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Effect of *Aspergillus niger* fermented soybean meal and sunflower oil cake on growth, carcass composition and haemolymph indices in *Penaeus vannamei* Boone, 1931

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ABSTRACT

Commercial solvent extracted soybean meal (SBM) and sunflower oil cake (SFC) were fermented using the fungus, *Aspergillus niger*. A 45-day indoor feeding trial was carried out to assess the effect of these fermented ingredients on growth, carcass composition and haemolymph indices in *Penaeus vannamei*. Nine iso-nitrogenous diets were formulated by replacing fishmeal (*w*/w) with untreated/fermented SBM (200, 250, 300. 350 and 400 g/kg) and SFC (0, 25, 50, 75 and 100 g/kg). Each diet was randomly allotted to three tanks holding 20 shrimp per tank under flow-through culture condition (1.5 ml/min). Results revealed that there was no significant difference in the growth between animals fed on diets containing fermented SBM up to 350 g/kg and fermented SFC up to 50 g/kg and those fed on the control diet, whereas, the level of inclusion was 250 and 25 g/kg for the respective untreated materials. However, the broken-line analysis indicated that maximum inclusion level of fermented SBM and fermented SFC was 325.1 and 32.6 g/kg respectively. Better feed efficiency measures were obtained with diets formulated using the fermented ingredients when compared to those formulated using the respective untreated ingredients. A significant increase was observed in ether extract levels of shrimp fed on the diets containing test ingredients when compared to those fed on control diet. Haemolymph indices showed a significant difference in total protein, glucose, cholesterol and triglycerides levels between the dietary treatments. The results of this study indicate that the fungal fermented ingredients could be used as potential protein sources rather than untreated materials in the diet of *P. vannamei*.

1. Introduction

Shrimp feed exceedingly relies upon fishmeal because of its wholesome attributes such as excellent amino acid profiles, higher palatability and digestibility. Increasing demand and diminishing availability of fishmeal in recent years has brought about an increase in its cost. This phenomenon has generated an interest in identifying appropriate optional protein sources as substitutes for fishmeal. Plant protein sources, particularly certain oilseed cakes/meals are considered to be suitable alternatives to fishmeal because of their wide availability, sustainability and also reasonable price (Gatlin et al., 2007). Among the oilseed cakes, soybean meal (SBM) is a promising ingredient. The global production of SBM is around 226 million metric tons (Mt) and India accounts for around 4% of the total production (USDA, 2016). The usage of SBM has been studied in numerous aquatic species (Refstie et al., 1998; Amaya et al., 2007) however, SBM as a major or sole protein source in the diet of shrimp is restricted because of inadequate levels of sulphur containing amino acids and the presence of anti-nutritional factors, particularly trypsin inhibitor (Shiu et al., 2015). Similarly, the usage of other oilseed meals has also been examined in various aquatic species for increasing the affordability of feed ingredients (Davis and Arnold, 2002; Thiessen et al., 2004). One among them is the sunflower oil cake (SFC). The worldwide production of SFC is 17.9 Mt., with India contributing around 3.8% (USDA, 2016). In our earlier study, SFC was assessed as a protein source in the diet of *Penaeus monodon* and the results revealed that the inclusion of SFC beyond 2.5% in the diet of *P. monodon* reduced the digestibility and growth due to the higher content of fiber fractions (Dayal et al., 2011). Hence, the nutritional quality of the plant protein sources needs to be enhanced for higher levels of inclusion and better utilization in the diet of shrimp.

Solid state fermentation is an economically viable processing technique adopted in recent years, which in part or totally eliminates the limitations related to plant protein sources (Shi et al., 2015). In our previous studies (Jannathulla et al., 2017a, 2017b), it was found that plant protein sources fermented with the fungus, *Aspergillus niger* had a lower level of anti-nutritional factors and fiber fractions than the

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respective unfermented ingredients. This result is in agreement with various findings (Hong et al., 2004; Shi et al., 2015). Nonetheless, the greater part of previous reports on fermentation has been confined to assessing restricted nutrients, without focusing on growth parameters in candidate shrimp species. The present work, therefore, aimed to study the impact of fermentation using the fungus, *A. niger* on the utilization of SBM (high protein with low fiber) and SFC (low protein with high fiber) in the diet of *P. vannamei*.

2. Materials and methods

2.1. Fermentation methodology

The fungus, *A. niger* listed under GRAS notifications (Generally Recognized As Safe) by FDA (GRAS Notice No. 35, 2010) was used for fermentation. The ATCC (6275) culture of *A. niger* acquired from Himedia Laboratories (Mumbai, India) was grown on potato dextrose agar (PDA) for five days at 35 ± 1 °C in an incubator. Tween 80 (0.1%) was used to harvest the fungal spores and the suspension was approximately adjusted to 1×10^7 spores/ml. Meanwhile, the commercial solvent extracted SBM and SFC were purchased from nearby markets ($n = 6$) in and around Chennai, India and were ground to a particle size of < 500 μm. Both the substrates were hydrated with water to bring the moisture content to 60–65% and then subsequently, autoclaved at 121 °C for 15 min. After cooling to room temperature, the autoclaved substrates were inoculated with 5% *A. niger* suspension. Fermentation was carried out in a 500 ml Erlenmeyer flask plugged with cotton to facilitate air exchange at 35 ± 1 °C in an incubator for three days, with three sets of replications (Shi et al., 2015). Post fermentation, all the samples were dried at 50 °C for 48 h to bring down the moisture content to below 10%. In order to have a representative sample, all the replicates of an ingredient was pooled to avoid a possible variation. The fermented materials were ground to fine particles of < 250 μm and stored at 4 °C until further use. The nutritional composition of test ingredients (untreated and fermented SBM and SFC) and Fishmeal are presented in Table 1 ($n = 6$).

2.2. Experimental diets

The experimental diets were formulated based on the nutritional requirement of *P. vannamei* using locally available ingredients. While preparing the diets, all the dry solid ingredients listed in the formulae (Tables 2 and 3) were ground to fine particles using an electric grinder and passed through 250 μm sieves. Oil sources and feed additives were added to the ground materials and blended for 20 min in an electric blender for homogenization. The homogenized mash was hydrated with water at the rate of 500 ml/kg of mash and made into a dough. The dough was then steamed at atmospheric pressure for 5 min, cooled and pelletized in a tabletop pelletizer having a 2 mm diameter die (Dayal et al., 2003). The pellets were dried in a forced air oven at 60 °C for 12 h and stored in a refrigerator (4 °C) until use. The test ingredients, untreated and fermented SBM were serially included at the rate of 200 (control), 250, 300, 350 and 400 g/kg, whereas untreated and fermented SFC were included at the rate of 0 (control), 25, 50, 75 and 100 g/kg by replacing fishmeal (*w*/w basis).

