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Research Article

Replacement of commercial feed with fermented soybean meal for *Penaeus vannamei* during the nursery phase

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ABSTRACT. Shrimp farming is evolving towards intensification to enhance productivity and optimize resource usage. The growth of this sector is closely linked to the availability of high-quality postlarvae and juveniles. Intensive nurseries in two-phase systems play a crucial role in improving survival and growth rates during the grow out phase. However, this approach necessitates skilled labor and feeds with high crude protein content, which are expensive and potentially risky to water quality, and therefore to animal health. Therefore, it is essential to develop strategies that improve this farming system. The objective of this study was to evaluate the effect of replacing commercial feed with different levels of fermented soybean meal (FSB; 0, 25, 50, 75, and 100%) using a commercial probiotic composed of a mixture of microorganisms during the culture of *Penaeus vannamei* postlarvae in an intensive nursery. The water quality, zooplankton density, and standard bacterial plate count of *Vibrio* spp. in water culture were evaluated. Daily weight gain, average final weight, and final productivity were affected by the 75% replacement. Shrimp fed with up to 50% replacement exhibited superior growth. Survival was not impacted by the replacement levels, remaining above 79%. Total zooplankton density was higher in the FSB 25% treatment ($P < 0.05$). The *Vibrio* spp. standard plate count was influenced by the different replacement levels, with 50% replacement reaching the highest levels ($P < 0.05$). Results showed that FSB can replace up to 50% of commercial feed for postlarvae during intensive nursery, maintaining growth rates similar to those achieved with commercial feed protocols.

Keywords: postlarvae; vegetable bran; fermentation; probiotic; shrimp; aquaculture

INTRODUCTION

The adoption of intensive systems enables productivity gains through the use of high stocking densities. In these systems, higher levels of technology are observed, including more efficient and modern aeration mechanisms, advanced water quality control technologies, and high-quality commercial feeds with substantial inclusion of fishmeal and high protein content (Tierney et al. 2020, Wasielesky et al. 2020, Nunes et al. 2021). High productivity in aquaculture can be achieved; however, it also comes with an increased risk of mortality. This risk is often associated with high population densities and the use of high feeding rates, which can lead to the accumulation of organic matter, thereby enabling the development of bacterial diseases, such as vibriosis (Zokaeifar et al. 2012). Additionally, operating these systems typically incurs higher costs

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due to the need for value-added inputs, such as probiotics and formulated feeds, as well as aeration systems, among other factors (Browdy et al. 2016, Peixoto et al. 2017, Supriyono et al. 2021). Fermented vegetable bran can be utilized as a strategy to mitigate production costs while simultaneously improving the cultivation environment. This approach enables a reduction in costs through the total or partial replacement of commercially formulated feeds in the feeding of cultivated organisms, as well as stimulating the production of zooplankton, such as copepods and rotifers, which serve as supplementary food sources, thereby reducing feed conversion (Romano 2017). Additionally, fermentation using probiotic organisms facilitates their dissemination in the cultivation environment, thereby positively impacting the system's ecology and leading to improved control over both water and sediment quality (Dauda et al. 2017, Romano et al. 2018, Albuquerque 2019, Leite et al. 2020).

The use of fermented vegetable brans, especially soybean meal, has been investigated in marine shrimp farming across both semi-intensive and intensive systems (Abd El-Naby et al. 2024). Its applications include serving as a fertilizer for natural food production and water quality control through probiotic dissemination and C/N ratio management, as well as its inclusion in animal nutrition (Shiu et al. 2015, Dossou et al. 2018, Van Nguyen et al. 2018, Albuquerque 2019, Leite et al. 2020). Research has demonstrated the effectiveness of fermented soybean meal (FSB) as a primary protein source in formulated diets for *Penaeus vannamei* postlarvae (PL) in intensive nurseries. Its use can positively impact weight gain and productivity, while also enhancing both the immunological and health status of farmed animals, potentially accelerating their reaction to pathogens, such as bacteria from genus *Vibrio* (Wang et al. 2016, Guo et al. 2018, Van Nguyen et al. 2018, Galkanda-Arachchige & Davis 2020). Additionally, this strategy enables feed cost reduction, given the lower price of this vegetable input. Fermenting soybean meal with probiotic organisms enhances its nutritional properties and reduces antinutritional factors (Xue et al. 2024). The substitution of fishmeal with FSB in formulated feeds has been extensively studied, with numerous studies confirming its efficiency as a primary protein source, even in intensive systems (Supriyati et al. 2015, Shiu et al. 2015, Sharawy et al. 2016).

