Overall evaluation of the replacement of fermented soybean to fish meal in juvenile white shrimp, *Litopenaeus vannamei* diet: growth, health status, and hepatopancreas histomorphology

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Abstract

This study was conducted to determine the effect of replacing fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks on the growth performance, feed utilization, immunological parameters, antioxidant enzyme assays and lipid peroxidation, digestive enzymes, and histopathological analysis of juvenile *Litopenaeus vannamei* (*L. vannamei*). By substituting 0.0%, 20%, 30%, and 40% FSBM for fishmeal (w/w), four isonitrogenous diets were generated. A total of 300 juvenile *L. vannamei* (1.59 ± 0.01 g) were randomly allocated to the experimental fiber tanks at a rate of fifteen shrimp per tank, with three replicates for each treatment. Growth performance and feed utilization decline considerably ($P < 0.05$) with increasing amounts of FM replacement with FSBM in diets. In comparison to the juveniles fed the other experimental diets, the diet containing a moderate level of FM replacement (20% FSBM) considerably enhanced growth performance and feed consumption during the feeding trial. The 20% FSBM-fed group had the highest protein content. In contrast, raising FSBM levels significantly increased lipid content ($P < 0.05$) compared to the control. However, there were no statistically significant differences ($P > 0.05$) across FSBM treatments. Hemolymph plasma total protein (TP) concentration and lysozyme activity were substantially greater ($P < 0.05$) in 20% FSBM compared to 40% FSBM ($P < 0.05$). In addition, 20% FSBM exhibits a substantial ($P < 0.05$) increase in the activity of antioxidant enzymes (CAT SOD, GPX, and GR). In contrast, the control and 30% FSBM groups had considerably more lipid peroxidation markers (MDA) than the 20% and 40% FSBM groups. Hepatopancreas amylase activity was considerably elevated ($P < 0.05$) in the control group and with 40% FSBM. In addition, hepatopancreas and intestinal protease and lipase activity increased significantly by 20% FSBM. Considerably, more B cells were present in the 40% FSBM diet than in the control diet; however, they were significantly less prevalent in the 20% and 30% FSBM diets ($P < 0.05$).

Keywords Fermented soybean meal (FSBM) · Growth performance · Health status · Digestive enzymes · Histopathological analysis · Juvenile *Litopenaeus vannamei*

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Introduction

Crustaceans are a common species that contribute significantly to the aquaculture industry. Because of high protein and minerals, it provides for healthy living, and shrimp has emerged as the favorite and fastest growing species in the crustacean aquaculture industry (Bondad-Reantaso et al. 2012). Pacific whiteleg shrimp (*Litopenaeus vannamei*; *L. vannamei*) is an important economic species in aquaculture (Pauly and Froese 2012). In 2020, whiteleg shrimp was ranked as the top-produced species with 5.81 Mmt (FAO 2022). The selection of shrimp species for culture is highly dependent on their ability to accept a wide range of feed formulations and ingredients (Chi et al. 2009). Despite its numerous advantages, the aquaculture industry faces numerous challenges; the most pressing of which is the demand for and supply of fishmeal (FM), the industry's primary protein source in artificial aquatic and marine shrimp diets. FM has long been used in the aquafeed formulation as a source of high-quality protein with its high digestibility and good amino acid (AA) profile (Sookying et al. 2013). However, reliance on FM has been identified as a significant impediment to the aquaculture industry's long-term development (Tacon and Metian 2008) because of its consistent price increase due to its limited supply and increased incorporation in livestock and aquaculture feed (Zhang et al. 2018). As a result, pursuing less expensive and more sustainable alternative protein sources of both animal and plant origin to reduce FM levels in aquafeed without impairing growth performance is a major ongoing global interest (Ding et al. 2015; Faggio et al. 2015; Dossou et al. 2018; Hoseinifar et al. 2011; Ishwarya et al. 2018; Nath et al. 2018). Over the last few decades, a wide range of protein ingredients has been studied as potential FM replacers, with plant proteins receiving the most attention as the most viable candidates due to their lower cost and abundant availability (Gatlin III et al. 2007; Dossou et al. 2018; Abd El-Naby et al. 2022). Plant protein sources such as soybean meal (SBM) are the primary options when replacing FM in aquafeed. However, SBM has several nutritional drawbacks, including the presence of anti-nutritional factors (ANFs) that reduce feed utilization, absorption, and feed conversion ratio (Kikuchi 1999), in addition to the amino acid imbalance and lower protein content when compared to FM. Thermal and mechanical processes, soaking, germination/malting, and fermentation have all been used to reduce the ANF content of SM and improve its nutrients, bioavailability, and nutritional value (Hotz and Gibson 2007). Among these methods, fermentation has been proposed as the most cost-effective for improving the nutritional quality of SBM not only through the biodegradation of ANFs (such as trypsin inhibitors, oligosaccharides, and phytic acid), proteins, and fibers but also through the production of probiotics and prebiotics, which may improve palatability, nutrient digestibility, and immune function (Hong et al. 2004). Fermented soybean meal (FSBM) was produced by fermenting SBM; many microorganisms were tested for SBM fermentation, and it was revealed that the nutritional value of the produced FSBM varies depending on the type of microorganism (Feizi et al. 2022). Thus, the goal of this study is to assess the effects of long-term feeding fermented soybean meal in different levels of replacement to the fish meal on juvenile *L. vannamei*. Growth performance, feed utilization, immunological parameters, antioxidant enzyme assays, lipid peroxidation, digestive enzymes, and histopathological analysis were measured for providing accurate evaluation.