In general, the control diet was formulated as a fishmeal-based diet which did not have test ingredients, in order to evaluate the effect of plant protein sources as an alternative to fishmeal. Hence, in the present study, SFC was not included in the control diet as it is one of the test ingredients (Table 3). SBM currently represents one of the predominant protein sources of choice in shrimp feed formulation and is included at levels higher than 20% in commercially available feeds because of its protein/amino acid composition, consistency of supply and cost. Consequently, 200 g/kg of SBM was included in the control diet as done in commercial formulations with the assumption that the outcome of the present study could have more viable applications. Since the real aim of the present study was the application aspect of fermentation in aquafeed technology, untreated SBM was, subsequently replaced by fermented SBM to assess whether the impact of fermentation was valuable enough to warrant the discontinuation of use of untreated SBM. Since untreated and fermented ingredients used in the present investigation showed diverse protein levels (Table 1), fishmeal was replaced with test ingredients on a *w*/w basis for better correlation of treatments to assess the specific effect of fermentation on shrimp growth and nutrient utilization. However, the experimental diets were formulated to be iso-nitrogenous by adjusting the nitrogen content with corn gluten meal. Besides, substitution of fishmeal with test ingredients brought about a reduction not only in the level of protein but also in that of lipid, as both the test ingredients were solvent extracted (Table 1). Fishmeal used in the present study contained 105.31 g/kg lipid, although the test plant protein sources had < 17.34 g/kg of lipid. Hence, palm oil was included in the test diets to compensate the lipid loss due to fishmeal substitution. However, 20 g/kg of fish oil and 10 g/kg of lecithin were included in test diets as done in the control diet. Proximate and essential amino acid compositions of experimental diets ($n = 3$) are given in Table 2 (SBM) and Table 3 (SFC).

2.3. Experimental design

The feeding trials were conducted for 45 days in a flow-through aquaculture system (1.5 ml/min) at the Muttukadu Experimental Station of ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. The juveniles procured from a farm near Chennai, India were acclimatized for two weeks to the experimental conditions. During acclimatization, shrimp were fed on a diet containing 37% crude protein. The juveniles of *P. vannamei* (3.08 ± 0.07 g) were randomly stocked in 500 l (1.31 × 0.64 × 0.73 m) oval-shaped fiberglass reinforced plastics (FRP) tanks at the rate of twenty shrimps per tank, with three replications for each treatment. The shrimp were fed the respective experimental diet thrice a day (7.00 AM, 12.30 PM and 5.30 PM) and the amount of diet provided was adjusted according to survival, body weight and intake. The uneaten feed particles (if any) were expelled from the tank after an hour of feeding and were dried overnight at 60 °C in a hot air oven to compute the feed intake on a daily basis. Shrimp were maintained under natural photoperiodicity of 12 h l: 12 h D. At the end of the growth trial, weight gain (WG), daily growth coefficient (DGC), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent protein utilization (APU) and survival for each dietary treatment were determined.

$$WG\ (\%) = [\text{Initial weight (g)} - \text{Final weight (g)}]/\text{Initial weight (g)} \times 100$$

$$DGC = [\text{Final body weight}^{1/3} - \text{Initial body weight}^{1/3}]/\text{Days of experiment} \times 100$$

$$FCR = \text{Feed intake (g)}/\text{Weight gain (g)}$$

$$PER = \text{Weight gain (g)}/\text{Protein intake (g)}$$

$$APU = \text{Protein gain (g)}/\text{Protein intake (g)}$$

$$\text{Survival}\ (\%) = \text{Final number of animals}/\text{Initial number of animals} \times 100$$

Haemolymph samples were acquired from five shrimps in each replication of a treatment (fifteen shrimp per treatment) through the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on a 1 ml syringe containing 0.3 ml of anticoagulant solution. In order to have a representative sample, all the three replicates in a treatment were pooled to avoid possible variations, and represented as one replicate. Haemolymph indices such as total protein, glucose, cholesterol and triglycerides were estimated using the respective commercial kits obtained from Sigma-Aldrich (Code No: TP0100, GAHK20, MAK043 and TR0100) in a UV-spectrophotometer (Shimadzu, UV-1800) at 595, 340, 570 and 540 nm, respectively,

Table 1
Chemical composition of fishmeal and test ingredients used in the present study (g/kg dry matter basis).

Particulars	Fishmeal	Test ingredients			
		SBM[1]	FSBM[2]	SFC[3]	FSFC[4]
Chemical composition					
Crude protein	631.67 ± 6.05	524.04 ± 7.37^{b}	598.51 ± 5.34^{a}	356.07 ± 1.41^{y}	375.44 ± 1.65$^{\times}$
Ether extract	105.31 ± 3.40	10.91 ± 0.42^{a}	7.77 ± 0.30^{b}	17.34 ± 0.49$^{\times}$	15.63 ± 1.00^{y}
Crude fiber	5.39 ± 0.33	69.57 ± 2.55^{a}	67.74 ± 2.37^{b}	288.51 ± 7.28$^{\times}$	264.11 ± 7.01^{y}
Neutral detergent fiber	10.04 ± 0.23	119.24 ± 1.26^{a}	114.73 ± 0.83^{b}	438.61 ± 5.21$^{\times}$	403.66 ± 3.75^{y}
Acid detergent fiber	8.16 ± 0.11	78.26 ± 2.36^{a}	73.91 ± 1.66^{b}	275.94 ± 2.03$^{\times}$	257.22 ± 2.90^{y}
Nitrogen free extract	68.06 ± 8.36	320.04 ± 5.58^{a}	246.01 ± 7.60^{b}	259.74 ± 8.79$^{\times}$	264.27 ± 7.81$^{\times}$
Total ash	189.57 ± 5.11	75.44 ± 4.28^{a}	79.97 ± 3.27^{a}	78.34 ± 2.80$^{\times}$	80.55 ± 1.74$^{\times}$
Essential amino acids					
Arginine	43.77 ± 0.75	30.04 ± 2.23^{b}	40.71 ± 2.97^{a}	16.15 ± 1.19^{y}	18.80 ± 0.99$^{\times}$
Histidine	16.94 ± 0.52	17.50 ± 1.69^{b}	19.46 ± 0.99^{a}	4.69 ± 0.58^{y}	5.64 ± 0.64$^{\times}$
Isoleucine	29.65 ± 0.53	27.26 ± 0.59^{b}	29.04 ± 0.52^{a}	33.65 ± 0.58$^{\times}$	33.70 ± 0.65$^{\times}$
Leucine	50.83 ± 0.83	39.27 ± 0.76^{a}	40.09 ± 0.60^{a}	14.59 ± 0.77^{y}	18.70 ± 1.10$^{\times}$
Lysine	52.95 ± 0.60	12.49 ± 1.62^{b}	40.08 ± 2.24^{a}	11.80 ± 1.88^{y}	23.10 ± 2.80$^{\times}$
Methionine	19.06 ± 0.29	7.41 ± 1.13^{b}	9.90 ± 0.75^{a}	17.00 ± 1.59$^{\times}$	17.75 ± 0.94$^{\times}$
Phenylalanine	27.53 ± 0.55	20.25 ± 1.00^{b}	25.24 ± 1.11^{a}	16.04 ± 0.90^{y}	17.20 ± 0.55$^{\times}$
Threonine	28.95 ± 1.05	17.15 ± 1.17^{b}	19.09 ± 0.53^{a}	10.25 ± 1.07^{y}	15.00 ± 1.28$^{\times}$
Tryptophan	7.06 ± 0.29	6.70 ± 0.47^{b}	7.70 ± 0.39^{a}	4.20 ± 0.45$^{\times}$	4.40 ± 0.34$^{\times}$
Valine	34.59 ± 0.93	16.29 ± 0.75^{b}	17.55 ± 1.06^{a}	14.80 ± 0.96$^{\times}$	14.90 ± 1.05$^{\times}$
Anti-nutritional factors					
Trypsin inhibitor	–	2.41 ± 0.03^{a}	0.14 ± 0.02^{b}	nd[5]	nd[5]
Phytic acid	–	13.36 ± 0.23^{a}	6.53 ± 0.15^{b}	nd[5]	nd[5]
Tannin	–	nd[5]	nd[5]	8.79 ± 0.17$^{\times}$	6.10 ± 0.09^{y}
Saponin	–	10.03 ± 0.01^{a}	2.10 ± 0.06^{b}	6.42 ± 0.37$^{\times}$	2.17 ± 0.21^{y}

