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Solid-state fermentation converts rice bran into a high-protein feed ingredient for *Penaeus monodon*

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Fermented rice bran (FRB) was evaluated as an alternative protein source to soybean meal (SM) in practical diets for juvenile black tiger shrimp, *Penaeus monodon*. This feed ingredient was tested in a feeding trial to replace soybean meal in *P. monodon* diets at 0% (T0), 12.5% (T12.5), 25% (T25), 37.5% (T37.5), and 50% (T50). Five iso-nitrogenous and iso-caloric experimental diets containing 44% crude protein were fed to groups of juvenile shrimp (0.47 ± 0.002 g) randomly assigned to twenty 60-l rectangular tanks equipped with a recirculating seawater system. Each dietary treatment was run in 4 replicates, and the feeding trial lasted 50 days. Results show significant improvement in weight gain, specific growth rate, and protein efficiency ratio in treatment T12.5 and T25. Treatments with higher levels of SM replacement with FRB exhibited similar growth indices to those of the control group. Polynomial regression analysis indicates that the optimum replacement of soybean meal with FRB for optimum growth is 21.08%. The apparent dry matter and protein digestibility coefficients of FRB are 83.05 ± 0.02% and 87.20 ± 0.30%, respectively. There were no significant differences in the whole-body composition (dry matter, protein, lipid, ash) among treatments of shrimp fed with FRB replacement. The data suggest that FRB replacement of dietary soybean meal is feasible at 50% without affecting the growth performance but may promote growth at 21.08% replacement of *P. monodon*.

KEYWORDS

solid-state fermentation, alternative protein source, *Penaeus monodon*, rice bran, *Trichoderma harzianum*, soybean meal

1 Introduction

The global aquaculture production of giant black tiger shrimp (*Penaeus monodon*) in 2021 was 695,674 metric tons, valued at $ 5.9 billion, with more than 99% of production coming from Asian countries (FAOSTAT, 2024). The intensive farming of *P. monodon* in Asia has experienced sluggish growth at an annual rate of 0.9% in the past ten years (FAOSTAT, 2024). Similar to other Asian countries, the production of shrimp in the Philippines is declining, with an annual growth rate of -4.8% (PSA, 2024). Aside from diseases, the major constraint of shrimp aquaculture is feed cost. Feed ingredients used in commercial giant black tiger shrimp diets in many developing countries in Asia are mostly imported.

Among the imported feed ingredients, soybean meal (SBM) is the most important feed-protein source used in shrimp feeds (Brown et al., 2008). However, livestock and aquaculture industries compete in the use of SBM, and this has resulted in an increase in prices and erratic supply (Traifalgar et al., 2019). Since shrimp feed accounts for more than 50% of production cost (Hung and Quy, 2013), there is currently great interest in reducing feed costs using locally available feed ingredients such as rice bran.

One locally available feed ingredient is rice bran, which is cheap and available in large quantities. This material is produced as a by-product of the rice milling process and is mainly used as an energy source in animal feed (Phongthai et al., 2017). The Philippines is the 7th largest rice producer in the world and contributes 2.5% of global rice production (World Agricultural Production, 2024). Since rice bran accounts for about 8 to 11% of the grain, approximately 87 million metric tons are produced annually and could be a cheaper source of feed protein in shrimp diets (FAO, 2023). However, rice products are not normally used in shrimp feeds because they are similarly priced with wheat products but have no feed-binding properties (Akiyama et al., 1992). The limitation of its use is also attributed to its high fiber content (12.4-27.8%), low protein (7.8%), and the presence of anti-nutritional factors (Luh, 1991; Tillman et al., 2005; Hertrampf, 2006).

The fungus *Trichoderma* is renowned for its ability to colonize diverse ecological niches, including soil, roots, and leaves, and is particularly noted for its secretion of substantial quantities of cellulases. This fungus has been documented to be a fast colonizer of cellulosic substrates and does not produce aflatoxins (Ahmed et al., 2009; Bulgari et al., 2023). Furthermore, it is known that this fungus can grow and utilize cellulosic rice stalks and rice milling by-products as substrates and convert them into fungal biomass. Additionally, *Trichoderma harzianum* has been previously reported to be used in the fermentation of rice by-products, such as rice husk and rice polishing (Ahmed et al., 2017; Sala et al., 2019).

Several studies have been conducted to improve the quality of rice bran and increase its utilization as a feed ingredient (Schmidt and Furlong, 2012; Kang et al., 2015; Supriyati et al., 2015; Hong et al., 2016). Among this technique is biomass transformation through solid-state fermentation (SSF). Fermentation of rice bran increases its nutrient availability through changes arising from microorganisms' metabolic activity (da Silveirai and Furlong, 2007; Jang and Yang, 2008), increases protein and soluble sugars, and reduces complex carbohydrates (Iyayi and Aderolu, 2004). However, to this date, there are no published reports on the use of fermented rice bran (FRB) in shrimp feeds, and information regarding feed value and biological testing in aquatic animals is limited. In the present study, we evaluated the feed value of SSF rice bran as a replacement for soybean meal in the diet of juvenile *P. monodon*.

2 Materials and method

2.1 Rice bran fermentation

2.1.1 Microbial inoculum

Fungus *T. harzianum* was sourced from the Bureau of Soils and Water Management, Department of Agriculture Regional Field Office 6, Iloilo City, Philippines. The fungus was inoculated on Petri dishes containing potato dextrose agar medium (PDA), incubated at 30°C for seven days, and stored at four °C. Spores were collected aseptically, and the count was determined by serial dilution followed by the spread plating method. Spore count was expressed as colony-forming units (CFU) per milliliter.