Although scientific literature encompasses research on the development and evaluation of strategies for using soybean meal in shrimp nutrition, the vast majority of studies focus on its use as an ingredient in formulated feeds (Zhou et al. 2015). However, more recently, the use of this ingredient as a direct feed source for farmed shrimp has been explored. Fermentation of these ingredients by probiotic microorganisms, such as *Lactobacillus* and *Bacillus*, promotes the degradation of antinutritional compounds and the production of beneficial metabolites, such as organic acids, digestive enzymes and bioactive peptides, which improve nutrient digestibility, intestinal health and water quality (Zhao et al. 2020, Li et al. 2021). Studies have demonstrated the positive effects of incorporating fermented ingredients into shrimp diets, leading to enhanced growth, improved immune response, and increased microbial stability in the culture environment (Yang et al. 2020). However, challenges such as standardizing final product quality, production costs, and possible environmental impacts still require further investigation (Zhang et al. 2019). According to Albuquerque (2019) using the fermentation process with the commercial probiotic BM-PRO™ increased the crude protein, total carbohydrate, and lipid content of FSB, including its use as an exclusive food source during the entire growout phase for the marine shrimp *P. vannamei*, providing performance results similar to those obtained with commercially formulated feed. However, there remains a lack of evaluation of FSB as an exclusive nutritional element for postlarvae during the intensive nursery phase.

MATERIALS AND METHODS

Experimental design

The study lasted 23 days and was conducted at the Aquacultura Fortaleza S.A. (AquaFort) farm, located on the Coreaú River between the municipalities of Camocim and Granja, in Ceará State, Brazil. The trial was conducted in circular tanks with a bottom area of 385 m², a water column of 1.3 m, and a useful volume of 500 m³ each. PL were stocked at an average initial weight of 0.003 ± 0.001 g (BK-2000, GEHAKA, São Paulo, Brazil) at a density of 9,000 PL m^{-3}.

The trial employed a completely randomized experimental design with five treatments, each using FSB at varying concentrations (0, 25, 50, 75, and 100%) in triplicate. These treatments represented the replacement levels (%) of commercial feed with FSB in the feeding protocol. The control was represented by the absence of FSB (FSB0%); only commercial feed is offered. During the trial, PL were fed a combination of three commercial diets with different grain sizes, each suited to the corresponding PL stage (Table 2). Food

was offered 12 times a day, at 2-h intervals between each feeding (Table 3).

Raising conditions

The tanks were supplied with previously filtered water (salinity at 46) and provided with supplementary aeration through a diffused air system, using flexible microperforated hoses placed at the bottom of each tank. A 7.5 HP air blower powered each tank's aeration. Initially, the tanks were fertilized with 50 g m^{-3} of fermented rice bran (FRB) and 0.5 g m^{-3} of the commercial probiotic BM-PRO™ (Biotrends Soluções Biotecnológicas, Eusébio, CE, Brazil) for three consecutive days before stocking. Throughout the experimental period, daily applications of FRB (10 g m^{-3}) were added directly to the water. After the third day of the experiment, partial water exchanges (approximately 5% of the total volume) were performed to prevent nitrogen peaks and the accumulation of organic matter. Water replacements with filtered water were carried out to maintain the system's water level and salinity.

Vegetable bran fermentation with probiotic

The fermentation processes for soybean and rice bran used in the feeding and fertilization protocols, respectively, were carried out using BM-PRO™ (Biotrends Biotech Solutions, Eusébio, CE, Brazil), according to the manufacturer's recommendations and based on previous studies conducted by the company. The choice of this commercial probiotic was based on the fermenting microorganisms, such as bacteria and yeast, present in its composition (Table 1). The application of fermented rice bran is a strategy aimed at creating conditions like the natural environment, with the goal of controlling water quality (Pimentel et al. 2025a).

Fermentation of rice bran

Rice bran fermentation was performed following the protocol for a 250 L total volume culture. Two hundred fifty grams of BM-PRO™ were hydrated in 10 L of water for 4 h. After activation, the microbial culture was inoculated into a previously prepared mixture consisting of 25 kg of rice bran, 213 L of water with salinity at 46, and 1.75 kg of sodium bicarbonate ($NaHCO_3$). The culture underwent fermentation for 40 h before being applied to the culture tanks. In all experimental units, the application of FRB was performed according to the following protocol: three doses of 50 g m^{-3} of bran were administered for three days, before population, in conjunction with three doses of 250 g of the commercial probiotic used in the bran fermentation process.