All the values are mean ± SD of six observations
Values with the same superscript letters in the same row between untreated and the respective fermented samples are not significant ($P > 0.05$).
[1] Untreated soybean meal.
[2] Fermented soybean meal.
[3] Untreated sunflower oil cake.
[4] Fermented sunflower oil cake.
[5] Not detected.

according to the accredited methodologies given by Sigma-Aldrich (http://www.sigmaaldrich. com/content/dam/sigma-aldrich/docs/Sigma/Bulletin/(Code No)bul.pdf). The remaining whole shrimps, not used for haemolymph collection, were used for carcass composition analysis.

2.4. Water quality parameters

Ultraviolet treated water was used for the experiments and water quality parameters viz., salinity (19 to 21 g/l), temperature (26.5 to 28.5 °C), dissolved oxygen (5.8 to 7.8 mg/l), pH (8 to 8.5) and total ammonia-nitrogen (< 0.1 mg/l) were measured periodically by standard methods (APHA, 2012) and recorded.

2.5. Biochemical analysis

Proximate composition of test ingredients, experimental diets and shrimp carcass in terms of moisture, crude protein, ether extract, crude fiber and total ash were analyzed according to the methods of AOAC (1997). Anti-nutritional factors, such as trypsin inhibitor (Kakade et al., 1974), saponin (Wang et al., 2007), phytic acid (Davies and Reid, 1979) and tannin (Price et al., 1978) were analyzed by standard methods. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated following Van Soest et al. (1991) method.

Amino acid profiles were analyzed using pre-column HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolyzing the samples with 6 N hydrochloric acid in a sealed tube for 22 h at 110 °C in a vacuum oven (Finlayson, 1964). The acid was evaporated using a vacuum rotary evaporator and the residue was placed in a diluent (0.1 N hydrochloric acid) and then filtered using 0.2 µm membrane syringe filter. Separation of amino acids was done on a column (YMC-Triart C18, RRHD 1.8 µm, 2.1 × 100 mm dimension) under gradient elution using phosphate buffer (20 mmol) as mobile phase A and combination of acetonitrile: methanol: water (45:40:15) as mobile phase B at the flow rate of 0.3 ml/min. The gradient was achieved by setting the concentration of mobile phase B at 11 to 13% for 3 min, 31% for 5 min, 37% for 15 min, 70% for 20 min and 100% for 25 min. Mercaptopropionic acid, O-pthaladehyde and fluorenylmethoxycarbonyl chloride were used as derivatizing agents. Amino acids were qualified and quantified by a fluorescent detector (RF-20AXS) using the amino acid mixer as an external standard (Sigma Aldrich, Cat. No: AAS18-5ML). Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). The partial oxidation of methionine during acid digestion was prevented by using 0.1% phenol (Jajic et al., 2013).

2.6. Statistical analysis

Data on chemical composition of test ingredients were statistically evaluated using the *t*-test to find the effect of fermentation. The other experimental data viz., feed composition, growth performance, carcass composition and haemolymph indices of shrimp were subjected to one way analysis of variance (ANOVA) and a multiple comparison of treatments was done using Tukey's test to detect significant difference between the treatments. Prior to statistical evaluation, the data were checked for determining the homogeneity of variance after ascertaining the normal distribution. SPSS ver. 17.0 was used for data analysis and all the statistical tests were evaluated at 5% of significance ($P < 0.05$). Regression analysis of the broken-line model was performed to calculate the maximum inclusion level of fermented ingredients by replacing fishmeal in the diet of *P. vannamei.*

Table 2
Ingredient, proximate and essential amino acid composition of experimental diets containing graded levels of untreated and fermented soybean meal by replacing fishmeal (g/kg as fed basis).