2.1.2 Fermentation

Freshly milled rice bran was obtained from the Iloilo Rice Processing Complex in Pototan, Iloilo, Philippines. Broken rice and rice hull were separated from the bran using a 0.4 mm sieve and were kept at -20 ˚C until use. Rice bran was subjected to a solid-state fermentation system as previously described (Schmidt and Furlong, 2012). Trays (15 cm x 10 cm x 3.5 cm) containing 2 cm layers of rice bran portions (sterilized at 121°C for 15 min) were moistened with nutrient solution (0.8 g/l ammonium chloride). *T. harzianum* spores were added to an initial concentration of 3 x 10^6 spores/g of bran. Sterile distilled water was added to the medium to adjust the humidity to 50%. The trays were placed in a fermentation chamber composed of a plastic-coated insulated rectangular metal box with a dimension of 1.0 x 0.5 x 1.5 m with a temperature of 30 ˚C and relative humidity of 60%. After 96 hours of incubation, the fermented biomass was sterilized, oven-dried at 60°C, ground, sieved in 0.20 mm, and stored at -20°C.

2.2 Animals and experimental design

The experiment was conducted at the hatchery complex of the Institute of Aquaculture (IA), College of Fisheries and Ocean Sciences (CFOS), University of the Philippines Visayas (UPV) in Miagao, Iloilo, Philippines. Good quality and disease-free *P. monodon* post larvae (PL 15) were obtained from a private hatchery in Guimbal, Iloilo, Philippines. Samples were submitted to the Fish Health Section of the Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department for PCR analysis. The post larvae were acclimated and stocked in a 50-ton canvass pond for 30 days with a stocking density of 100

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pieces/m^3. Prior to stocking, tilapia "green water" was inoculated from the adjacent tank, and aeration was provided. Shrimp were fed with live artemia for seven days and a commercial diet at a feeding rate of 25% of total body weight three times per day. Feeding trays were used to monitor the growth and health of shrimp. A commercial probiotic, BZT ® Aquaculture (*Lactobacillus plantarum, Bacillus subtilis, Bacillus licheniformis*, and *Saccharomyces cerevisiae*), was applied once a week at one ppm to control ammonia, nitrate, and nitrite levels. No water exchange was done during the nursery phase. Water parameters were monitored regularly, and optimum levels were maintained. At the end of the nursery phase, juveniles were collected using a scoop net and transferred to the experimental set-up.

The juveniles (0.47 ± 0.002 g) were randomly distributed into twenty (20) units of 60-L plastic tanks with fifteen shrimp per tank and were acclimated for seven days. This set-up comprised five dietary treatments in four replicates arranged in a complete randomized design (CRD). Three artificial shelters fabricated using plastic sticks were placed in each tank to reduce cannibalism. The set-up ran on a recirculating system, and aeration was provided. Water temperature, salinity, dissolved oxygen (DO), and pH were monitored daily. Ammonia, nitrite, and nitrate were monitored two times per week using test kits (API Saltwater Master Test Kit, MARS Fishcare, USA). All of these water quality parameters were maintained at the optimum levels throughout the experimental period.

2.3 Experimental diet and feeding

Five isonitrogenous diets (44% protein and 12.4% lipid) were formulated by replacing 0,12.5, 25, 37.5, and 50% of soybean meal weight with FRB in the *P. monodon* diet. The composition of the experimental diets is shown in Table 1. Diet's proximate composition analysis was determined following the official methods stipulated in AOAC (1990).

TABLE 1 Feed formulation (%) and proximate composition (% dry weight) of different experimental diets.

Ingredients	Experimental diets				
	T0	T12.5	T25	T37.5	T50
Soybean meal [a]	45.0	39.4	33.7	28.1	22.5
FRB	0.0	5.6	11.3	16.9	22.5
Fish meal [b]	10.0	10.0	10.0	10.0	10.0
Shrimp meal [c]	10.0	10.0	10.0	10.0	10.0
Corn starch	12.0	10.0	8.0	6.0	4.0
Pro-en K[d]	8.0	10.0	12.0	14.0	16.0
Gluten[e]	5.0	5.0	5.0	5.0	5.0
Fish oil[f]	5.0	5.0	5.0	5.0	5.0
Lecithin[g]	1.0	1.0	1.0	1.0	1.0
Vitamin mix[h]	2.0	2.0	2.0	2.0	2.0
Mineral mix[i]	2.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0
Proximate composition (% dry weight)					
Dry Matter	87.7	89.1	87.9	87.1	88.4
Crude Protein	44.3	44.9	44.3	43.5	43.8
Crude Lipid	12.3	12.4	12.5	12.5	12.4
Crude Fiber	2.8	2.9	2.9	3.0	3.1
Ash	9.1	8.9	8.6	9.0	9.3
NFE	19.2	20.0	19.6	19.1	19.8
Carbohydrate	22.0	22.9	22.5	22.1	22.9
Gross Energy (kcal/kg)	4338	4346	4348	4350	4355

[a]Dehulled, defatted soybean meal, [b]70% Danish Fish Meal, [c]*Acetes* sp., [d]Protein enriched sweet potato, [e]Corn Gluten, [f]Cod liver oil, [g]70% Lecithin-Soy, [h]Vitamin premix contributed the following per kg of feed: B-carotene, 36 mg/kg; cholecalciferol, 3 mg/kg; thiamin, 72 mg/kg; riboflavin, 144 mg/kg; pyridoxine, 132 mg/kg; cyanocobalamine, 0.4 mg/kg; ά-tocopherol, 330 mg/kg; menadione, 48 mg/kg; niacin, 288 mg/kg; pantothenic acid, 80 mg/kg; biotin, 0.4 mg/kg; folic acid, 24 mg/kg; inositol, 600 mg/kg; stay C, 2000 mg/kg, [i]Mineral premix contributed the following per kg of feed: P, 2400 mg/kg; Ca, 2400 mg/kg; Mg, 300 mg/kg; Fe, 30 mg/kg; Zn, 84 mg/kg; Cu, 42 mg/kg; K, 1500 mg/kg; Co, 22 mg/kg; Mn, 32 mg/kg; Se, 0.02 mg/kg; Mo, 0.01 mg/kg; Al, 0.5 mg/kg; I, 8 mg/kg.
T0= Control (0% FRB); T12.5 = 12.5% FRB; T25 = 25% FRB; T37.5 = 37.5% FRB; T50 = 50% FRB.