Materials and methods

Ethical statement

The Animal Research and Ethics Committee of the National Institute of Oceanography and Fisheries in Suez, Egypt, approved the research work plan and permitted it to work with *Litopenaeus vannamei* post-larvae. All experiments were done by the committee's rules.

Solid-state fermentation of soybean meal preparation

Commercial soybean meal (SBM) was obtained from a company in Egypt's Zagazig province and ground to a particle size of 500 m. The modified method of Yabaya et al. (2009) was used to perform fermented SBM. Briefly, 2 kg of SBM was combined with 1.1 L of distilled water (50% moisture), and 60.5 mg of commercial dry yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) of cell density 3×10^6 cell g^1 (Fermipan®, GB ingredients, China). In a Hobart food mixer, all the ingredients were homogenized for 15 min. The mixture was incubated for 48 h at 40 °C, the ideal growth temperature for *S. cerevisiae*, in a 10-L glass jar covered with aluminum foil. Ten grams of the solid-state fermented soybean meal (SSF-SBM) with yeast was sampled at 0, 12, 24, and 48 h of fermentation to assess the anti-nutritional factors (ANFs) and chemical composition content. Finally, the SSF-SBM was dried to a constant weight at 70 °C.

Diet formulation

A control diet with FM (FSBM0) as the primary source of protein and three experimental diets in which the FM in the control diet was replaced by FSBM at 20, 30, and 40% resulted in four isonitrogenous (41.2 g/kg crude protein and 8.2 g/kg isocaloric diets) (Table 1). The dry ingredients and fish oil were combined to make a stiff dough by adding water. Using a meat grinder, the mixtures were formed into 1.6-mm-diameter pellets. In a forced-air oven set at 40 °C, pellets were dried. Before use, fully dried diets were put in plastic bags and kept at 18 °C.

Amino acid analysis

Following procedures recommended by the Association of Official Analytical Chemists (AOAC 1990) and the protocol of Llames and Fontaine (1994), the amino acid profile of FSBM was determined using an amino acid analyzer (LA8080, Amino SAYA, Hitachi High-Tech, Japan).

Feeding trial

L. vannamei juveniles were obtained from the El-Sahaba hatchery in Damietta, Egypt, and transported to the National Institute of Oceanography and Fisheries' Invertebrates Laboratory in Suez, Egypt. The juveniles (1.59 ± 0.01 g) were acclimated for 1 week before being distributed at random into fifteen fiberglass tanks (40 L volume). Compressed air was used to provide continuous aeration. For 12 weeks, shrimp juveniles were fed the four test diets in triplicate (0.0, 20%, 30%, and 40% FSBM, respectively) to apparent satiation

Table 1 Ingredients and chemical analysis of the experimental diets (on a dry matter basis with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM)

Ingredients	Control	FSBM levels (%)		
	0.0	20	30	40
Fish meal (72% protein)	32.0	25.6	22.4	19.2
Fermented soybean meal (48% protein)	0.0	9.4	14.1	18.8
Soybean meal (42% protein)	35.0	35.0	35.0	35.0
Wheat flour (15% protein)	21.6	18.3	16.7	15.1
Cod fish oil	3.4	3.7	3.8	3.9
Di–calcium phosphate	2.0	2.0	2.0	2.0
Soy lecithin	1.0	1.0	1.0	1.0
Vitamins and Minerals premix[2]	3.0	3.0	3.0	3.0
Brewer's yeast	2.0	2.0	2.0	2.0
Proximate chemical analysis g/kg diet				
Dry matter	922.3	925.3	923.5	922.9
Crude protein	412.0	412.8	413.0	413.2
Crude lipid	82.8	82.6	82.4	79.6
Ash	10.18	11.15	11.23	11.41
Fiber	33.1	33.3	33.7	33.2
NFE[3]	461.92	460.15	459.67	462.59
GE (KcJ/kg diet)[4]	19.30	19.28	19.27	19.22

[1]Vitamin and mineral mixture each 1 kg of mixture contains 4800 IU vitamin A, 2400 IU cholecalciferol (vitamin D), 40 g vitamin E, 8 g vitamin K, 4.0 g vitamin B12, 4.0 g vitamin B2, 6 g vitamin B6, 4.0 g pantothenic acid, 8.0 g nicotinic acid, 400 mg folic acid, 20 mg biotin, 200 gm choline, 4 g copper, 0.4 g iodine, 12 g iron, 22 g manganese, 22 g zinc, 0.04 g selenium, 1.2 mg folic acid, 12 mg niacin, 26 mg D-calcium pantothenate, 6 mg pyridoxine HCl, 7.2 mg ribo £ avin, 1.2 mg thiamine HCl, 3077 mg sodium chloride (NaCl, 39% Na, 61% Cl), 65 mg ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20% Fe), 89 mg manganese sulfate ($MnSO_4$, 36% Mn), 150 mg zinc sulfate ($ZnSO_4. 7H_2O$, 40% Zn), 28 mg copper sulfate ($CuSO_4 _5H_2O$, 25% Cu), 11 mg potassium iodide (KI, 24% K, 76% I), 1000 mg Celite AW521 (acid-washed diatomaceous earth silica). w% on a dry matter (DM) basis

[2]Nitrogen-free extract (calculated by difference) = 100 − (protein + lipid + ash + fiber)

[3]Gross energy was calculated using the factors as follows: protein, 23 MJ/kg; lipid, 35 MJ/kg; carbohydrates, 15 MJ/kg (Molina-Poveda et al. 2015)

twice daily. Dietary amounts were adjusted based on survival and body weight. Approximately 10% of the water volume in each aquarium was changed out daily with fresh aerated and filtered marine water after removing the accumulated wastes.