Ingredients	Control diet (CNT)	Diets with test ingredients							
		SBM 250	SBM 300	SBM 350	SBM 400	FSBM 250	FSBM 300	FSBM 350	FSBM 400
Fishmeal[1]	250	200	150	100	50	200	150	100	50
SBM[2]	200	250	300	350	400	–	–	–	–
FSBM[3]	–	–	–	–	–	250	300	350	400
Acetes[4]	80	80	80	80	80	80	80	80	80
Squid meal	15	15	15	15	15	15	15	15	15
Corn gluten	20	33	40	47	54	24	28	32	36
Sesame cake	50	50	50	50	50	50	50	50	50
Wheat flour	324	306	294	282	270	315	306	297	288
Fish oil[1]	20	20	20	20	20	20	20	20	20
Palm oil	–	5	10	15	20	5	10	15	20
Lecithin	10	10	10	10	10	10	10	10	10
Pre-mix[5]	20	20	20	20	20	20	20	20	20
Binder[6]	10	10	10	10	10	10	10	10	10
BHT[7]	1	1	1	1	1	1	1	1	1
Proximate composition									
Moisture	87.6 ± 1.3^{a}	88.2 ± 0.6^{a}	86.2 ± 1.0^{a}	86.7 ± 1.8^{a}	87.6 ± 1.2^{a}	88.2 ± 0.7^{a}	87.0 ± 1.3^{a}	87.1 ± 0.2^{a}	87.3 ± 1.2^{a}
Crude protein	374.4 ± 5.3^{a}	367.4 ± 8.3^{a}	363.8 ± 9.9^{a}	364.6 ± 3.4^{a}	365.6 ± 2.5^{a}	372.6 ± 5.0^{a}	371.4 ± 7.2^{a}	369.8 ± 4.4^{a}	376.5 ± 6.7^{a}
Ether extract	67.6 ± 1.0^{a}	69.4 ± 0.9^{a}	70.1 ± 1.4^{a}	71.1 ± 0.9^{a}	70.1 ± 2.0^{a}	69.8 ± 2.6^{a}	71.1 ± 0.6^{a}	71.5 ± 1.5^{a}	72.2 ± 2.0^{a}
Crude fiber	29.8 ± 1.5^{f}	30.1 ± 1.2^{f}	33.7 ± 1.9^{d}	36.3 ± 1.5^{c}	38.7 ± 1.5^{a}	29.8 ± 0.9^{f}	32.6 ± 1.1^{e}	36.2 ± 1.0^{c}	37.6 ± 1.2^{b}
NDF[8]	291.9 ± 1.7^{g}	297.3 ± 3.7^{f}	333.1 ± 2.6^{d}	356.2 ± 4.1^{c}	381.4 ± 0.5^{a}	291.1 ± 2.4^{g}	319.5 ± 2.3^{e}	354.0 ± 1.8^{c}	367.5 ± 3.8^{b}
ADF[9]	124.6 ± 2.0^{g}	128.8 ± 4.0^{f}	142.7 ± 3.4^{d}	155.4 ± 3.0^{b}	165.4 ± 2.7^{a}	125.0 ± 7.0^{g}	132.3 ± 2.9^{e}	148.4 ± 2.4^{c}	155.0 ± 3.4^{b}
NFE[10]	297.1 ± 8.5^{e}	311.3 ± 11.8^{cd}	324.0 ± 11.5^{ab}	324.9 ± 6.8^{ab}	327.1 ± 4.3^{a}	305.4 ± 10.5^{de}	315.1 ± 13.7^{bcd}	319.6 ± 6.9^{abc}	315.5 ± 10.8^{bcd}
Total ash	143.5 ± 2.3^{a}	133.6 ± 2.8^{b}	122.2 ± 1.6^{c}	116.4 ± 1.3^{d}	110.9 ± 1.5^{e}	134.2 ± 3.0^{b}	122.8 ± 3.6^{c}	115.8 ± 1.7^{d}	110.9 ± 2.7^{e}
Essential amino acids									
Arginine	23.1 ± 0.6^{a}	23.8 ± 1.7^{a}	24.7 ± 1.0^{a}	25.1 ± 1.1^{a}	23.7 ± 0.9^{a}	24.2 ± 1.2^{a}	26.4 ± 1.7^{a}	23.9 ± 1.4^{a}	25.3 ± 1.7^{a}
Histidine	8.8 ± 0.6^{a}	8.6 ± 0.8^{a}	9.0 ± 0.7^{a}	9.1 ± 0.6^{a}	8.7 ± 0.3^{a}	9.1 ± 0.5^{a}	9.4 ± 0.6^{a}	9.3 ± 0.5^{a}	10.1 ± 1.1^{a}
Isoleucine	15.3 ± 0.9^{a}	15.6 ± 1.1^{a}	15.2 ± 0.9^{a}	15.7 ± 0.6^{a}	16.2 ± 0.6^{a}	15.6 ± 0.4^{a}	15.8 ± 0.2^{a}	16.9 ± 1.0^{a}	16.3 ± 0.8^{a}
Leucine	26.4 ± 0.9^{a}	27.5 ± 1.2^{a}	27.9 ± 2.0^{a}	26.9 ± 1.8^{a}	28.8 ± 2.1^{a}	26.7 ± 2.1^{a}	27.3 ± 1.5^{a}	27.2 ± 1.6^{a}	27.5 ± 2.9^{a}
Lysine	21.4 ± 1.1^{a}	20.9 ± 0.9^{a}	21.1 ± 1.2^{a}	19.7 ± 2.0^{a}	19.1 ± 2.0^{a}	21.2 ± 2.1^{a}	22.2 ± 2.1^{a}	20.8 ± 2.3^{a}	21.4 ± 2.6^{a}
Methionine	8.4 ± 0.4^{a}	8.2 ± 0.3^{a}	7.7 ± 0.4^{abcd}	6.9 ± 0.7^{e}	7.0 ± 0.5^{de}	8.0 ± 0.2^{ab}	7.8 ± 0.3^{abc}	7.4 ± 0.8^{bcde}	7.2 ± 0.3^{cde}
Phenylalanine	17.3 ± 0.5^{a}	17.9 ± 1.0^{a}	18.7 ± 0.7^{a}	18.3 ± 0.4^{a}	19.1 ± 0.9^{a}	17.6 ± 1.2^{a}	18.7 ± 2.0^{a}	18.1 ± 1.0^{a}	19.1 ± 1.2^{a}
Threonine	14.3 ± 0.6^{a}	13.9 ± 1.0^{a}	14.2 ± 1.3^{a}	14.1 ± 0.9^{a}	13.5 ± 0.1^{a}	14.1 ± 0.4^{a}	13.9 ± 0.1 ^{a}	14.7 ± 0.8^{a}	13.5 ± 0.9^{a}
Tryptophan	4.2 ± 0.4^{a}	3.9 ± 0.3^{a}	3.8 ± 0.4^{a}	4.3 ± 0.5^{a}	4.1 ± 0.3^{a}	4.2 ± 0.4^{a}	4.8 ± 0.3^{a}	4.4 ± 0.5^{a}	4.7 ± 0.3^{a}
Valine	17.1 ± 0.5^{a}	16.7 ± 0.7^{a}	17.2 ± 0.6^{a}	17.8 ± 0.8^{a}	17.1 ± 0.9^{a}	16.7 ± 0.9^{a}	16.8 ± 0.8^{a}	15.7 ± 0.9^{a}	15.3 ± 1.0^{a}

All the values are mean ± SD of three observations
Values with the same superscript letters in the same row are not significantly different ($P > 0.05$).
[1] Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India.
[2] Untreated soybean meal.
[3] Fermented soybean meal.
[4] Mantis shrimp used as a protein source.
[5] Pre-mix (g/kg): Thiamine hydrochloride (25.50 g), riboflavin (25.00 g), prydoxine hydrochloride (50.00 g), cyanogobalamine (0.10 g), menadione (5.00 g), all-trans tocopherol acetate (99.00 g), retinyl acetate (10.00 g), vitamin D (50 g), nicitinic acid (101.00 g), D-Ca-pantothenate (61.00 g), biotin (25.00 g), folic acid (6.25 g), inositol (153.06 g), ferric citrate (13.70 g), $ZnSO_4.7H_2O$ (28.28 g), $MgSO_4.7H_2O$ (0.12 g), MnSO4·H2O (12.43 g), $CuSO_4.5\ H_2O$ (19.84 g), $CoC_{12}.6H_2O$ (4.07 g), KIO_4 (0.03 g), KCl (15.33 g), Na_2SeO_3 (0.02 g).
[6] Pegabind, Bentoli AgriNutrition Asia Pvt. Ltd., Singapore.
[7] Butylated hydroxytoluene: Sigma Aldrich (Cat. No: PHR1117).
[8] Neutral detergent fiber.
[9] Acid detergent fiber.
[10] Nitrogen free extract (Calculated by difference).

3. Results

The crude protein and amino acid contents were higher in the fermented ingredients than in the respective untreated materials. The increase due to fungal fermentation was almost double in limiting amino acids, methionine and lysine. A marginal reduction was observed in crude fiber, NDF and ADF. All the analyzed anti-nutritional factors found to be much lower in fermented ingredients than in the respective untreated ingredient. However, the nutritional composition of fishmeal (Table 1) was comparatively better than fermented SBM and SFC. No significant differences were observed in moisture, crude protein and ether extract levels of experimental feeds due to the substitution of fishmeal with test ingredients, while a gradual increase in crude fiber, NDF and ADF, and a gradual decrease in total ash content were observed (Tables 2 and 3).

The growth performance, carcass composition and haemolymph indices of *P. vannamei* are depicted in Table 4 for SBM and Table 5 for SFC. The inclusion level of untreated SBM and SFC were limited to 250 and 25 g/kg, respectively, whereas the inclusion level could significantly ($P < 0.05$) be increased up to 350 g/kg for fermented SBM and 50 g/kg for fermented SFC in the diet of *P. vannamei*, without compromising on growth performance. A gradual increase in FCR was observed with increasing inclusion levels of both untreated and fermented plant protein sources. However, shrimp fed on fermented ingredients showed lower FCR than those fed with the comparable levels of untreated ingredients. Among all the treatments, PER and APU were higher in shrimp fed with diets having fermented ingredients, as compared to those fed on diets having the respective level of untreated ingredients. There was no significant difference in survival among the treatments (83.33 to 96.67%). The correlation between weight gain and inclusion level of fermented ingredients was performed using the broken-line regression analysis, which indicated that the maximum

Table 3
Ingredient, proximate and essential amino acid composition of experimental diets containing graded levels of untreated and fermented sunflower oil cake by replacing fishmeal (g/kg as fed basis).