Once all of the dry ingredients were mixed by hand, soy lecithin and fish oil were added. Water was then gradually added (500 ml/kg) until the resulting dough could be easily extruded. The moist mixture was pelleted in a 2-mm diameter meat grinder. The 10 cm long "spaghetti-like" strands were oven-dried at 60°C for 18-24 hours. After drying, strands were broken, sieved to the appropriate size, packed in sealed plastic bags, and stored at -20°C until use.

Shrimp were fed ad libitum three times per day (8:00 AM, 12:00 NN, and 4:00 PM) for 50 days. Excess feeds were siphoned, dried, weighed every morning, and subtracted from the daily feed intake.

2.4 Evaluation of growth performance

The weight of shrimp was recorded at 10-day intervals for 50 days. At the end of the experiment, growth performance was evaluated and calculated by following these equations:

$$Percent\ \ Weight\ \ Gain\ \ (\%WG) = \frac{final\ \ weight\ \ (g) - initial\ \ weight\ \ (g)}{initial\ \ weight\ \ (g)} \times 100$$

$$Specific\ \ Growth\ \ Rate\ \ (SGR) = \frac{ln(final\ \ weight\ \ (g)) - ln(initial\ \ weight\ \ (g))}{number\ \ of\ \ days} \times 100$$

$$Feed\ \ Conversion\ \ Ratio\ \ (FCR) = \frac{total\ \ feed\ \ intake\ \ (g)}{weight\ \ gain\ \ (g)}$$

$$Percent\ \ Survival\ \ (\%\ \ S) = \frac{final\ \ number\ \ of\ \ fish}{initial\ \ number\ \ of\ \ fish} \times 100$$

$$Protein\ \ Efficiency\ \ Ratio\ \ (PER) = \frac{weight\ \ gain\ \ (g)}{protein\ \ intake\ \ (g)} \times 100$$

$$Average\ \ Daily\ \ feed\ \ intake\ \ (ADFI) = \frac{Supplied\ \ feeds}{days}$$

At the conclusion of the feeding trial, all shrimp samples from each tank were pooled, anesthetized at cold temperature, freeze-dried, and ground for approximate analyses of the whole-body composition following the standard methods (AOAC, 1990). Nutrient retention of *P. monodon* was calculated by the following equation:

$$Protein\ \ Retention = \frac{protein\ \ gain\ \ of\ \ fish\ \ (g)}{protein\ \ intake\ \ from\ \ feeds\ \ (g)} \times 100$$

$$Lipid\ \ Retention = \frac{lipid\ \ gain\ \ of\ \ fish\ \ (g)}{lipid\ \ intake\ \ from\ \ feeds\ \ (g)} \times 100$$

2.5 Ingredient digestibility

Apparent digestibility coefficients for dry matter (ADMD) and crude protein (APD) of FRB as feed ingredient were measured using 1% chromium oxide (Cr_2O_3) as an inert marker. The method described by Cho et al. (1982) and Bautista-Teruel et al. (2003) was adapted using a combination of a reference diet to test ingredients in a proportion of 70:30 (Table 2).

In the digestibility assessment of the test ingredient, 25 shrimp were stocked in 250-l conical fiberglass tanks, with each dietary treatment run in triplicate. The experimental set-up was a flow-through culture system equipped with continuous aeration. Water temperature and salinity were maintained at 24–28°C and 30–34 ppt, respectively. Prior to the digestibility experiment, the shrimp were acclimated for seven days. The shrimp were fed with experimental diets ad libitum three times daily. One hour after feeding, uneaten feed and feces were removed, and fecal materials from the collection chamber were collected two hours after feeding. Collected feces were gently rinsed with distilled water, damp dried on filter paper, and oven-dried at 60°C. The dried feces were ground to a fine and homogeneous powder and stored in a -20°C freezer until analyzed.

Chromic oxide was analyzed in the feces and two diets using Ultraviolet-Visible-Near Infrared (UV-VIS-NIR) (Furukawa, 1966; Divakaran et al., 2002). Crude protein in the feces and two diets were analyzed following the official method stipulated in AOAC (1990).

Dry matter and protein apparent digestibility coefficients (ADCs) of diets were calculated using the following equation (Cho, 1979):

$$\%\ ADC_{diet} = 100 - \left[100 \times \left(\frac{C_{diet}}{N_{diet}}\right) \times \left(\frac{N_{feces}}{C_{feces}}\right)\right]$$

where:
C_{diet} = % chromic oxide in diet,
N_{diet} = % nutrient in diet,
N_{feces} = % nutrient in feces,
C_{feces} = % chromic oxide in feces

TABLE 2 Composition of reference and test diets for *in vivo* digestibility experiment in shrimp, *P. monodon* juveniles (g/100g feed).