Fermentation of soybean bran

Soybean bran fermentation was performed using the following method for a total of 100 kg of soybean bran. Five hundred grams of BM-PRO™ were hydrated in 25 L of water salinity (46) for 4 h. After microbial activation, 75 L of water was added, and the mixture was homogenized, totaling 100 L of culture. This culture was then gradually inoculated into 100 kg of soybean bran. This homogeneous mixture remained in the fermentation process for 48 h, covered and protected from the sunlight. The production of fermented soybean bran was carried out according to daily feed demand.

Water quality monitoring

Water quality parameters were recorded twice daily (07:00 and 17:00 h), except for alkalinity, which was measured once a day at 07:00 h. Dissolved oxygen and temperature were recorded using a probe (Instrutherm, MO900, Brazil); pH was measured with a portable pH meter (Akso, model AK90, Brazil); and salinity was measured with a probe (Akso, AR8012, Brazil). Ammonia, nitrite, and alkalinity levels were determined using a colorimetric test with a photometer reading (YSI, EcoSense 9500, USA).

Zooplankton density analysis

A volume of 20 L of water was collected daily from each experimental unit. After homogenization, three 1 mL aliquots were taken from each sample, fixed in a 4% formalin solution, and analyzed under an optical microscope (Olympus, CX21, Japan) at 4× magnification using a Sedgewick-Rafter chamber (Utermöhl 1958). The densities of copepods and rotifers were then determined based on zooplankton counts.

***Vibrio* spp. monitoring**

Water samples were collected daily using sterilized 50 mL Falcon tubes. A two-fold serial dilution (10^{-1} and 10^{-2}) was performed by suspending 1 mL of each sample in 9 mL of 2.5% (m/v) saline solution. After this, 0.1 mL aliquots of the 10^{-1} dilution were taken and evenly spread on plates containing thiosulfate-citrate-bile-sucrose agar (TCBS), using a Drigalsky loop, following the Spread Plate technique. The plates were incubated upside down at 35°C for 24 h. Bacterial colonies were then counted using the Standard Plate Count (SPC) method (Downes & Ito 2001), and results were expressed as colony-forming units per milliliter (CFU mL^{-1}).

Table 1. Biological composition of the BM-PRO™ commercial probiotic. Source: Biotrends Biological Solutions. CFU: colony-forming units.

Species	Concentration (CFU kg^{-1})
Bacillus subtilis	4.0×10^{11}
Bacillus licheniformis	4.0×10^{11}
Bacillus pumilus	4.0×10^{11}
Lactobacillus plantarum	2.0×10^{11}
Lactobacillus acidophilus	1.0×10^{11}
Saccharomyces cerevisiae	4.0×10^{11}
Total microorganism counting	1.9×10^{12}

Table 2. Composition of different commercial feeds indicated for each post-larval stage of the marine shrimp *P. vannamei* under intensive nursery. [1]Bern Aqua NV, Olen, Belgium, [2]Epicore BioNetworks Inc., Eastampton, NJ, USA, [3]Zeigler Bros Inc., Garners, PA., USA. NI: not informed.

Commercial feed	MeM[1]	Epibal[2]	PL Raceway 40-9[3]	PL Raceway 40-9[3]
Granulometry (µm)	200-300	300	400-600	600-850
Warranty levels				
Crude protein (%)	60.0	49.0	40.0	40.0
Lipids (%)	15.0	14.0	9.0	9.0
Ashes (%)	14.5	12.0	3.0	3.0
Maximum humidity (%)	8.0	10.0	10.0	10.0
Fibers (%)	1.7	4.0	13.0	13.0
Phosphorus (%)	2.3	NI	1.1	1.1

Growth performance

At the end of the experiment, the following performance parameters of the shrimp were evaluated:

Survival rate (SR, %): (final number of PL / initial number of PL) × 100;

Final weight (FW, g): total weight of PL sampled / total number of PL sampled;

Yield (kg m^{-3}): final biomass (kg) / volume (m^3);

Daily weight gain (DWG, g d^{-1}): (final average weight – initial average weight)/reared days;

Specific growth rate (SGR, % d^{-1}): [(ln FW - ln IW)/ experimental time (d)]× 100; and

Feed conversion factor (FCR): feed offered (kg) / biomass gain (kg).

Statistical analysis

The data obtained in the study were evaluated for normality using the Shapiro-Wilk test and for homoscedasticity using Levene's test. A one-way ANOVA followed by Tukey's *post-hoc* test was used to compare the means. The tests were performed according to Zar (2010), with a significance level of 5% ($P < 0.05$).