Ingredients	Control diet (CNT)	Diets with test ingredients							
		SFC 25	SFC 50	SFC 75	SFC 100	FSFC 25	FSFC 50	FSFC 75	FSFC 100
Fishmeal[1]	250	225	200	175	150	225	200	175	150
SFC[2]	–	25	50	75	100	–	–	–	–
FSFC[3]	–	–	–	–	–	25	50	75	100
Acetes[4]	80	80	80	80	80	80	80	80	80
Squid meal	15	15	15	15	15	15	15	15	15
Soybean meal	200	200	200	200	200	200	200	200	200
Corn gluten	20	29	39	49	59	27	34	42	50
Sesame cake	50	50	50	50	50	50	50	50	50
Wheat flour	324	313	301	289	277	315	306	296	286
Fish oil[1]	20	20	20	20	20	20	20	20	20
Palm oil	–	2	4	6	8	2	4	6	8
Lecithin	10	10	10	10	10	10	10	10	10
Pre-mix[5]	20	20	20	20	20	20	20	20	20
Binder[6]	10	10	10	10	10	10	10	10	10
BHT[7]	1	1	1	1	1	1	1	1	1
Proximate composition									
Moisture	87.6 ± 1.3^{a}	86.5 ± 1.5^{a}	78.9 ± 7.2^{a}	80.1 ± 8.5^{a}	82.3 ± 4.0^{a}	84.5 ± 1.3^{a}	86.7 ± 1.1^{a}	78.5 ± 6.9^{a}	79.5 ± 6.4^{a}
Crude protein	374.4 ± 5.3^{a}	375.3 ± 8.8^{a}	380.4 ± 9.1^{a}	367.4 ± 7.9^{a}	370.8 ± 6.9^{a}	374.3 ± 6.4^{a}	381.7 ± 8.3^{a}	369.9 ± 5.3^{a}	371.7 ± 7.4^{a}
Ether extract	67.6 ± 1.0^{a}	70.0 ± 5.3^{a}	71.5 ± 1.3^{a}	69.8 ± 1.8^{a}	70.1 ± 0.6^{a}	71.3 ± 1.6^{a}	70.5 ± 1.0^{a}	69.7 ± 2.0^{a}	68.5 ± 1.2^{a}
Crude fiber	29.8 ± 1.5^{e}	34.6 ± 2.5^{d}	40.9 ± 2.6^{c}	47.2 ± 1.7^{b}	53.4 ± 1.6^{a}	34.4 ± 1.0^{d}	40.4 ± 2.4^{c}	46.4 ± 2.9^{b}	52.4 ± 1.2^{a}
NDF[8]	291.9 ± 1.7^{i}	345.0 ± 0.9^{g}	403.8 ± 4.6^{e}	465.8 ± 1.9^{c}	528.7 ± 1.2^{a}	334.8 ± 1.2^{h}	394.8 ± 4.7^{f}	452.7 ± 1.5^{d}	513.5 ± 2.9^{b}
ADF[9]	124.6 ± 2.0^{i}	149.2 ± 3.0^{g}	172.6 ± 2.0^{e}	201.7 ± 1.7^{c}	226.8 ± 3.8^{a}	144.2 ± 3.4^{h}	163.2 ± 3.8^{f}	192.5 ± 2.2^{d}	216.6 ± 1.1^{b}
NFE[10]	297.1 ± 8.5^{a}	303.2 ± 17.6^{a}	300.7 ± 27.9^{a}	310.8 ± 36.8^{a}	301.5 ± 14.0^{a}	305.0 ± 12.6^{a}	292.8 ± 13.2^{a}	310.3 ± 18.9^{a}	305.4 ± 17.2^{a}
Total ash	143.5 ± 2.3^{a}	130.4 ± 1.5^{b}	127.6 ± 2.0^{c}	124.7 ± 2.2^{d}	121.8 ± 1.5^{e}	130.6 ± 2.4^{b}	127.9 ± 0.8^{c}	125.2 ± 3.3^{d}	122.5 ± 2.3^{e}
Essential amino acids									
Arginine	23.1 ± 0.6^{a}	22.8 ± 0.9^{a}	26.4 ± 2.9^{a}	22.3 ± 1.7^{a}	22.1 ± 1.3^{a}	24.8 ± 1.4^{a}	22.6 ± 0.8^{a}	23.7 ± 1.0^{a}	22.7 ± 1.8^{a}
Histidine	8.8 ± 0.6^{a}	8.7 ± 0.4^{a}	8.3 ± 0.5^{a}	8.9 ± 0.4^{a}	7.8 ± 0.7^{a}	8.7 ± 0.8^{a}	9.1 ± 0.8^{a}	8.5 ± 0.5^{a}	8.1 ± 0.3^{a}
Isoleucine	15.3 ± 0.9^{a}	15.8 ± 1.0^{a}	16.8 ± 1.0^{a}	16.4 ± 1.3^{a}	17.2 ± 0.7^{a}	16.2 ± 0.8^{a}	15.5 ± 0.8^{a}	16.5 ± 0.9^{a}	16.9 ± 0.5^{a}
Leucine	26.4 ± 0.9^{a}	27.1 ± 1.6^{a}	26.8 ± 0.8^{a}	25.9 ± 1.1^{a}	27.2 ± 0.9^{a}	26.7 ± 2.3^{a}	26.6 ± 0.8^{a}	27.3 ± 0.5^{a}	27.7 ± 1.3^{a}
Lysine	21.4 ± 1.1^{a}	20.7 ± 1.3^{a}	20.4 ± 0.9^{a}	18.6 ± 1.2^{a}	19.4 ± 0.6^{a}	21.0 ± 1.4^{a}	19.6 ± 1.9^{a}	22.7 ± 2.3^{a}	20.9 ± 0.9^{a}
Methionine	8.4 ± 0.4^{a}	7.8 ± 0.6^{a}	7.3 ± 2.1^{a}	8.4 ± 0.6^{a}	8.9 ± 0.3^{a}	8.1 ± 0.6^{a}	7.7 ± 0.7^{a}	7.4 ± 0.4^{a}	8.8 ± 0.4^{a}
Phenylalanine	17.3 ± 0.5^{a}	17.1 ± 1.1^{a}	17.7 ± 0.8^{a}	18.0 ± 0.5^{a}	18.2 ± 0.4^{a}	17.8 ± 0.6^{a}	17.3 ± 1.1^{a}	18.0 ± 0.8^{a}	18.1 ± 0.8^{a}
Threonine	14.3 ± 0.6^{a}	15.1 ± 0.5^{a}	14.8 ± 0.8^{a}	14.4 ± 0.5^{a}	13.7 ± 0.4^{a}	14.0 ± 0.7^{a}	14.4 ± 0.6^{a}	15.2 ± 1.4^{a}	14.3 ± 1.2^{a}
Tryptophan	4.2 ± 0.4^{a}	3.8 ± 0.9^{a}	4.3 ± 1.2^{a}	4.4 ± 0.4^{a}	4.7 ± 0.9^{a}	4.4 ± 0.6^{a}	4.8 ± 0.7^{a}	5.1 ± 0.4^{a}	3.9 ± 0.7^{a}
Valine	17.1 ± 0.5^{a}	17.4 ± 0.9^{a}	16.8 ± 1.0^{a}	17.3 ± 1.3^{a}	17.8 ± 1.1^{a}	16.9 ± 1.1^{a}	17.4 ± 1.9^{a}	16.6 ± 1.1^{a}	17.0 ± 1.0^{a}