Ingredient	Reference	Test Diet (70% Reference:30% FRB)
Fish meal	25.0	17.5
Shrimp meal	10.0	7.0
Squid meal	10.0	7.0
Soybean meal	30.0	21.0
Corn starch	11.0	7.4
Gluten	5.0	3.5
Fish oil	4.0	2.8
Mineral mix	2.0	1.4
Vitamin mix	2.0	1.4
Cr_2O_3	1.0	1.0
FRB	–	30.0
Total	100.0	100.0

ADCs of ingredients were calculated using the equation (Bureau and Hua, 2006):

$$\%\,ADC_{ingredient} = ADC_{testdiet} + \left[\left(ADC_{testdiet} - ADC_{ref\,.\,diet}\right) \times \left[\frac{0.7 \times N_{ref}\,(as\ \ is)}{0.3 \times N_{ingr}\,(as\ \ is)}\right]\right]$$

where:

$ADC_{testdiet}$ = Apparent Digestibility Coefficient of test diet,

$ADC_{ref.diet}$ = Apparent Digestibility Coefficient of reference diet,

N_{ref} (as is) = nutrient in the reference diet,

N_{ingr} (as is) = nutrient in the test diet

2.6 Proximate composition analysis

The FRB proximate composition analysis was determined by following the official methods stipulated in AOAC (1990). Crude protein was determined by the Kjeldahl method (Foss Tecator Digestion and Foss Kjeltec 8200 Auto Distillation Unit), while crude lipid was determined by Soxhlet extraction (Foss Soxtec 2050 Automatic System). Moisture was determined by a moisture analyzer (Mettler Toledo Halogen), and crude fiber was determined by the ceramic fiber filter method (Foss Fibertec 2010 System). The ash content was analyzed by furnace combustion (AOAC, 1984). Nitrogen-free extract (NFE) was calculated (Aksnes and Opstvedt, 1998), and NFE plus fiber is expressed as the total carbohydrate content. The gross energy of the diets and feces was also calculated (NRC, 2011).

2.7 Amino acid analysis

The FRB, experimental diets, and shrimp tail muscle amino acid profile were analyzed using Shimadzu High-Performance Liquid Chromatograph LC-10A/C-R7A Amino Acid Analysis System following the method detailed in AOAC Official Method 994.12 and Llames and Fontaine (1994) at the Nutrition Laboratory, IA, CFOS, UPV. The Chemical Score was calculated based on the methodologies outlined by Traifalgar et al. (2019) and Peñaflorida (1989) utilizing the essential amino acid requirements of *P. monodon*. The formula is presented below.

$$Chemical\ \ Score\ (\%) = \frac{{}^{A}/_{E}\ \ of\ \ FRB}{{}^{A}/_{E}\ \ of\ \ shrimp} \times 100$$

$$Where: {}^{A}/_{E} = {}^{essential\ \ amino\ \ acid}/_{total\ \ essential\ \ amino\ \ acid} \times 100$$

The Chemical Score Index (CSI) was the score of the lowest essential amino acid. The Essential Amino Acid Index (EAAI) of the feedstuffs was determined using the following formula:

$$Essential\ \ Amino\ \ Acid\ \ Index\ (EAAI) = \sqrt[n]{\frac{aa_1}{AA_1} \times \frac{aa_2}{AA_1} \times \ldots \times \frac{aa_n}{AA_n}}$$

where:

aa_1 = the A/E (essential amino acid/total essential amino acid) ratio in the feed

AA_1 = the A/E ratio in shrimp

n = number of essential amino acids

The EAAI was patterned after the formula for fish nutrition research (Castell and Tiews, 1980), with whole egg as the reference protein. In this study, however, whole juvenile *P. monodon* was used as the reference protein (Deshimaru and Shigeno, 1972).

2.8 Statistical analysis

The results of three replicate samples were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS version 26. Differences between treatments were evaluated by Tukey's test. In the case of two replicate samples, the independent samples t-test was used to analyze the means. Values were considered statistically significant at $P< 0.05$.

3 Results

3.1 Proximate composition and amino acid profile of rice bran, FRB, and SBM

After fermentation, rice bran crude protein and crude lipid increased by 169.2% (12.7 to 34.2%) and 40.3% (14.4 to 20.2%), respectively (Table 3). Conversely, rice bran crude fiber, ash, and NFE decreased by 87.1% (16.3 to 2.1%), 82.3% (12.4 to 2.2%), and 9.9% (34.4 to 31.0%), respectively. The proximate analyses of dehulled and defatted soybean meal used in diet formulation showed 12.0% moisture, 48.0% crude protein, 3.1% crude lipid, 3.0% crude fiber, 6.3% ash, and 27.6% NFE.

Generally, there was an increase in the amount of amino acid in FRB compared to unfermented rice bran except for tryptophan (Table 4). All known essential amino acids for shrimp were found present in the FRB. The EAAI of FRB was 85.98, and the CSI was 25.20, with tryptophan as the limiting amino acid (Table 5). Using EAAI, FRB was rated as a good-quality protein material.

3.2 Apparent digestibility coefficients

The apparent dry matter (ADMD), protein (APD), and ingredient (ADI) digestibility coefficients of FRB by *P. monodon*

TABLE 3 Proximate composition (%) of rice bran (RB), fermented rice bran (FRB), and soybean meal (SBM).

Nutrient Composition	RB	FRB	SBM
Moisture	9.8	10.3	12.0
Crude Protein	12.7	34.2	48.0
Crude Lipid	14.4	20.2	3.1
Crude Fiber	16.3	2.1	3.0
Ash	12.4	2.2	6.3
NFE	34.4	31.0	27.6
Total	100.0	100.0	100.0

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are presented in Table 6. The FRB was found to be highly digestible with ADMD and APD coefficients at 83.05 ± 0.02% and 87.20 ± 0.30%, respectively. ADI coefficients were also high at 85.19 ± 0.08% in dry matter and 90.67 ± 0.78% in protein. Generally, the apparent digestibility coefficients of FRB were comparable to soybean meal and higher than unfermented rice bran.