RESULTS

Microbiological evaluation of the soybean meal fermentation process

The growth of microbial communities during the fermentation of soybean meal, utilizing the microorganisms and nutrients of the probiotic BM-PRO® is shown (Fig. 1). *Lactobacillus* spp. and yeasts grew during the fermentation process of soybean meal with the probiotic, increasing from a logarithmic scale of 10^6 to 10^9 CFU g^{-1} and from 10^6 to 10^8 CFU g^{-1}, respectively, within the first 24 h. Total heterotrophs (*Bacillus* spp.) remained viable and metabolically active at the logarithmic scale of 10^6 CFU g^{-1}. The exponential growth of *Lactobacillus* spp. was accompanied by a gradual decrease in the pH of the soybean meal, indicating stability from the sixth day onward. The pH values during the fermentation of soybean meal are shown (Fig. 2).

Table 3. Feeding rate (%) based on the estimated weight (g) of *Penaeus vannamei* postlarvae (PL) reared in the nursery phase under commercial conditions. [1]Bern Aqua NV, Olen, Belgium, [2]Epicore BioNetworks Inc., Eastampton, NJ, USA, [3]Zeigler Bros Inc., Garners, PA, USA.

Rearing days	Weight (mg)	Feeding rate (%)	Commercial feed (%)			
			MeM[1]	Epibal[2]	PL Raceway 40-9[3]	PL Raceway 40-9[3]
			200-300	300	400-600	600-850
1	0	35	100	-	-	-
2	0	34	100	-	-	-
3	0	33	100	-	-	-
4	0.01	32	100	-	-	-
5	0.01	31	10	90	-	-
6	0.01	30	40	60	-	-
7	0.01	29	30	50	20	-
8	0.01	28	-	60	40	-
9	0.01	27	-	60	40	-
10	0.02	26	-	30	40	30
11	0.03	25	-	30	40	30
12	0.04	24	-	30	40	30
13	0.04	23	-	30	40	30
14	0.05	22	-	-	50	50
15	0.06	21	-	-	50	50
16	0.08	20	-	-	50	50
17	0.10	19	-	-	50	50
18	0.11	18	-	-	50	50
19	0.13	17	-	-	50	50
20	0.13	16	-	-	50	50
21	0.14	15	-	-	50	50
22	0.17	14	-	-	50	50
23	0.14	13	-	-	-	100

Water quality

The results of the physical and chemical parameters evaluated throughout the experimental period are shown in Table 4. No significant variations in pH ($P > 0.05$) were observed between treatments. Oxygen levels in FSB0% were higher ($P < 0.05$) and remained above 7 mg L^{-1}. Meanwhile, the temperature remained above 30ºC in all treatments, being higher in FSBJ100% ($P < 0.05$) at both measurement times. Ammonia levels remained above 1 mg L^{-1} in all treatments; however, the lowest ammonia concentration ($P < 0.05$) occurred with a higher level of feed replacement by FSB (FSB100%). Alkalinity levels remained above 150 mg L^{-1} of $CaCO_3$ in all treatments, with the highest levels in FSB100% ($P < 0.05$). This same behavior was also observed for nitrite in FSB100% ($P < 0.05$).

Zooplankton density

The results of the average density of zooplankton groups are shown in Figure 3. The different concentrations of FSB influenced both copepod density (Fig. 3a) and total zooplankton (Fig. 3c), with FSB25% leading to a significant concentration of these organisms ($P < 0.05$). In contrast, rotifer density (Fig. 3b) was not significantly affected by the treatments ($P > 0.05$).

***Vibrio* spp. monitoring**

Results for standard plate counts of *Vibrio* spp. in the culture water of *P. vannamei* postlarvae are shown (Fig. 4). The treatments influenced the SPC, with FSB50% presenting the highest concentration of *Vibrio* (2,265 CFU mL^{-1}).