Physicochemical characteristics of water

Daily partial exchange of 10% of the water kept the water quality parameters within the acceptable ranges during the experimental trial. Dissolved oxygen levels were kept at 5.74 ± 0.50 mg/L using an oxygen meter (HANNA, HI 9146-04/10), pH was kept at 7.5 ± 0.3 using a pH meter (Adwa, AD-11), unionized ammonia concentration was kept at 0.22 ± 0.04 mg/L using DREL/2 HACH kits (HACH Co., Loveland, Co.), and salinity was kept at

30.56 ± 0.80 (Bioevopeak Co., Ltd., China), while the water temperature was maintained at a range of 28.52 ± 0.92 °C.

Growth performance and feed utilization

Weight in (g) and length in (cm) of each post larvae (PL) were measured at the start of the experiment and every 2 weeks for the next 12 weeks. All shrimp were weighed to determine their final weight at the end of the experiment, which was then compared to their initial weight on the first day. By counting the individuals in each aquarium, the survival rates of the PL were also estimated.

Feed and body composition chemical analysis

The standard methods of AOAC (2012) were used to determine the moisture, crude protein, crude lipid, and ash content of diets and shrimp samples. The dry matter of the samples was determined by drying them to a constant weight at 105 °C. Crude protein was calculated by multiplying nitrogen by 6.25 using the Kjeldahl method (Kjeltec TM8400, FOSS, Sweden). The Soxhlet method was used to determine crude lipid after diethyl ether extraction (Buchi 36680, Switzerland), after 16 h of combustion in a muffle furnace at 550 °C. According to Goering and van Soest (1970), the crude fiber was estimated. Gross energy was computed according to the standards of NRC (1993).

Immunological parameters

Hemolymph samples were taken from the ventral sinus of the recently molted shrimp using 1-mL syringes and a 27-G needle containing anticoagulant (1:1), according to El Asely et al. (2010). The molting stages were identified through periodic observation of the flock and were confirmed through microscopic examination of the epidermal retraction following Robertson et al. (1987).

Hemolymph plasma was obtained by centrifugation to the hemolymph-anticoagulant mix at (10,000 g/4 °C) for 5 min, according to Lamela et al. (2005). The obtained plasma was stored at − 20 °C.

The total protein of hemolymph plasma was determined following Bradford (1976) at 595 nm, where the bovine serum albumin was used as standard.

Lysozyme activity was determined through measurement of the decrease in the absorbance of lysis to the *Micrococcus lysodeikticus* cells (Sigma-Aldrich, Cat. no. LY0100) at a wavelength of 450 nm following the manufacturer instructions and the protocol of Sotelo-Mundo et al. (2003).

Antioxidant enzyme assays and lipid peroxidation

The shrimp were euthanized following the American Veterinary Medical Association (AVMA) guidelines for animal euthanasia (Leary et al. 2013). Shrimp were immersed in ice for 15 min till no motion was noticed. Hepatopancreas was dissected and kept in cold phosphate-buffered saline (PBS), and then, it was homogenized. Tissue homogenate was centrifuged at 12,000 × g for 12 min at 4 °C. The supernatant was removed and stored at − 75 °C. Catalase (CAT), superoxide dismutase (SOD), and glutathione

peroxidase (Gpx) activities were measured photometrically using a microplate reader and the commercially available kits (Abcam; Catalase-ab83464, SOD-ab65354, GPx-ab219926) at the wavelength (OD 570 nm, OD 450 nm, and OD 405 nm) respectively, while GR activity was measured fluorometrically at Ex/Em = OD420/480 nm using kits (Abcam; GR-ab83461). Colorimetric lipid peroxidation assay kit (Abcam, ab118970) was used to detect the content of malondialdehyde (MDA) in the hepatopancreas tissue homogenate at OD = 532 nm.

Digestive enzyme assay

Hepatopancreas and intestines were weighed and homogenized in cold PBS. Tissue homogenates were centrifuged at $18,894 \times g$ at 4 °C for 5 min. The supernatant was stored at − 20 °C until analysis. Amylase, lipase, and protease activities were determined using Abcam kits following the manufacturer's instructions. Amylase activity was detected using colorimetric kits (ab102523) measured at OD = 405 nm. Lipase activity was assayed using kits (Abcam, ab102524), where lipase hydrolyzes a triglyceride substrate to form glycerol which is quantified enzymatically by monitoring a linked change in the absorbance of a probe (OD = 570nm). Lipase activity was calculated as nanomole of glycerol per milligram of protein. Protease activity was estimated by Protease Activity Assay Kit (fluorometric—green) (ab112152) with fluorescence intensity at Ex/Em = 490/525 nm.

Histopathological analysis

The hepatopancreas of three shrimp/replicate/group were dissected and then fixed for 24 h in Davidson's fixative before being transferred to 70% ethanol for standard histological processing (Bell and Lightner 1988). The tissue sections (4–5m) were stained with hematoxylin-eosin, examined using a light microscope (Olympus CX 41, Japan), and photographed using an Olympus E-620 digital camera. Using Image J software equipped with a cell counter plugin, the number of R cells (Restzellen or resorptive cell) and B cells (Blasenzellen or blister cell) as well as tubule diameter were determined in 20 randomly selected tubules from each treatment (Romano et al. 2015).