All the values are mean ± SD of three observations
Values with the same superscript letters in the same row are not significantly different ($P > 0.05$).
[1] Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India.
[2] Raw sunflower oil cake.
[3] Fermented sunflower oil cake.
[4] Mantis shrimp used as a protein source.
[5] Pre-mix (g/kg): Thiamine hydrochloride (25.50 g), riboflavin (25.00 g), prydoxine hydrochloride (50.00 g), cyanogobalamine (0.10 g), menadione (5.00 g), all-trans tocopherol acetate (99.00 g), retinyl acetate (10.00 g), vitamin D (50 g), nicitinic acid (101.00 g), D-Ca-pantothenate (61.00 g), biotin (25.00 g), folic acid (6.25 g), inositol (153.06 g), ferric citrate (13.70 g), $ZnSO_4.7H_2O$ (28.28 g), $MgSO_4.7H_2O$ (0.12 g), $MnSO4{\cdot}H2O$ (12.43 g), $CuSO_4.5\ H_2O$ (19.84 g), $CoC_{12}.6H_2O$ (4.07 g), KIO_4 (0.03 g), KCl (15.33 g), Na_2SeO_3 (0.02 g).
[6] Pegabind, Bentoli AgriNutrition Asia Pvt. Ltd., Singapore.
[7] Butylated hydroxytoluene: Sigma Aldrich (Cat. No: PHR1117).
[8] Neutral detergent fiber.
[9] Acid detergent fiber.
[10] Nitrogen free extract (Calculated by difference).

inclusion level of fermented SBM was 325.1 g/kg (Fig. 1) and fermented SFC was 32.6 g/kg (Fig. 2). Carcass lipid was significantly ($P < 0.05$) higher in the test groups compared to the control, whereas there was no significant differences in crude protein and ash content among the treatments. Increasing fishmeal substitution with test ingredients gradually decreased the level of haemolymph total protein, cholesterol and triglycerides whereas; a reverse trend was observed for haemolymph glucose.

4. Discussion

Substitution of fishmeal with plant protein sources is still quite a challenging task for nutritionists due to the associated constraints, such as deficiency of certain essential amino acids, higher content of fiber fractions and anti-nutritional factors. Hence, the method of fungal fermentation was adopted to enhance the nutritional quality of plant protein sources in the present study. Untreated SBM and SFC could be included at 250 and 25 g/kg, respectively, with no deleterious effect on the shrimp; growth rate and feed efficiency significantly ($P < 0.05$) reduced when the inclusion increased above the levels mentioned. However, shrimp fed on diets formulated with the fermented ingredients performed much better compared to those fed with diets containing the respective level of untreated ingredients. Lim and Dominy (1990) reported that *P. vannamei* fed with diet having 40% of the marine protein sources replaced using SBM showed growth equivalent to that of the control group, whereas 100% replacement showed a poor growth response due to the presence of trypsin inhibitor. The untreated SBM used in the present study had 2.41, 13.36 and 10.03 g/kg of trypsin inhibitor, phytic acid and saponin (Table 3), respectively. Swick and Ivey (1992) reported that phytic acid impairs the growth rate of aquatic species by forming phytic acid–protein complexes. Since 50 to 80% of phosphorus exists as phytate-bound

Table 4
Growth performances, carcass composition and haemolymph indices of *P. vannamei* fed with experimental diets having graded levels of untreated and fermented soybean meal by replacing fishmeal.

Particulars	Control diet (CNT)	Diets with test ingredients								SEM	*P*-value
		SBM 250	SBM 300	SBM 350	SBM 400	FSBM 250	FSBM 300	FSBM 350	FSBM 400		
Initial wt (g)	3.06^{a}	3.13^{a}	3.06^{a}	3.04^{a}	3.06^{a}	3.05^{a}	3.08^{a}	3.08^{a}	3.10^{a}	0.003	0.832
Final wt (g)	9.52^{a}	9.78^{a}	8.81^{b}	8.48^{bc}	8.23^{c}	9.67^{a}	9.60^{a}	9.35^{a}	8.79^{b}	0.049	< 0.001
Weight gain (%)	211.65^{a}	212.73^{a}	188.15^{b}	179.44^{bc}	169.41^{c}	216.48^{a}	211.80^{a}	203.25^{a}	183.96^{b}	39.238	< 0.001
DGC[1]	1.48^{a}	1.50^{a}	1.36^{b}	1.31^{bc}	1.26^{c}	1.51^{a}	1.49^{a}	1.44^{a}	1.35^{b}	0.001	< 0.001
FCR[2]	1.86^{c}	1.81^{c}	2.09^{b}	2.20^{ab}	2.32^{a}	1.82^{c}	1.84^{c}	1.92^{c}	2.11^{b}	0.003	< 0.001
PER[3]	1.44^{a}	1.51^{a}	1.32^{bc}	1.24^{cd}	1.18^{d}	1.48^{a}	1.46^{a}	1.41^{ab}	1.26^{cd}	0.002	< 0.001
APU[4]	23.59^{a}	24.75^{a}	21.60^{bc}	20.40^{cd}	19.35^{d}	24.23^{a}	23.98^{a}	23.15^{ab}	20.67^{cd}	0.546	< 0.001
Survival (%)	93.33^{a}	86.67^{a}	93.33^{a}	86.67^{a}	96.67^{a}	90.00^{a}	86.67^{a}	93.33^{a}	86.67^{a}	40.628	0.730
Carcass composition (g/kg wet basis)											
Moisture	78.10^{a}	78.61^{a}	78.56^{a}	78.58^{a}	78.85^{a}	78.62^{a}	77.38^{a}	78.27^{a}	78.66^{a}	0.167	0.107
Crude protein	16.34^{a}	15.99^{a}	16.03^{a}	15.95^{a}	15.78^{a}	15.92^{a}	16.86^{a}	16.13^{a}	15.90^{a}	0.112	0.180
Ether extract	0.88^{b}	1.08^{a}	1.02^{a}	1.02^{a}	1.03^{a}	0.99^{a}	1.07^{a}	1.07^{a}	1.06^{a}	0.002	0.026
Total ash	2.91^{a}	2.92^{a}	2.89^{a}	2.76^{a}	2.86^{a}	2.86^{a}	3.01^{a}	3.01^{a}	2.91^{a}	0.006	0.503
Haemolymph indices											
Total protein (g/dl)	9.28^{a}	8.18^{c}	8.19^{c}	7.47^{d}	6.23^{e}	8.84^{ab}	8.10^{c}	8.25^{bc}	6.35^{e}	0.074	< 0.001
Glucose (g/dl)	1.45^{d}	1.55^{cd}	1.61^{bc}	1.67^{bc}	1.81^{a}	1.48^{d}	1.66^{b}	1.65^{bc}	1.81^{a}	0.002	< 0.001
Cholesterol (mg/dl)	23.98^{a}	22.40^{b}	19.58^{c}	17.34^{ef}	15.93^{fg}	21.82^{b}	19.45^{cd}	17.99^{de}	15.71^{g}	0.447	< 0.001
Triglycerides (mg/dl)	65.52^{a}	61.23^{ab}	56.09^{c}	46.41^{d}	35.86^{e}	65.14^{a}	56.90^{bc}	43.72^{d}	37.67^{e}	3.729	< 0.001

All the values are mean ± SD of three observations.
Values with the same superscript letters in the same row are not significantly different ($P > 0.05$) from each other.
[1] Daily growth coefficient.
[2] Feed conversion ratio.
[3] Protein efficiency ratio.
[4] Apparent protein utilization.