3.3 Amino acids composition of experimental diets

The amino acid profile of the experimental diets, as detailed in Table 7, adequately meets the essential amino acid requirements of *P. monodon*. This means that the composition of amino acids present in the diets sufficiently fulfills the nutritional needs of *P. monodon*.

3.4 Growth performance of *P. monodon*

Significant differences (P< 0.05) were observed among the treatments in the growth parameters of *P. monodon* (Table 8). In terms of weight gain (%) and SGR, T25 was significantly higher (P< 0.05) than T0 but not significantly higher (P > 0.05) than T12.5. PER was significantly higher (P< 0.05) in T12.5 and T25 among treatments. Conversely, FCR was significantly lower in the same treatments compared to T0, T37.5, and T50. The DFI was highest in T25 at 30.7 ± 0.18 mg/day. There were no significant differences (P > 0.05) in survival among treatments. Generally, 0% and 50% replacement levels were observed to be significantly similar in terms of weight gain, SGR, PER, FCR, DFI, and survival.

The second-order polynomial regression analysis of weight gain suggests an FRB replacement level of 21.08% would provide the maximum growth for *P. monodon* juvenile (Figure 1).

TABLE 4 Amino acid profile (% AA in protein) of rice bran (RB), fermented rice bran (FRB), and soybean meal (SBM).

Amino Acid	RB	FRB	SBM
Essential Amino Acid			
Phenylalanine	0.50	2.11	5.18
Valine	0.64	1.59	4.41
Tryptophan	0.17	0.05	0.43
Threonine	0.46	1.38	3.79
Isoleucine	0.44	0.93	4.50
Methionine	0.31	0.80	1.39
Histidine	0.34	1.60	2.90
Arginine	0.99	1.04	5.98
Leucine	0.94	2.76	7.17
Lysine	0.45	1.76	5.78
Non-essential amino acid			
Tyrosine	0.47	1.82	2.72
Serine	0.62	1.47	5.21
Aspartine	1.08	2.99	11.07
Glutamic acid	2.08	4.87	18.72
Glycine	0.58	2.02	3.99
Alanine	0.72	2.57	4.00
Proline	0.60	nd*	5.40
Cystine	0.28	nd*	1.17

*not detected.

3.5 Proximate and amino acid composition of shrimp carcass

There were no significant differences (P > 0.05) in the whole-body composition of shrimp fed with different levels of FRB (Table 9). The crude protein, crude lipid, ash, and moisture were found to be similar among treatments.

After the feeding trial, it was determined that the T50 diet was comparable to the T0 diet in terms of growth performance and shrimp whole body composition. The amino acid profile of shrimp tail muscle fed with T0 and T50 diets was then analyzed for comparison. The histidine, arginine, and lysine values were reported to be significantly higher (P< 0.05) in *P. monodon* fed with a T50 diet compared to T0 (Table 10). However, no significant differences (P > 0.05) were observed in the total amino acids, essential amino acids, and non-essential amino acids of *P. monodon* when fed with T50 compared to T0.

3.6 Nutrient retention

Protein retention in shrimp fed with increasing FRB replacement levels was found to be significantly different (P< 0.05) among treatments (Figure 2). Shrimp fed with the T25 diet exhibited the highest protein retention (12.46 ± 0.05), followed by those fed with the T12.5 diet (10.35 ± 0.11), both of which were significantly higher than those fed with the T0 diet (9.35 ± 0.08), T37.5 diet (9.32 ± 0.3), and T50 diet (8.97 ± 0.04). The protein retention of shrimp fed with the T37.5 diet and T50 diet was not significantly different than the T0 diet. In contrast, FRB dietary protein replacement did not significantly (P > 0.05) influence lipid retention of *P. monodon* juveniles.

3.7 Water quality parameters

The mean water temperature was 24.84 ± 0.03°C (range: 24.8-25.8°C) at 0800H while 27.14 ± 0.18°C (range: 25.8-27.8°C) at 1600H. The lowest recorded water temperature was at 24.8°C, while the highest was at 27.8°C. The mean dissolved oxygen (DO) was 4.84 ± 0.07 mg/l (range 4.75-6.25 mg/l), the mean salinity was 15.14 ± 0.06 ppt (range: 14-16 ppt), and the mean pH was 8.20 ± 0.01 (range: 8.12-8.30). The average ammonia was 0.47 ± 0.12 ppm (range: 0.25-1.00 ppm), average nitrite was 0.57 ± 0.44 ppm (range: 0-2 ppm), and average nitrate was 1.5 ± 0.33 ppm (range: 0-10 ppm).

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TABLE 5 Essential Amino Acid Index (EAAI) and Chemical Score Index (CSI) of fermented rice bran (FRB).