Statistical analysis

SPSS software (ver. 17.0) was used to conduct the statistical analysis. All data were given as mean ± standard error (S.E.). Using one-way ANOVA and Tukey's post hoc multiple comparisons, statistical significance was determined. P-values < 0.05 were deemed statistically significant.

Results

Essential amino acid content

In the current study, the partial replacement of FM with FSBM influenced the aa profile in the diet, with an improvement in the contents of some amino acids such as arginine, lysine,

Table 2 Essential amino acid profile (g/kg) experimental diets

Items	FM				
FSBM-FM (%)	0.0	20	30	40	
Essential amino acid					Requirements for shrimps
Arginine	24.2	24.0	32.9	32.8	20
Histidine	13.1	12.6	12.2	12.0	10
Lysine	27.1	26.6	26.2	25.9	22
Methionine	10.8	10.1	9.5	8.9	9
Leucine	27.9	28.0	28.1	28.1	19
Isoleucine	18.2	18.4	18.4	18.4	11
Threonine	16.4	16.0	15.7	15.4	15
Phenylalanine	18.7	18.8	19.0	19.1	14
Valine	20.6	20.1	19.8	19.5	14

FM fishmeal, *FSBM* fermented soybean meal

Table 3 Growth performance of *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
Initial weight (g)	1.58 ± 0.01	1.57 ± 0.01	1.57 ± 0.01	1.56 ± 0.01
Final weight (g)	10.79 ± 0.17[bc]	15.09 ± 0.42[a]	11.75 ± 0.39[b]	9.82 ± 0.20[c]
Weight gain (g)	9.21 ± 0.17[bc]	13.52 ± 0.41[a]	10.18 ± 0.38[b]	8.26 ± 0.21[c]
RBWG (%)	583.46 ± 12.41[c]	861.30 ± 26.08[a]	647.98 ± 21.38[b]	530.53 ± 18.18[c]
SGR (%g/day)	2.28 ± 0.02[c]	2.69 ± 0.03[a]	2.39 ± 0.03[b]	2.19 ± 0.03[d]
Survival rate (%)	100	100	93.3±3.85	93.3±3.85

Means having the same letter in the same row is not significantly different at $P < 0.05$. Weight gain (g) = W1–W0; weight gain (%) = 100 (W1–W0)/W1; where Ln, natural log; W0, initial body weight (g); W1, final body weight (g); and T, time (day); specific growth rate (SGR%/day) = ((Ln W1–Ln W0)/T) × 100; survival rate (%) = 100 × (fish no. at the end/fish no. stocked at the beginning)

FM fishmeal, *FSBM* fermented soybean meal

leucine, isoleucine, phenylalanine, threonine, and valine in shrimp diets and a decrease in the contents of some amino acids such as histidine and methionine (Table 2).

Growth performance and feed utilization

At the end of the feeding trial, the survival of *L. vannamei* juveniles was reduced with no significant difference between feeding groups. There was no significant difference in growth performance or feed utilization parameters between the four experimental groups ($P > 0.05$) (Table 3). The 20% FSBM diet had significantly improved growth performance in terms of FBW, BWG, RBWG, and SGR ($P < 0.05$), followed by the groups fed the 30, 0.0, and 40% FSBM diets, respectively, while there were no statistically significant

Table 4 Feed utilization of *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
Feed intake (g feed/fish)	14.06 ± 0.40^b	18.50 ± 0.50^a	14.81 ± 0.18^b	14.45 ± 0.42^b
FCR	1.52 ± 0.05^b	1.37 ± 0.04^c	1.46 ± 0.06^{bc}	1.74 ± 0.01^a
FER	65.77 ± 2.42^b	73.16 ± 2.21^a	68.83 ± 3.13^{ab}	57.20 ± 0.46^c
PER	1.75 ± 0.05^a	1.90 ± 0.08^a	1.77 ± 0.08^a	1.52 ± 0.04^b
APU (%)	5.85 ± 0.23^{bc}	7.98 ± 0.38^a	6.41 ± 0.59^b	4.82 ± 0.12^c
EU (%)	2.16 ± 0.16^{bc}	3.58 ± 0.19^a	2.57 ± 0.29^b	1.66 ± 0.08^c

Means having the same letter in the same row is not significantly different at $P < 0.05$. Feed intake (FI) = total feed intake per tank/number of fish; feed conversion ratio (FCR) = feed intake (g)/body weight gain (g); protein efficiency ratio (PER) = weight gain (g)/total protein intake (g); apparent protein utilization (APU (%)) = 100 (protein gain in fish (g)/protein intake in diet (g)); energy utilization (EU (%)) = 100 (gross energy gain (g)/gross energy intake (g)

FM fishmeal, *FSBM* fermented soybean meal

Table 5 Whole body composition (% of fresh weight basis) of *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
Moisture	75.64 ± 0.48	76.08 ± 0.31	76.19 ± 0.21	76.46 ± 0.08
Crude protein	20.36 ± 0.09^c	21.99 ± 0.02^a	21.18 ± 0.27^b	21.06 ± 0.09^b
Total lipids	0.80 ± 0.01^b	0.85 ± 0.02^a	0.86 ± 0.02^a	0.86 ± 0.01^a
Ash	1.46 ± 0.02^a	1.36 ± 0.01^{ab}	1.35 ± 0.06^{ab}	1.34 ± 0.02^b

Means having the same letter in the same row is not significantly different at $P < 0.05$

FM fishmeal, *FSBM* fermented soybean meal

differences in these parameters between the 30, 0.0, and 40% FSBM diets. The lowest was found at 40% FSBM.

