6.60 g kg^{-1}). Increasing calcium is beneficial since it plays various important roles, especially bone formation, muscle contraction and blood clotting (Jannathulla *et al.,* 2017). However, other elements like magnesium, phosphorus, potassium and sodium were not affected in shrimp carcass due to fishmeal substitution using FSBM (Table 6). Jannathulla *et al.* (2017) suggested that this could be due to the extraction of most of the minerals from the rearing system (water) by shrimps.

The results of the present study clearly indicated that *P. vannamei* can be successfully farmed with a feed containing minimum inclusion of fishmeal, without having any negative impacts on immune response and carcass composition. Additionally, an enhanced digestive enzymatic activity of shrimp was observed with fermented soybean meal. In conclusion, the analysed parameters of *P. vannamei* in this study exhibited satisfactory results with respect to the inclusion of fermented ingredients in substituting fishmeal. Hence, fermented plant proteins, in particular fermented soybean meal could be considered as a suitable ingredient in the shrimp feed industry, with great potential to reduce the pressure on fishmeal demand. The present study also suggests further investigations on upscaling the fermentation technique before advocating for commercial applications.

Table 5. Major fatty acid composition of *P. vannamei* fed experimental diets having graded levels of fermented soybean meal (mg kg^{-1} wet basis)

Particulars	Control (CNT)	Diets with fermented soybean meal (FSBM)				SEM	P value
		FSBM 250	FSBM 300	FSBM 350	FSBM 400		
C16:0	1490.42^{a}	1134.68^{bc}	1183.66^{bc}	1074.03^{c}	1287.81^{b}	4199.857	0.002
C18:0	1010.15^{ab}	926.86^{bc}	839.49^{c}	704.08^{d}	1027.56^{ab}	1565.898	0.000
C14:1	28.53^{a}	18.60^{b}	19.90^{b}	17.33^{b}	14.29^{b}	10.727	0.032
C16:1	176.15^{ab}	206.50^{a}	141.88^{bc}	94.48^{c}	108.1^{1c}	378.264	0.003
C18:1	1016.16^{a}	1156.03^{a}	1126.15^{a}	813.90^{a}	800.08^{a}	18457.645	0.111
C18:2	974.17^{a}	896.05^{b}	761.86^{c}	703.81^{c}	708.17^{c}	869.251	0.000
C18:3	83.81^{a}	77.81^{a}	87.88^{a}	60.73^{a}	85.42^{a}	131.611	0.271
C20:4	419.49^{a}	377.72^{ab}	346.84^{ab}	292.92^{bc}	258.25^{c}	1254.654	0.018
C20:5	833.16^{a}	811.57^{a}	817.49^{a}	648.00^{b}	594.69^{b}	755.263	0.000
C22:6	505.07^{ab}	517.53^{ab}	451.22^{b}	349.24^{c}	316.47^{c}	672.687	0.000

Means with the same superscript letters in the row do not differ significantly ($p>0.05$)

Table 6. Macro mineral composition of *P. vannamei* fed experimental diets having graded levels of fermented soybean meal (g kg^{-1} wet basis)

Particulars	Control (CNT)	Diets with fermented soybean meal (FSBM)				SEM	P-value
		FSBM 250	FSBM 300	FSBM 350	FSBM 400		
Ca	6.60^{c}	7.37^{a}	7.31^{a}	7.35^{a}	7.06^{b}	0.008	0.000
K	2.98^{a}	2.95^{a}	2.91^{a}	2.97^{a}	2.93^{a}	0.002	0.613
Mg	0.60^{a}	0.62^{a}	0.64^{a}	0.61^{a}	0.64^{a}	0.000	0.440
Na	1.63^{a}	1.60^{a}	1.55^{a}	1.59^{a}	1.52^{a}	0.003	0.403
P	2.41^{a}	2.52^{a}	2.50^{a}	2.45^{a}	2.50^{a}	0.006	0.660

Means with the same superscript letters in the row do not differ significantly ($p>0.05$)

Acknowledgements

This work was supported by Indian Council Agricultural Research (ICAR), New Delhi, India through the project, National Innovation in Climate Resilient Agriculture (NICRA-2049/3001). Authors are thankful to Dr. K. K. Vijayan, Director, ICAR-CIBA, Chennai, India for providing all the facilities to carry out the present work. Thanks are also to D. Vasanthakumar, E. Amala Claret and R. Yuvapuspha, Technicians, ICAR-CIBA, Chennai fort heir assistance in conducting the experiments.

References

AOAC 1997. *Official methods of analysis,* 18th edn. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington DC, USA.