Essential Amino Acid	FRB (% AA in CP)	*P. monodon* requirement (% AA in CP)	A/E[a]		A/E Ratio[b]	Chemical Score
			FRB	*P. monodon*		
Arginine	1.04	5.30	7.41	15.96	0.46	46.42
Histidine	1.60	2.20	11.44	6.63	1.73	172.60
Isoleucine	0.93	2.70	6.65	8.13	0.82	81.76
Leucine	2.76	4.30	19.68	12.95	1.52	151.93
Lysine	1.76	5.20	12.56	15.66	0.80	80.17
Phenylalanine	2.11	3.70	15.03	11.14	1.35	134.82
Methionine	0.80	2.40	5.72	7.23	0.79	79.06
Threonine	1.38	3.50	9.83	10.54	0.93	93.22
Tryptophan	0.05	0.50	0.38	1.51	0.25	25.20
Valine	1.59	3.40	11.32	10.24	1.11	110.54
Total	14.01	33.20				
EAAI	85.98					
CSI	25.20					

[a]A/E= essential amino acid/total essential amino acid x 100.
[b]A/E Ratio = A/E of FRB/A/E of shrimp,

4 Discussion

The nutritional composition of rice bran was improved after fermentation by *T. harzianum*. The protein content was increased to about 34.5%, and the fiber was decreased to about 2.1%. Rice bran fermentation has been shown to improve its nutritional composition. The application of solid-state fermentation (SSF) by *T. harzianum* using rice polishing as a substrate was demonstrated in the study of Ahmed et al. (2017), where higher proteins were also attained. Solid-state fermentation appears to improve the nutritional value by improving the digestibility and increasing the protein content of the biomass material. Nutrient enrichment of rice bran was also reported in other studies using different types of fungus, *Trichoderma viride* (Iyayi and Aderolu, 2004), *Rhizopus oryzae* (Oliveira et al., 2010; Schmidt and Furlong, 2012), *Pleurotus sapidus* (Omarini et al., 2019), *Trichoderma longibrachiatum* and *Aspergillus niger* (Hong et al., 2016). Improvement of nutritional composition was also revealed in other agro-industrial waste like fermented sweet potato meal (Traifalgar et al., 2019), fermented copra meal (Apines-Amar et al., 2016), and fermented palm kernel cake (Yana et al., 2010). The improvement in protein content due to fermentation has been associated with the bioconversion of starchy substrates into protein-rich microbial cellular components (Jaganmohan et al., 2013).

TABLE 6 Apparent digestibility coefficients (ADC) for dry matter, protein, and ingredients of fermented rice bran (FRB) by *P. monodon*.

Apparent Digestibility Coefficients	FRB	Rice Bran[a]	SBM[a]
% ADMD	83.05 ± 0.02	40.0 ± 1.50	60.1 ± 1.40
% APD	87.20 ± 0.30	76.4 ± 0.80	90.4 ± 0.90
% ADI (Dry Matter)	85.19 ± 0.08		
% ADI (Protein)	90.67 ± 0.78		

[a]values reported by Akiyama et al. (1989) in *L. vannamei*.

In the present study, fermentation decreased the fiber content of rice bran by about 7-fold compared to the unfermented rice bran. The content of total dietary fiber (TDF) in rice bran is approximately 20-30%, and nearly 90% of that content consists of insoluble dietary fiber (IDF) comprising of cellulose, hemicellulose, insoluble β-glucan, and arabinoxylans (Lai et al., 2007; Zhao et al., 2018). The high content of these IDF in rice bran is responsible for the low nutritional value and limited use of this biomass in feeds (Dodd and Cann, 2009). Fermentation using fungi has been known to decrease the IDF of agricultural biomass since these organisms are known producers of xylanase enzymes that are more active than those produced by yeasts and bacteria (Ravindra, 2001; Polizeli et al., 2005). Among these fungi, *T. harzianum* has been reported to produce cellulases and hemicellulases that are active in hydrolyzing plant cellulose. This may explain the decreased fiber content of rice bran due to fermentation in the present study (Kim et al., 2003; Gottschalk et al., 2010; Pathak et al., 2014).

The nutritional quality of a feed ingredient for farmed animals is quantified based on the digestibility coefficient that measures the availability of nutrients for assimilation. Results suggest that the SSF of rice bran has improved the digestibility coefficient of this ingredient. The apparent ingredient digestibility coefficient for dry matter and protein of *P. monodon* was about 85% and 90%, respectively. These values are higher than those of raw, unprocessed rice bran and soybean meal in *L. vannamei*

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TABLE 7 Essential amino acid composition (g/100 g sample) of experimental diets with comparison to *P. monodon* requirement.

Amino Acid	FRB	T0	T12.5	T25	T37.5	T50	*P. monodon* requirement [a]
Phenylalanine	5.42	1.97	2.14	2.30	2.47	2.63	1.70
Valine	5.75	1.86	2.06	2.25	2.45	2.64	1.35
Tryptophan	0.11	0.49	0.46	0.43	0.39	0.36	0.20
Threonine	4.91	1.57	1.75	1.92	2.09	2.26	1.40
Isoleucine	3.02	1.70	1.75	1.80	1.85	1.90	1.01
Methionine	2.28	0.85	0.94	1.03	1.12	1.20	0.89
Histidine	4.39	0.93	1.10	1.27	1.44	1.61	0.80
Arginine	2.53	2.78	2.73	2.68	2.63	2.58	1.85
Leucine	8.93	3.35	3.64	3.93	4.22	4.51	1.70
Lysine	5.11	2.48	2.61	2.74	2.86	2.99	2.08

[a] (Millamena et al., 1996a, Millamena et al., 1996b, Millamena et al., 1997, Millamena et al., 1998, Millamena et al., 1999).
T0= Control (0% FRB); T12.5 = 12.5% FRB; T25 = 25% FRB; T37.5 = 37.5% FRB; T50 = 50% FRB.

TABLE 8 Growth performance of *P. monodon* fed with different levels of fermented rice bran (FRB).