As shown in Table 4, the low replacement level (20% FSBM) significantly increased feed intake, resulting in the greatest growth performance relative to the other replacements. In addition, 20% replacement exhibited the lowest FCR ($P < 0.05$) compared to those fed a 40% FSBM diet. Conversely, the low replacement level group (20% FSBM) significantly improved ($P < 0.05$) FER, PER, APU, and EU compared to the high replacement level group. Forty-percent FSBM diet produced the least amount of feed utilization.

Proximate body composition analysis

Table 5 presents the approximate composition of the entire body of *L. vannamei* juveniles. The present investigation revealed no statistically significant variations ($P > 0.05$) in the moisture content of *L. vannamei* shrimp across all dietary regimens, with moisture levels ranging from 75.64 to 76.46%. The group fed 20% FSBM had the highest protein content (21.99%), while the group fed 0% FSBM had the lowest protein level (20.36%). It was

Table 6 Hemolymph total protein (T. protein) and lysozyme (LZM) of *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks (mean $\pm$ SD, n =5)

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
T. protein	0.69 ± 0.08^b	0.78 ± 0.03^a	0.67 ± 0.01^b	0.47 ± 0.05^c
LZM	309.0 ± 2.64^b	336.0 ± 3.46^a	253.33 ± 6.69^{bc}	255.66 ± 4.33^c

Means having the same letter in the same row is not significantly different at $P < 0.05$

FM fishmeal, *FSBM* fermented soybean meal

Table 7 The antioxidant activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), and malondialdehyde (MDA) in *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks (mean $\pm$ SD, n =5)

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
CAT (U/mg)	7.54 ± 0.34^c	$23.12 \pm .87^a$	1.04 ± 0.15^d	15.30 ± 0.60^b
SOD (U/mg)	16.96 ± 1.16^c	78.43 ± 2.15^a	53.06 ± 6.73^b	12.32 ± 2.80^c
GPX (U/mg)	0.82 ± 0.04^c	1.85 ± 0.08^a	0.31 ± 0.03^d	1.37 ± 0.07^b
GR (mg/g)	37.86 ± 2.63^c	108.98 ± 4.64^a	13.74 ± 0.92^d	77.20 ± 3.14^b
MDA (nmol/gm)	14.27 ± 0.86^b	0.53 ± 0.04^d	29.84 ± 1.19^a	6.42 ± 0.80^c

Means having the same letter in the same row is not significantly different at $P < 0.05$

FM fishmeal, *FSBM* fermented soybean meal

noticed that raising FSBM levels significantly increased lipid content ($P < 0.05$) compared to the control. However, there were no statistically significant changes ($P > 0.05$) among FSBM treatments. In contrast, as the amount of dietary FSBM increased from 0 to 40% FSBM, ash concentrations tended to decrease. The highest significant levels of ash content were recorded in the control diet (0.0% FSBM).

Immunological parameters

Hemolymph plasma TP concentration was substantially greater ($P < 0.05$) in 20% FSBM, followed by 30% FSBM and 0% FSBM, with 40% FSBM having the lowest concentration (Table 6). Similarly, lysozyme activity was greater in 20% FSBM than in other FSBM concentrations (Table 6).

Antioxidant enzyme assays and lipid peroxidation

The obtained results presented in Table 7 showed a significant increase ($P < 0.05$) in the activity of hepatopancreas catalase enzyme in 20% FSBM, and the same pattern was recorded in the other measured antioxidant enzymes (SOD, GPX, and GR). On the other

hand, significantly low catalase activity was recorded in 30% FSBM, followed by control and 40% FSBM. In contrast, the content of lipid peroxidation marker (MDA) was significantly higher ($P < 0.05$) in control and 30% FSBM than in 20 and 40% FSBM levels.

Digestive enzyme assay

Hepatopancreas amylase activity was significantly high ($P < 0.05$) in 40% FSBM and control followed by 30% FSBM and 20% FSBM (Table 8). While protease activity showed a significant increase in 20% FSBM, its lowest activity was recorded in 40% FSBM. While protease activity showed a significant increase in the 20% FSBM diet, its lowest activity was recorded in the 40% FSBM diet. Contrarily, the values of lipase activity were significantly higher ($P < 0.05$) in 20% of FSBM, and the lowest activity was recorded in 40% of FSBM (Table 8).

On the other hand, intestinal amylase activity was significantly high ($P < 0.05$) in 0.0% FSBM, while 20% FSBM was the lowest (Table 8). Protease activity showed a significant increase in 20% FSBM, and its lowest activity was recorded at 40% FSBM. Contrarily, the values of lipase activity were significantly higher ($P < 0.05$) in 20% of FSBM, and the lowest activity was recorded in 40% of FSBM (Table 8).

Gross pathology

Gross pathology revealed that shrimp given 30 and 40% FSBM exhibited a pale white distal hepatopancreas and a slightly shrunken stomach (Fig. 1a, b).