Table 5
Growth performances, carcass composition and haemolymph indices of *P. vannamei* fed with experimental diets having graded levels of untreated and fermented sunflower oil cake by replacing fishmeal.

Particulars	Control diet (CNT)	Diets with test ingredients								SEM	*P*-value
		SFC 25	SFC 50	SFC 75	SFC 100	FSFC 25	FSFC 50	FSFC 75	FSFC 100		
Initial wt (g)	3.06^{a}	3.07^{a}	3.13^{a}	3.05^{a}	3.08^{a}	3.13^{a}	3.13^{a}	3.05^{a}	3.08^{a}	0.004	0.871
Final wt (g)	9.52^{a}	9.38^{ab}	9.15^{bc}	8.75^{de}	8.58^{e}	9.65^{a}	9.45^{ab}	8.93^{cd}	8.81^{de}	0.020	< 0.001
Weight gain (%)	211.65^{a}	205.50^{ab}	192.54^{bc}	186.64^{cd}	178.69^{d}	208.66^{a}	202.57^{ab}	192.41^{bcd}	185.93^{cd}	36.655	< 0.001
DGC[1]	1.48^{a}	1.45^{ab}	1.40^{bc}	1.35^{cd}	1.31^{d}	1.48^{a}	1.45^{ab}	1.38^{cd}	1.35^{cd}	0.001	0.001
FCR[2]	1.86^{e}	1.90^{de}	1.99^{cd}	2.11^{ab}	2.18^{a}	1.84^{e}	1.90^{e}	2.04^{bc}	2.10^{ab}	0.002	< 0.001
PER[3]	1.44^{ab}	1.40^{ab}	1.32^{cd}	1.29^{de}	1.24^{e}	1.45^{ab}	1.38^{bc}	1.32^{cd}	1.28^{de}	0.001	< 0.001
APU[4]	23.59^{ab}	23.16^{ab}	21.80^{cd}	21.37^{de}	20.45^{e}	24.01^{ab}	22.84^{bc}	21.87^{cd}	21.22^{de}	0.225	< 0.001
Survival (%)	93.33^{a}	96.67^{a}	90.00^{a}	86.67^{a}	83.33^{a}	86.67^{a}	93.33^{a}	86.67^{a}	90.00^{a}	58.270	0.816
Carcass composition (g/kg wet basis)											
Moisture	78.10^{a}	78.61^{a}	78.60^{a}	78.70^{a}	78.59^{a}	78.57^{a}	78.09^{a}	77.78^{a}	78.48^{a}	0.100	0.160
Crude protein	16.34^{a}	15.98^{a}	15.98^{a}	15.84^{a}	15.93^{a}	15.93^{a}	16.28^{a}	16.42^{a}	15.97^{a}	0.064	0.349
Ether extract	0.88^{c}	1.02^{ab}	0.97^{ab}	0.95^{bc}	0.98^{ab}	0.95^{bc}	1.00^{ab}	1.04^{a}	1.00^{ab}	0.001	0.029
Total ash	2.91^{a}	2.91^{a}	2.88^{a}	2.72^{a}	2.89^{a}	2.86^{a}	2.91^{a}	3.08^{a}	2.92^{a}	0.007	0.249
Haemolymph indices											
Total protein (g/dl)	9.28^{a}	8.48^{bc}	7.95^{c}	7.32^{d}	6.62^{e}	8.84^{ab}	8.17^{c}	8.29^{bc}	7.06^{de}	0.069	< 0.001
Glucose (g/dl)	1.45^{d}	1.52^{d}	1.61^{c}	1.69^{bc}	1.81^{a}	1.42^{d}	1.64^{c}	1.68^{c}	1.77^{ab}	0.002	< 0.001
Cholesterol (mg/dl)	23.98^{a}	22.19^{b}	19.25^{c}	17.67^{d}	16.11^{ef}	21.78^{b}	20.12^{c}	17.65^{de}	14.97^{f}	0.464	< 0.001
Triglycerides (mg/dl)	65.52^{a}	61.80^{a}	54.70^{b}	46.75^{c}	35.55^{d}	64.04^{a}	55.42^{b}	43.40^{c}	36.26^{d}	4.135	< 0.001

All the values are mean ± SD of three observations.
Values with the same superscript letters in the same row are not significantly different ($P > 0.05$).
[1] Daily growth coefficient.
[2] Feed conversion ratio.
[3] Protein efficiency ratio.
[4] Apparent protein utilization.

phosphate in untreated SBM (Ravindran et al., 1995), it is not readily accessible to mono-gastric animals, including shrimp due to the lack of the enzyme phytase (Wu et al., 2009). This would reduce the availability of other essential elements, mainly calcium, magnesium and potassium notwithstanding phosphorous (Liener, 1994). Decreased feeding rate, growth and molting frequency have been reported in *P. japonicus* exposed to > 0.1 mg/l of saponin (Chen et al., 1996). A similar deleterious impact was reported in *Macrobrachium rosenbergii* (Yeh et al., 2006). In our study, the fungus *A. niger* fermentation significantly ($P < 0.05$) reduced trypsin inhibitor, phytic acid and saponin by 94.2, 51.1 and 79.1%, respectively in SBM (Table 1). Makinde and Akinoso (2014) reported that phosphorus availability increases due

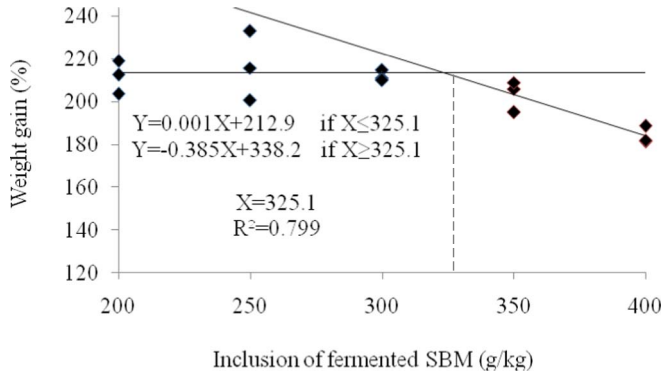

Fig. 1. Estimation of optimal substitution level of fishmeal with fermented SBM in the diet of *P. vannamei* using broken line analysis.