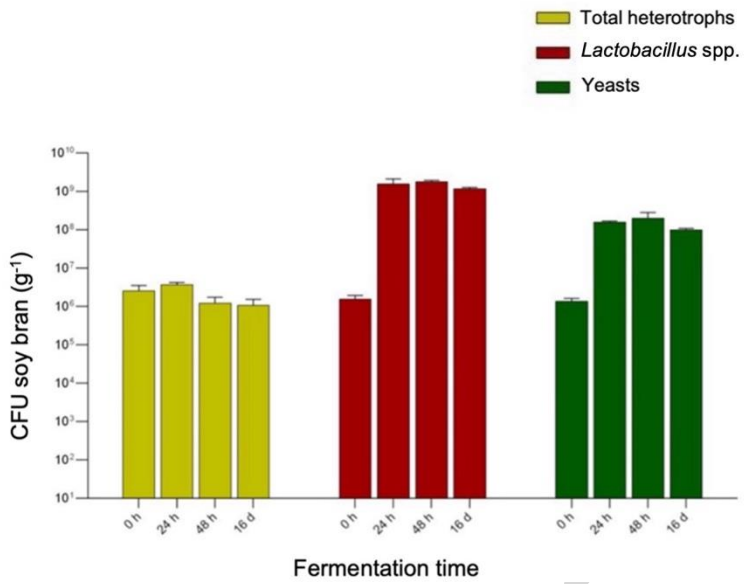

Figure 1. Growth of microbial communities of the probiotic BM-PRO™ during the soybean meal fermentation process. CFU: colonies forming units; h: hours; d: days.

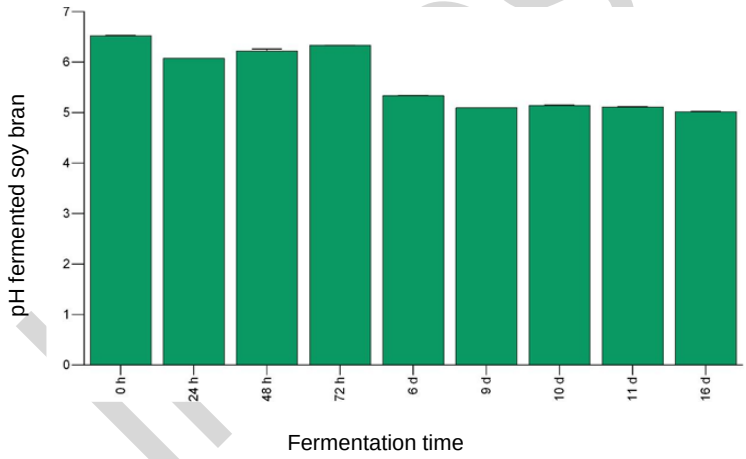

Figure 2. Evaluation of pH values during fermentation of soybean meal with BM-PRO™ probiotic.

Growth performance

Overall, the varying concentrations of FSB replacing commercial feed influenced FW, DWG, and productivity ($P < 0.05$), but they did not affect survival, SGR, and FCR ($P > 0.05$) as shown in Table 5. The PL fed with up to 50% replacement of commercial feed with FSB showed superior performance compared to the PL fed with 100% replacement ($P < 0.05$).

DISCUSSION

The exponential growth of *Lactobacillus* spp. is accompanied by the production of organic acids, mainly lactic acid, which gradually lowers the pH of the soybean meal from an initial value of 6.5 to 5.0. Under specific fermentation conditions and depending on the scale, the pH can decrease further to as low as 4.5 (Santos et al. 2019). In our study, the high morphological similarity of microorganism colonies contained in commercial probiotics (*Bacillus licheniformis*, *B. pumilus*, *B. subtilius*, *Lactobacillus acidophilus*, *L. planctarum*, *Lactobacillus* spp., and *Saccharomyces cervisiae*), during the soybean fermentation process, associated with significant microbial growth, demonstrated the predominance of probiotic microorganisms.

Table 4. Physical and chemical parameters of the water used to cultivate *Penaeus vannamei* postlarvae fed at different levels of replacement of commercial feed with fermented soybean meal (FSB) during the intensive nursery phase under commercial conditions. DO: dissolved oxygen, T: temperature, NO_2: nitrite, TAN: total ammonia nitrogen. Values for triplicate groups are presented as mean ± standard deviation; different superscript letters between lines indicate significant differences (Tukey test; $P < 0.05$).