Histology of hepatopancreas

Figure 2a–d depicts the hepatopancreatic tubules of shrimp fed the control and experimental diets. Considerably, more B cells were present in the 40% FSBM diet than in the control diet; however, they were significantly less prevalent in the 20% and 30% FSBM diets ($P <$

Table 8 The hepatopancreas and intestinal digestive enzymes (amylase, protease, and lipase) of *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks (mean ± SD, n =5)

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
Hepatopancreas digestive enzymes				
Amylase (U/L)	68.32 ± 4.07[a]	27.98 ± 1.74[c]	48.01 ± 3.29[bc]	68.32 ± 4.07[a]
Protease (ng/mg)	66.33 ± 3.17[c]	139.0 ± 4.16[a]	97.66 ± 5.04[b]	33.0 ± 3.60[d]
Lipase (U/L)	4.78 ± 1.20[c]	13.50 ± 0.50[a]	9.24 ± 0.31[b]	1.83 ± 0.27[d]
Intestinal digestive enzymes				
Amylase (U/L)	219.33 ± 17.9[a]	22.56 ± 4.84[d]	94.36 ± 3.77[c]	142.17 ± 3.46[b]
Protease (ng/mg)	50.0 ± 1.52[c]	124.33 ± 3.48[a]	67.33 ± 2.72b	35.0 ± 1.73[d]
Lipase (U/L)	7.67 ± 0.26[b]	13.67 ± 0.50[a]	9.24 ± 0.31b	1.83 ± 0.27[d]

Means having the same letter in the same row is not significantly different at $P < 0.05$

FM fishmeal, *FSBM* fermented soybean meal

Fig. 1 **a** 30% FSBM fed group; the distal part of the hepatopancreas with the fuzzy white zone (arrow). **b** The control group with normal hepatopancreas
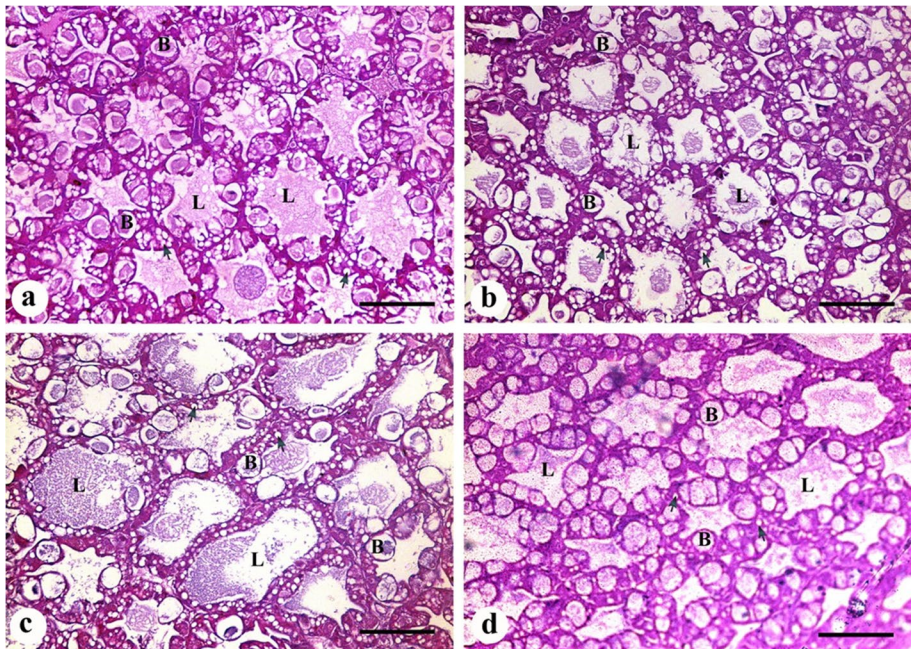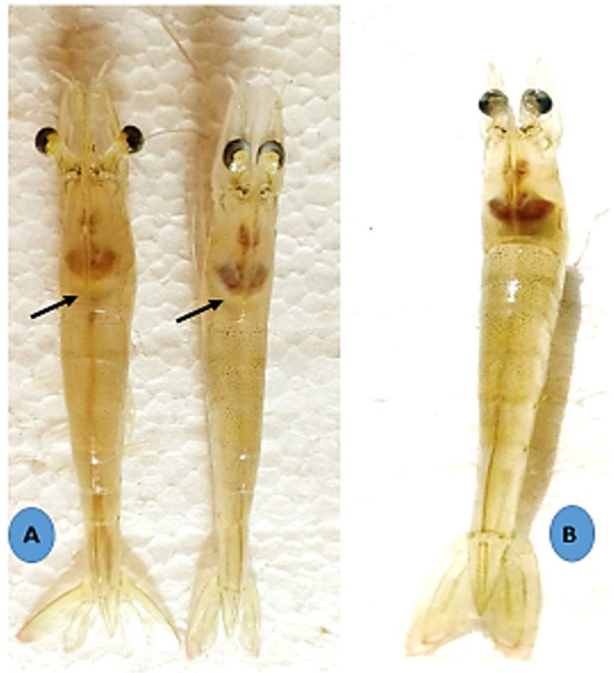

Fig. 2 Showing the histological normal structure of the hepatopancreas of shrimp *Litopenaeus vannamei* fed **a** the control diet and **b** 20, **c** 30, and **d** 40% partial fish meal replacement with *Saccharomyces cerevisiae* fermented soybean meal for 60 days. (B) B cell (small arrow) and R cell, (L) hepatopancreatic tubule, H&E staining, bar = 50 μm

0.05). In addition, 30% of FSBM-fed shrimp had considerably more R cells than the others. In terms of hepatopancreatic tubule diameters, the 30% and 40% FSBM diets had considerably larger tubules than the other diets ($P < 0.05$). The predominance of B cells and R cells in tubules, as well as the tubule diameter, is shown in Table 9.