Barros, M. M., Lim, C. and Klesius, P. H. 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus puctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture,* 207: 263-279. DOI: 10.1016/S0044-8486(01)00740-2

Becerra-Dorame, M. J., Mart ınez-Porchas, M., Martınez-Cordova, L. R., Rivas-Vegas, M. E., Lopez-El, J. A. and Porchas-Cornejo, M. A. 2012. Production response and digestive enzymatic activity of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) intensively pre-grown in microbial heterotrophic and autotrophic-based systems. *Sci. World J.,* 1–6. https://doi.org/10.1111/j.1365-2095.2010.00809.x.

Cheng, W. and Chen, J. C. 2001. Effects of intrinsic and extrinsic factors on the haemocyte profile of the prawn, *Macrobrachium rosenbergii. Fish Shellfish Immunol.,* 11: 53-63. DOI:10.1006/fsim.2000.0293.

Colvin, P. M. 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne Edwards). *Aquaculture,* 7: 315-326.

Dayal, J. S., Ponniah, A. G., Khan, H. I., Babu, E. M., Ambasankar, K. and Vasagam, K. P. 2013. Shrimps -a nutritional perspective. *Curr. Sci.,* 104: 1487-1491.

Dayal, J. S., Rajaram, V., Ambasankar, K. and Ahamad Ali, S. 2011. Sunflower oil cake as a replacement for fishmeal in feeds of tiger shrimp, *Penaeus monodon* reared in tanks and in net cages. *Indian J. Mar. Sci.,* 40; 460-470.

Deering, M. J., Fielder, D. R. and Hewitt, D. R. 1997. Growth and fatty acid composition of juvenile leader prawns, *Penaeus monodon*, fed different lipids. *Aquaculture,* 151: 131-141.

Ehigiator, F. A. R. and Oterai, E. A. 2012. Chemical composition and amino acid profile of a caridean prawns (*Macrobrachium vollenhoveanil*) from Ovia river and tropical periwinkle (*Tympanonus fusctus*) from Benin River. Edo State, Nigeria. *Int. J. Res. Rev. App. Sci.,* 11: 162-167.

FAO 2015. *Fisheries and aquaculture department. summary tables of fishery statistics: Capture, aquaculture, commodity and food balance sheets.* Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/fishery/statistics/en/, 2015 (Accessed 17 July 2016).

Finlayson, A. J. 1964. Amino acid recovering in the analysis of some feed samples. *Can. J. Plant Sci.,* 45: 184-188.

Folch, J., Lees, M. and Sloane-Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.,* 226: 497-509.

Gonzalez-Felix, M. L., Gatlin, D. M., Lawrence, A. L. and Perez-Velazquez, M. 2002. Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. *Aquaculture,* 207: 151-167. DOI: 10.1016/S0044-8486(01)00797-9.

Iwasaki, M. and Harada, R. 1985. Proximate and amino acid composition of the roe and muscle of selected marine species. *J. Food Sci.,* 50: 1585-1587.

Jannathulla, R., Syama Dayal, J., Ambasankar, K. and Muralidhar,M. 2018a. Effect of *Aspergillus niger* fermented soybean meal and sunflower oil cake on growth, carcass composition and haemolymph indices in *Penaeus vannamei* Boone, 1931. *Aquaculture,* 486: 1-8.

Jannathulla, R., Syama Dayal, J. , Ambasankar, K., Eugine, A. C. and Muralidhar, M. 2018b. Fungus, *Aspergillus niger*, fermented groundnut oil cake as a fishmeal alternative in the diet of *Penaeus vannamei. Aquac. Res.,* 49: 2891-2902.

Jannathulla, R., Syama Dayal, J., Chitra, V., Ambasankar, K. and Muralidhar, M. 2017. Growth and carcass mineralization of Pacific whiteleg shrimp *Penaeus vannamei* Boone 1931 in response to water salinity. *Indian J. Fish.,* 64: 22-27. DOI: 10.21077/ijf.2017.64.2.59404-04.

Jannathulla, R., Syama Dayal, J., Vasanthakumar, D., Ambasankar, K. and Muralidhar, M. 2017. Effect of fermentation methods on amino acids, fiber fractions and anti-nutritional factors in different plant protein sources and essential amino acid index for *Penaeus vannamei* Boone, 1931. *Indian J. Fish.,* 64: 40-47. DOI: 10.21077/ijf.2017.64.2.60341-0.

Khan, H. I., Dayal, J. S., Ambasankar, K., Madhubabu, E. P., Jannathulla, R. and Rajaram, V. 2018. Enhancing the dietary value of palm oil in the presence of lysolecithin in tiger shrimp, *Penaeus monodon. Aquac. Int.,* 26: 509-522. DOI: 10.1007/s10499-017-0235-x.