Growth Parameters	T0	T12.5	T25	T37.5	T50
Weight Gain (%)	150.89 ± 6.77^{bc}	169.8 ± 0.56^{ab}	177.36 ± 0.95^{a}	150.08 ± 9.22^{bc}	139.79 ± 2.69^{c}
SGR	1.84 ± 0.05^{bc}	1.99 ± 0.00^{ab}	2.04 ± 0.01^{a}	1.83 ± 0.07^{bc}	1.75 ± 0.02^{c}
FCR	2.09 ± 0.04^{a}	1.7 ± 0.03^{b}	1.83 ± 0.00^{b}	2.1 ± 0.07^{a}	2.22 ± 0.05^{a}
PER	1.06 ± 0.02^{b}	1.29 ± 0.02^{a}	1.26 ± 0.00^{a}	1.11 ± 0.04^{b}	1.04 ± 0.02^{b}
DFI (mg/day)	29.53 ± 0.66^{ab}	27.14 ± 0.03^{b}	30.7 ± 0.18^{a}	29.29 ± 0.86^{ab}	29.16 ± 0.33^{ab}
Survival	80.00 ± 6.67	75.56 ± 2.22	88.89 ± 2.22	80.00 ± 0.00	80.00 ± 3.85

T0= Control (0% FRB); T12.5 = 12.5% FRB; T25 = 25% FRB; T37.5 = 37.5% FRB; T50 = 50% FRB.

(Akiyama, 1989; Akiyama et al., 1989). This improvement in digestibility could be attributed to the decrease in cellulosic polysaccharides that intervene in the enzymatic digestion of food nutrients (Singh et al., 2009). Enzymes secreted by *T. harzianum* may also explain the high protein digestibility index of the fermented rice bran in the present study (Polizeli et al., 2005).

The quality of protein in a fish diet depends on the amount and balance of amino acids, which impact growth and production costs.

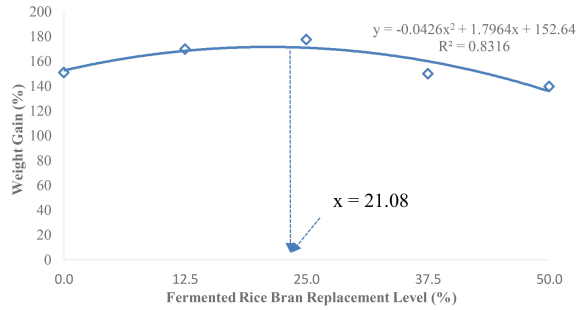

FIGURE 1
Optimum soybean meal replacement level of fermented rice bran (FRB) to achieve maximum growth in *P. monodon*.

Furthermore, besides its crucial role in protein synthesis and nitrogen balance, it also plays a significant part in vital metabolic processes in fish (Jobgen et al., 2006). Results of the present study showed that SSF with *T. harzianum* increased the protein content of rice bran about 3-fold compared to the raw material. The quantity of total amino acids in FRB was also increased as compared to the unfermented rice bran, indicating an improvement in the quality of protein. Similar improvements in the protein content and amino acid profile were observed when rice bran was fermented using *A. niger* (Putra et al., 2022). The beneficial effect of SSF was also reported in fermented copra meal (Dairo and Fasuyi, 2008), fermented sweet potato meal (Traifalgar et al., 2019), and fermented rice bran (Joseph et al., 2008). This improvement in protein content and quality has been associated with the microbial biomass that is known as natural protein concentrate as it contains highly digestible proteins with complete essential amino acids (Kurbanoğlu, 2001; Aggelopoulos et al., 2014).

The essential amino acid index of FRB was found to be high at 84%, rated as a good quality protein material, and comparable to soybean meal (Oser, 1959; Peñaflorida, 1989). The chemical score index of FRB showed tryptophan as the limiting amino acid. The amino acid profile of the fermented material is dictated by the microbial species and substrate used in the fermentation (Denardi-

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TABLE 9 Whole body composition (%) of *P. monodon* juveniles after the feeding trial.

Proximate Composition	T0	T12.5	T25	T37.5	T50
Moisture	78.07 ± 0.15	77.97± 0.15	77.52 ± 0.16	78.32± 0.14	78.10 ± 0.14
Crude Protein	73.25 ± 0.56	72.29 ± 0.61	74.51 ± 0.37	71.88 ± 0.96	72.83 ± 0.09
Crude Lipid	9.39 ± 0.42	8.89 ± 0.30	8.92 ± 0.42	10.00 ± 0.21	11.19 ± 0.98
Ash	21.54 ± 0.36	21.92 ± 0.68	22.03 ± 0.38	22.08 ± 1.09	21.71 ± 0.07

T0= Control (0% FRB); T12.5 = 12.5% FRB; T25 = 25% FRB; T37.5 = 37.5% FRB; T50 = 50% FRB.

Souza et al., 2018). *Trichoderma* utilizes tryptophan to form indole acetic acid, and the utilization of this amino acid by this fungus may explain the low tryptophan level in fermented rice bran (Kumar et al., 2017). In addition, deficiency in tryptophan among plant protein sources also agreed with the findings of the previous studies (Fetuga et al., 1973; Felker and Bandurski, 1977).

The use of soybean meal as a major plant protein source is considered a standard in aquatic animal nutrition. The results of the present study confirm the viability of FRB in replacing SBM in the diet of juvenile *P. monodon*. FRB substitution of 25% soybean meal in the diet of *P. monodon* showed significant improvement in weight gain, specific growth rate, FCR, and PER. However, no significant effect on growth performance was observed when replacing soybean meal at higher levels. This indicates that fermentation could improve the nutritional value of rice bran and be used as a partial replacement for soybean meal in the diet of *P. monodon*. The results of this study also showed higher replacement levels of FRB when compared to another study where only 20% of soybean meal was replaced by FRB utilizing *A. niger* in catfish diets (Putra et al., 2022). Other studies have also reported the positive effects of replacing soybean meal with fermented agro-industrial wastes on shrimp growth. For example, *L. vannamei* exhibited improved growth when fed diets containing fermented sweet potato meal (Traifalgar et al., 2019), while fermented copra meal was identified as a practical alternative protein source for black tiger shrimp diets (Apines-Amar et al., 2016). Moreover, our results align with previous studies on terrestrial animals, which have demonstrated enhancements in growth performance across various species; growth of broiler chickens fed with *Saccharomyces cerevisiae* FRB (Kang et al., 2015), higher egg production in layers-fed diet with *T. viride* FRB (Iyayi and Aderolu, 2004) and reduced feed cost in pigs fed with *T. longibrachiatum, A. niger, Pichia kudriavzevii* and *Lactobacillus buchneri* FRB (Hong et al., 2016).