Discussion

Plant proteins especially soya bean (SB) could be a promising source of protein in aquatic animal feed; however, they are high in cellulose, which is difficult for fish and other monogastric animals to digest (Gatlin III et al. 2007). Researchers are working on bioprocessing SB in other products such as soybean meal (SBM), soy protein isolate (SPI), fermented soybean meal (FSBM), and soybean protein concentrate (SPC) in shrimp diets in order to reduce ANFs (Abdul Kader et al. 2012; Gamboa-delgado et al. 2013). In addition, fermentation of SB has been reported to increase its protein content (Teng et al. 2012), promote antibacterial and antioxidant activities (He et al. 2013; Akbari and Wu 2015), and diminish immunoglobulin E immunological activity (Song et al. 2008).

The findings of this study indicate that 20% of FM can be substituted with FSBM without harming the health of shrimp. In addition, juvenile *L. vannamei* given 20% FBSM displayed enhanced growth performance and feed utilization, despite the fact that the amino acid composition of all experimental groups was identical. In addition, this improvement may be attributable to the improved lipid digestibility of FSBM (Refstie et al. 2005) and the reduction of anti-nutritional components during fermentation (Su et al. 2018). In contrast, the growth of shrimp fed a diet containing 40% FSBM was diminished. This decline may be attributable to the presence of non-digestible oligosaccharides, decreased protein digestibility, or a nutritional imbalance (Sharawy et al. 2016).

Our study's growth performance results were comparable to those of earlier research conducted on other crustacean species. According to the findings of Ding et al. (2015), the optimal growth performance of *M. nipponense* was achieved when 25% of the FM was substituted with FSM. Research on *F. indicus* suggests that replacing up to 28.57% of FM with FSM is significantly more economical (Sharawy et al. 2016). Shao et al. (2018) observed that a meal with a moderate FSM replacement level (20% of FM protein) efficiently boosted the growth of juvenile white shrimp and that a replacement level of up to 40% had no influence on shrimp growth performance.

Table 9 B-cell and R-cell prevalence (number/tubule) and tubule diameter (μm) (mean ± SE) from the hepatopancreas of shrimp *Litopenaeus vannamei* post-larvae fed diets with different replacement ratios of fishmeal (FM) with fermented soybean meal (FSBM) for 12 weeks

Items	Control	FSBM levels (%)		
FSBM-FM (%)	0.0	20	30	40
B cells	6.1 ± 0.22[b]	3.2 ± 0.36[c]	3.3 ± 0.53[c]	11.3 ± 0.74[a]
R cells	45.6 ± 1.53[b]	49.4 ± 2.53[b]	68.3 ± 3.01[a]	45.2 ± 2.8[b]
Tubule diameter	25.6 ± 1.67[b]	22.9 ± 1.3[b]	49.1 ± 2.7[a]	46.1 ± 3.08[a]

Means having the same letter in the same row is not significantly different at $P < 0.05$

FM fish meal, *FSBM* fermented soybean meal

In the present investigation, there were no statistically significant differences ($P > 0.05$) in the moisture content of *L. vannamei* shrimp between diets, while the addition of *S. cerevisiae* increased the protein content of FSBM-fed groups significantly. The same rise in body lipid content was observed with increased dietary FSBM, which included more carbohydrates than FM (Makkar et al. 2007). According to Kaushik et al. (2004), one of the major factors leading to increased lipid retention is an increase in dietary plant protein, which is associated with an increase in hepatic lipogenic enzyme activity, imbalances in dietary amino acid content, and higher whole-body lipid levels in sea bass and salmonids. In addition, Sharawy et al. (2016) reported that there were statistically significant variations ($P < 0.05$) in the protein, dry matter, lipid, and ash content of shrimp fed different experimental diets compared to control diets containing 0.0% protein (FSBM). In our study, ash contents tended to decrease as FSBM levels increased from 0 to 40% of the meal.

Hemolymph metabolites serve as physiological, nutritional, and immunological stress indicators in crustaceans. In addition, it has been used to evaluate the nutritional health of shrimp, whose blood protein and glucose levels are very sensitive to the protein content of their diet (Rosas et al. 2001). In the current study, the inclusion of FSBM in the diet had a significant effect on the hemolymph total protein (TP) content, with the maximum TP concentration seen in groups fed 20% FSBM, followed by 30% FSBM. According to Shiu et al. (2015), the increased protein content of soya bean meal after fermentation may account for the observed results. On the other hand, the decreased TP concentration in 40% FSBM is due to the detrimental effect of high soya bean levels on the digestibility, absorption, and utilization of dietary protein (Gilani et al. 2012).

With its antibacterial effectiveness against bacterial infection, lysozyme activity is one of the most important indicators of shrimp immunity (Kaizu et al. 2011). The addition of *S. cerevisiae* to ferment SBM was also beneficial in regulating serum lysozyme activity. The results demonstrated a considerable increase in the lysozyme activity of 20% of FSBM-fed groups. The process involved in boosting the immune system of shrimp hinges on the protein recognition pattern of the circulating sugars which evoke the immune cells (Vargas-Albores and Yepiz-Plascencia 2000). It is assumed that the yeast harboring β-glucan effectively stimulated lysozyme synthesis. In contrast to what was expected, the lysozyme activity decreased as the concentration of FM-replacement FSBM increased. This was postulated as a result of the fatigue of lysozyme-producing cells from long-term exposure to the triggering agents, recommending the use of a low dose for a brief period of time for an effective response (El-Barbary et al. 2021; Babu et al. 2013; El Asely et al. 2011).