Le Moullac, G. and Haffner, P. 2000. Environmental factors affecting immune response in Crustacea. *Aquaculture,* 191: 121-131. https://doi.org/10.1016/S0044-8486(00)00422-1.

Lee, M. H. and Shiau, S. Y. 2002. Dietary copper requirement of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune responses. *Fish Shellfish Immunol.,* 13: 259-270. https://doi.org/10.1006/fsim.2001.0401.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* 193: 265-275.

Metcalfe, L. D., Schimitz, A. and Pelka, J. R. 1966. Rapid preparation of fatty acids esters from lipids for gas chromatographic analysis. *Annexe Chem.,* 38: 524-535. https://doi.org/10.1021/ac60235a044.

Montero, D., Robaina, L., Caballero, M. J., Gins, R. and Izquierdo, M. S. 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of re-feeding period with a 100% fish oil diet. *Aquaculture,* 248: 121-134. doi: 10.5713/ajas.2011.11470.

Moreno-Arias, A., Lopez-Elias, J. A., Miranda-Baeza, A., Rivas-Vega, M. E., Martínez-Cordova, L. R. and Ramirez-Suarez, J. C. 2017. Replacement of fishmeal by vegetable meal mix in the diets of *Litopenaeus vannamei* reared in low-salinity biofloc system: effect on digestive enzymatic activity. *Aquac. Nutr.,* 23: 236-245. https://doi.org/10.1111/anu.12384.

Pakravan, S., Akbarzadeh, A., Sajjadi, M. M., Hajimoradloo, A. and Noori, F. 2017. Partial and total replacement of fish meal by marine microalga *Spirulina platensis* in the diet of Pacific white shrimp, *Litopenaeus vannamei*: Growth, digestive enzyme activities, fatty acid composition and responses to ammonia and hypoxia stress. *Aquac. Res.,* 48: 5576-5586. https://doi.org/10.1111/are.13379.

Perazzolo, L. M. and Barracco, M. A. 1997. The prophenoloxidase activating system of the shrimp *Penaeus paulensis* and associated factors. *Develop. Comp. Immunol.,* 21: 385-395. DOI: 10.1016/S0145-305X(97)00022-0.

Rumsey, G. L., Siwicki, A. K., Anderson, D. P. and Bowser, P. R. 1994. Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein

utilization in rainbow trout. *Vet. Immunol. Immunopathol.,* 41: 323-339. DOI:10.1016/0165-2427(94)90105-8.

Sastry, C. S. P. and Tammuru, M. K. 1985. Spectrophotometric determination of tryptophan in protein. *J. Food Sci. Technol.,* 22: 146-147.

Sharawy, Z., Goda, A. M. A. S. and Hassaan, M. S. 2016. Partial or total replacement of fishmeal by solid state fermented soybean meal with *Saccharomyces cerevisiae* in diets for Indian prawn shrimp, *Fenneropenaeus indicus*, post-larvae. *Anim. Feed Sci. Technol.,* 212: 90-99. https://doi.org/10.1111/anu.12838.

Shiu, Y. L., Hsieh, S. L., Guei, W. C., Tsai, Y. T., Chiu, C. H. and Liu, C. H. 2015. Using *Bacillus subtilis* E20-fermented soybean meal as replacement for fish meal in the diet of orange-spotted grouper (*Epinephelus coioides*, Hamilton). *Aquac. Res.,* 46: 1403-1416. https://doi.org/10.1111/are.12294.

Sritunyalucksana, K. and Soderhall, K. 2000. The proPO and clotting system in crustaceans. *Aquaculture,* 191: 53-69.

Sun, H., Tang, J., Yao, X., Wu, Y., Wang, X. and Liu, Y. 2015. Effects of replacement of fishmeal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, *Litopenaeus vannamei* Boone, 1931. *Aquac. Res.,* 1-10. https://doi.org/10.1111/are.12711.

Sung, H. H. and Sun, R. 2002. Use of monoclonal antibodies to classify haemocyte subpopulations of tiger shrimp (*Penaeus monodon*). *J. Crust. Biol.,* 22: 337-344.

Xu, X. L., Ji, W. J., Castell, J. D. and O'dor, R. K. 1994. Influence of dietary lipid sources on fecundity, egg hatchability and fatty acid composition of Chinese prawn (*Penaeus chinensis*) broodstock. *Aquaculture,* 119: 359-370. https://doi.org/10.1016/0044-8486(94)90300-X.

Yang, Y., Xie, S., Lei, W., Zhu, X. and Yang, Y. 2004. Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and immune response of *Macrobrachium nipponense. Fish Shellfish Immunol.,* 17: 105-114. DOI:10.1016/j.fsi.2003.11.006.

Date of Receipt : 11.04.2019
Date of Acceptance : 30.07.2019