Results on the carcass composition indicate no negative influence of FRB in the nutritional composition of *P. monodon*. Furthermore, the protein retention in shrimp was improved when SBM was replaced up to 25%. However, higher SBM replacement showed retention levels similar to the control. This could be explained by the increased essential amino acids in the diets with FRB, which led to efficient protein retention. This result is in contrast to other studies where partial replacement of SBM by

TABLE 10 Amino acid content (% AA in protein) of *P. monodon* juveniles after the feeding trial.

Amino Acid (AA)	T0	T50
Essential Amino Acid (EAA)		
Phenylalanine	2.15 ± 0.11	2.52 ± 0.11
Valine	0.66 ± 0.19	0.74 ± 0.19
Tryptophan	0.01 ± 0.00	0.01 ± 0.00
Threonine	0.43 ± 0.08	0.47 ± 0.08
Isoleucine	0.56 ± 0.13	0.59 ± 0.13
Methionine	0.74 ± 0.12	0.96 ± 0.12
Histidine	1.54 ± 0.01^{a}	1.73 ± 0.02^{b}
Arginine	2.74 ± 0.02 ^{a}	3.46 ± 0.02^{b}
Leucine	2.08 ± 0.35	2.53 ± 0.35
Lysine	2.10 ± 0.01^{a}	2.62 ± 0.01^{b}
Total EAA	13.01 ± 0.96	15.64 ± 0.96
Non-essential amino acid (NEAA)		
Tyrosine	1.00 ± 0.10	1.30 ± 0.11
Serine	0.73 ± 0.15	0.88 ± 0.15
Aspartine	1.22 ± 0.21	1.26 ± 0.22
Glutamic acid	2.41 ± 0.44	2.57 ± 0.44
Glycine	3.12 ± 0.17	3.36 ± 0.16
Alanine	1.02 ± 0.15	0.89 ± 0.14
Total NEAA	9.50 ± 1.22	10.27 ± 1.23
Total AA	22.50 ± 2.17	25.91 ± 2.18

T0= Control (0% FRB); T50 = 50% FRB.

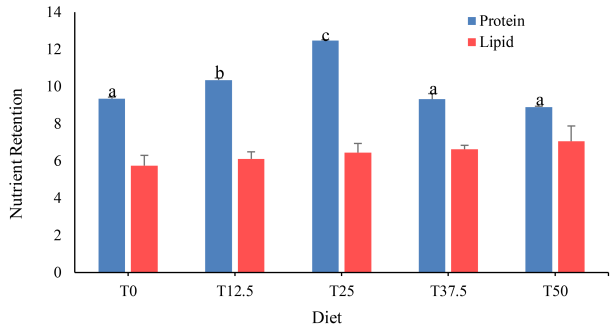

FIGURE 2
Nutrient retention of *Peneus monodon* fed with different levels of fermented rice bran (FRB).

fermented agro-industrial wastes showed no significant effect on the protein retention of shrimp (Apines-Amar et al., 2016; Traifalgar et al., 2019).

The amino acid analysis of *P. monodon* juveniles after the feeding trial showed that lysine in shrimp fed with 50% FRB replacement of SBM was significantly higher than control. Lysine, along with proline, alanine, glycine, serine, glutamic acid, and leucine, have been shown to be the important taste compounds of shrimp (Raksakulthai and Norman, 1992). The increase in these amino acids would further enhance its desirable flavor, and the decline can cause changes in the sensory characteristics of shrimp (Peralta et al., 2008). Furthermore, the glutamic acid, a substance responsible for the 'umami' taste in fish products (Lopetcharat et al., 2001; Kim et al., 2003) of 50% FRB-fed *P. monodon*, was higher than the control. These results suggest that FRB could improve the sensory characteristics of *P. monodon*, as shown by an increase in the amount of amino acid important to shrimp taste.

The present study has demonstrated that solid-state fermentation could improve the nutritional value of rice bran. Fermentation has increased the protein, decreased the fiber contents, enhanced the amino acid profile, and improved the digestibility coefficient of this feed ingredient. Nutritional evaluation tests through a feeding trial indicate that fermented rice bran could partially replace dietary soybean meal without affecting the growth performance and biochemical composition of *P. monodon*. Furthermore, a 25% replacement of SMB by FRB could improve the growth of *P. monodon.* It is recommended that 50% replacement of FRB could replace soybean meal without affecting the growth of shrimp. However, further research is required to explore the complete substitution of soybean meal with fermented rice bran. Given that rice bran is abundantly produced as a by-product of the rice industry in Asian countries, utilizing fermented rice bran as a feed ingredient represents a renewable and eco-friendly approach to achieving the sustainable production of *P. monodon* in the Asian region.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because this study adheres to the Philippine National Standard (PNS) on the Code of Good Aquaculture Practices (GAqP) for Shrimp and Crab (BAFS, 2017). Protocols on rearing, handling, and animal welfare, were strictly followed based on the guidelines stipulated in Philippine Republic Act Number 8485, known as the Animal Welfare Act of 1998.

Author contributions

FH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RT: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing. CD: Data curation, Writing – review & editing, Software.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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