In addition to exogenous sources of reactive oxygen species (ROS), regular cellular metabolism generates electrons that can alter the membrane protein structure, lipids, cell division, and apoptosis signaling pathway (Redza-Dutordoir and Averill-Bates 2016; Bauer and Bauer 1999). Antioxidants' function in cells is to maintain balance and scavenge excess reactive oxygen species (ROS) to mitigate their corrosive effect (Kurutas 2015).

In the present study, hepatopancreas CAT, SOD, Gpx, and GR activities increased significantly in the group fed 20% FSBM, indicating that the 20% substituted FSBM meal had a greater anti-oxidative effect than fish fed FM and the other two concentrations. Ding et al. (2015) detected a drop in CAT, SOD, and GSH-PX activities with increased FSM content in the diet of *Macrobrachium nipponense*. The results obtained were almost identical to those reported by Ding et al. which suggested that the antioxidative capacity of shrimp was compromised by the substitution of fishmeal. Xu et al. (2008) found that fish CAT activity fell dramatically from 30 to 20% when fishmeal was substituted. Despite the fact that Daiyong et al. (2009) discovered that the CAT activity of shrimp was unaffected, the SOD activity declined dramatically when fishmeal was

reduced from 25 to 20%. In addition to the enhanced flavonoid content created during soybean fermentation, tiny peptides, organic acids, and probiotics are also produced (Mukherjee et al. 2016). *Saccharomyces cerevisiae* produces vitamins and other metabolites that serve as exogenous antioxidant sources (Farid et al. 2019).

The hepatopancreatic MDA level of shrimp given 20% FSBM was much lower than that of shrimp fed the FM and other diets, demonstrating that the dietary replacement of FM with FSBM did not stimulate oxidative stress and was even successful at decreasing it. This could be attributable to the FSBM's high isoflavonoid content, which can neutralize free radicals and prevent lipid peroxidation (Yoon and Park 2014).

The hepatopancreas is responsible for the generation and release of digestive enzymes, the absorption of nutrients, and the mobilization and transport of nutrients such as lipids, glycogen, minerals, and organic compounds to muscle and other tissues in response to growth and reproductive needs (Ceccaldi 1989). The hepatopancreas secretes enormous quantities of digestive enzymes, such as amylases and proteases (Gamboa-delgado et al. 2003). Dietary content has a significant influence on digestive enzyme production and activity (Le Moullac et al. 1997; Guzman et al. 2001). In the present investigation, the substitution of fish meal with FSBM had a substantial effect on the activity of digestive enzymes; amylase activity was significantly higher in 40% FSBM and control diets than in other diets, indicating a higher carbohydrate content.

Interestingly, the digestive enzyme concentration in the intestine was substantially identical to that of the hepatopancreas, corroborating the findings of Córdova-Murueta et al. (2003).

The hepatopancreas is the most essential digestive organ in shrimp. Histologically, it consists of four distinct cell types contained within blind-ending tubules. E cells differentiate into R cells (nutrient absorption and storage), F cells (production of digestive enzymes), and B cells (presumed to be secretory in function) at the apex of the tubules (Gopinath and Paul Raj 2009).

In the present study, shrimp fed 30% partial fish meal replacement had significantly more R cells than other groups ($P < 0.05$); this increase in R cells, which are responsible for lipid storage in the hepatopancreas gland, may be indicative of an increase in energy reserve in the hepatopancreas as a result of the treatment. The B cells are large cells responsible for enzyme storage, and this study revealed that their prevalence was significantly higher in the 40% partial fish meal replacement group than in the control group but significantly lower in the 20% and 30% partial fish meal replacement groups ($P < 0.05$). In previous research, hypertrophied B cells were identified in shrimp fed moderate dosages of the mycotoxin deoxynivalenol (DON), indicating oxidative stress in shrimp (Xie et al. 2018). Also, the increased B-cell prevalence in *L. vannamei* has been observed to increase at low salinities, suggesting that this is a response to the increased nutrient use required for higher osmoregulatory functions (Li et al. 2008). Similar to our findings, Romano et al. (2015) concluded that while the prevalence of R cells was significantly higher in shrimp fed organic acid–blended diets, indicating greater energy reserves, the prevalence of B cells, which are primarily responsible for the secretion of digestive enzymes, was significantly lower. The 30% and 40% partial fish meal replacement groups had substantially larger hepatopancreatic tubule diameters than the other groups ($P < 0.05$). The increase in hepatopancreatic tubule diameter may be correlated with the greater prevalence of R cells and the resulting fat storage inside them (Johnston et al. 2003; Simon and James 2007; Pourmozaffar et al. 2019), which may be associated with the gross pathology picture of the hepatopancreas with the white fuzzy zone.

Conclusion

In conclusion, the current study demonstrates that 20% replacement of FM with FSBM improved growth performance, feed utilization, immunological parameters, antioxidant enzymes assays, lipid peroxidation, digestive enzymes, and histopathological analysis in juvenile *L. vannamei*, despite the fact that there was no difference in the amino acid content between the experimental groups. This improvement may also be attributable to the increased lipid digestibility of FSBM and the decrease in anti-nutritional components during fermentation.

Data availability Data of the present article will be available on request.

Declarations

Conflict of interest The authors declare no competing interests.

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