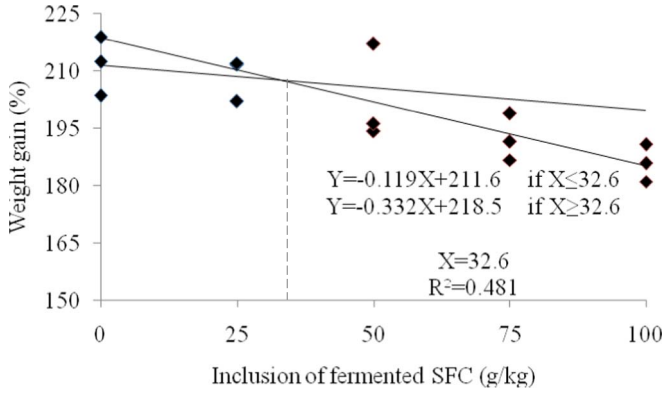

Fig. 2. Estimation of optimal substitution level of fishmeal with fermented SFC in the diet of *P. vannamei* using broken line analysis.

to degradation of phytic acid by the enzyme phytase produced during fermentation. Gull et al. (2013) reported 58 U/g phytase production during *A. niger* fermentation.

The challenging problem of digestibility due to the higher content of fiber and its fractions (Table 3) limits the usage of untreated SFC to 25 g/kg. This is in agreement with the findings of Dayal et al. (2011), who reported that growth performance and nutrient utilization significantly reduced when SFC was included beyond 2.5% in the diet of *P. monodon*. Lim et al. (1997) stated that *P. vannamei* fed on a diet containing canola meal, having a higher content of fiber (28%), exhibited depressed growth rate in contrast to those fed on low fiber canola meal (14%). The poor performance could be attributed to a decrease in the accessibility of entangled essential nutrients due to undigested fibrous components (Bureau et al., 1999). Besides, SFC also had anti-nutritional factors like tannin and saponin (Table 1). Krogdahl (1989) reported that the bitter taste of tannin decreased feed palatability, which lowered the feed intake and growth rate in Salmonids. Other deleterious effects of tannin have also been reported in various aquatic species (Maitra and Ray, 2003). In the present investigation, fiber fractions in SFC were markedly reduced with fermentation (Table 1), and tannin and saponin levels were also greatly reduced ($P < 0.05$) by 30.6% and 66.2%, respectively, compared to untreated SFC. In general, plant protein sources are deficient in one or more essential amino acids, particularly methionine, lysine and tryptophan that are a dietary pre-requisite of cultured species (De Silva et al., 1995). Hence, there is a need to supplement the respective deficient amino acid during feed formulation. However, Williams et al. (2001) have reported that supplementing crystalline amino acids into the diet resulted in higher leaching in water, faster degradation by enterocytes and micro flora in the gastrointestinal tract and extended rate of absorption compared to protein-bound amino acids. These drawbacks have been partly overcome by the methodology of fermentation using bacterial, fungal and yeast species (Ravindra, 2000). In the present study, an increase in the levels of limiting amino acids viz., methionine, lysine and tryptophan was noticed with the fungal fermentation (Table 1). This could likewise aid to enhance the inclusion levels of both fermented SBM and SFC in shrimp feed. From the present investigation, it seems that fermented SBM and fermented SFC could substitute 60 and 20% of dietary fishmeal, respectively with no negative effect on growth in *P. vannamei*. Sharawy et al. (2016) reported that the yeast fermented SBM could replace dietary fishmeal up to 50% in the diet of *P. indicus*. Our results are in concurrence with the findings of Shiu et al. (2015), who reported 37.42 and 61.67% of fishmeal substitution using untreated and bacterial fermented SBM, respectively in the diet of *P. vannamei*. Similar results were reported in *M. nipponense* (Ding et al., 2015), Nile tilapia (Hassaan et al., 2015) and *Labeo rohita* (Mukhopadhyay and Ray, 1999). In the present study, the feed and protein efficiency measures viz., FCR, PER and APU were better in shrimp fed diets containing fermented ingredients than in those fed on diets formulated with the respective level of untreated ingredients. No significant difference in survival among the treatements indicates the possibility of inclusion of the fermented ingredients even at higher levels.

Lipid deposition in the carcass was higher ($P < 0.05$) in shrimp fed test diets than those fed a control diet in our study. Kaushik et al. (2004) suggested that this could be due to upgraded hepatic lipogenesis. Menoyo et al. (2004) observed the higher activity of lipogenic enzymes in Gilthered seabream fed on a diet with vegetable oil substituting fish oil. In our study, palm oil was used to formulate isolipidic diets during fishmeal substitution, which could have resulted in increased carcass lipid. However, there was no difference in carcass moisture, protein and ash contents among the treatments. This is in agreement with findings of Yue et al. (2012) and Sun et al. (2015) while using a blend of SBM, groundnut oil cake, and fermented cottonseed meal as a fishmeal alternative in the diet of *P. vannamei*. Haemolymph protein was significantly ($P < 0.05$) lower in shrimp fed diets with test ingredients than those fed a control diet (CNT). This result is corroborated with the findings of Yun et al. (2017). The level of glucose in *P. vannamei* was gradually increased with the increasing fishmeal substitution using both the ingredients. Yun et al. (2017) suggested that this could partly be attributed to an increase in carbohydrate content due to the higher inclusion of plant protein sources. Both the analyzed lipid fractions (cholesterol and triglycerides) in haemolymph were significantly ($P < 0.05$) high in shrimp fed on a control diet (23.98 and 65.52 mg/dl, respectively). The levels gradually decreased to a range of 15.71–22.40 mg/dl for cholesterol and 35.86–61.23 mg/dl for triglycerides in shrimp fed SBM-based diets and an almost similar range was noticed among the shrimp fed SFC-based diets (14.97–22.19 mg/dl and 35.55–64.04 mg/dl). These results concur with earlier findings of Kaushik et al. (1995) in Rainbow trout and Venou et al. (2006) in Gilthered seabream. These authors suggested that the reduction in lipid fractions could be due to the hypolipidemic effect caused while substituting fishmeal using plant protein sources. Yet, interestingly, Yue et al. (2012) did not observed any difference in haemolymph triglyceride levels in *P. vannamei* fed on diets with fishmeal replaced using SBM and peanut meal.

The cost of feed formulation was computed based on the cost of ingredients prevailing in Indian markets. The cost of SBM increased from USD 646.2 to 861.5 per ton and SFC from USD 307.7 to 430.8 per ton due to fermentation. However, the total feed formulation cost reduced due to the inclusion of untreated and fermented plant protein sources in place of fishmeal. The price of fishmeal is USD 2169.8 per ton (World Bank Commodity Price Data, 2015). The inclusion of fermented SBM at the rate of 325.1 g/kg and fermented SFC at the rate of 32.6 g/kg by replacing fishmeal (*w*/w basis) reduced the total formulation cost by approximately USD 86 and 53 per ton respectively, while the reduction was between USD 44 and 26 per ton when the respective untreated ingredients were used.

5. Conclusion

From the present investigation, it observed that growth performance and nutrient utilization of plant protein sources could be better with fungal fermented ingredients compared to the respective untreated ingredients. Using *A. niger*, the inclusion levels of fermented SBM and SFC could be increased to 325.1 and 32.6 g/kg, respectively, as the inclusion levels of untreated SBM and SFC were 300 and 25 g/kg. The present investigation concluded that *A. niger* treated plant protein sources could be an appropriate option to fishmeal in the diet of *P. vannamei* rather using as such.

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