Treatment	pH	DO (mg L^{-1})	T (ºC)	NO_2 (mg L^{-1})	TAN (mg L^{-1})	Alcalinity (mg L^{-1} de $CaCO_3$)
FSB 0%	7.87 ± 0.18	7.87 ± 0.18^{a}	31.03 ± 0.39^{abc}	0.01 ± 0.01^{a}	2.63 ± 1.09^{b}	160.62 ± 20.96^{a}
FSB 25%	7.93 ± 0.14	4.82 ± 0.24^{b}	31.14 ± 0.44^{ac}	0.00 ± 0.00^{a}	4.15 ± 1.42^{c}	150.11 ± 25.01^{a}
FSB 50%	7.82 ± 0.20	5.28 ± 0.36^{c}	30.72 ± 0.57^{b}	0.08 ± 0.13^{b}	3.37 ± 1.24^{bc}	161.77 ± 22.55^{a}
FSB 75%	7.87 ± 0.24	5.44 ± 0.36^{c}	30.87 ± 0.36^{ab}	0.05 ± 0.03^{ab}	3.49 ± 1.27^{bc}	162.4 ± 34.14^{a}
FSB 100%	7.98 ± 0.22	6.35 ± 0.45^{d}	31.40 ± 0.60^{c}	0.10 ± 0.11^{b}	1.37 ± 1.21^{a}	187.83 ± 38.63^{b}

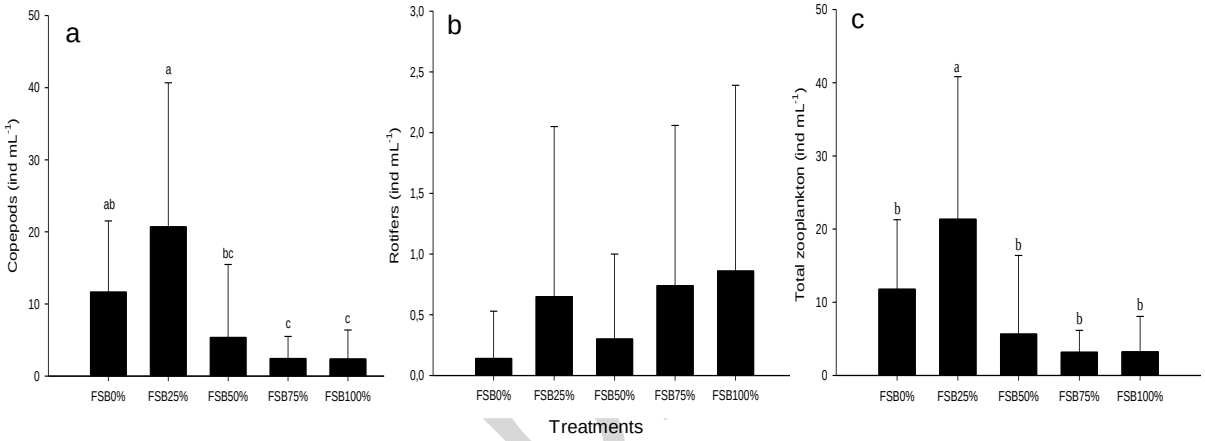

Figure 3. Density of microorganisms in the culture water of *Penaeus vannamei* postlarvae in intensive nurseries on a commercial scale: a) total zooplankton, b) copepods, and c) rotifers. The presence of different superscript indices between lines indicates a statistically significant difference between treatments ($P < 0.05$).

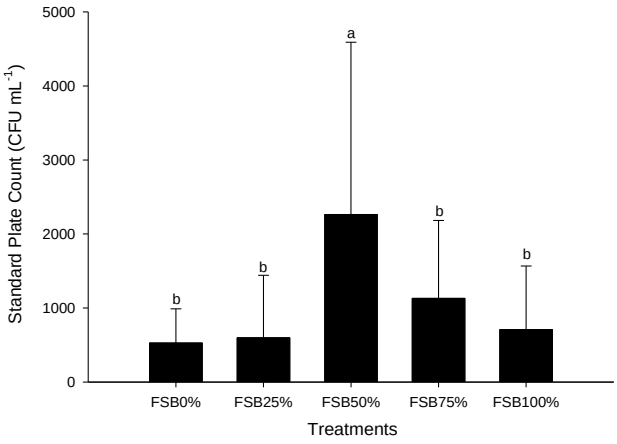

Figure 4. Standard Plate Count for *Vibrio* spp. in the culture water of *Penaeus vannamei* postlarvae in a nursery on a commercial scale. CFU: colonies forming units.

Heat was generated during the fermentation of plant materials (an exothermic reaction), and enzymes responsible for material hydrolysis were produced (Taherzadeh & Karimi 2007). Hydrolysates exhibit superior digestibility, making nutrients more readily assimilable for aquatic organisms (De Schrijver & Ollevier 2023). Plant material hydrolyzed through fermentation optimizes the cycling process in the environment by being more easily available to environmental microbial cascades, resulting in a reduced impact on the physical-chemical quality of both water and soil in production systems (Jiang et al. 2024). High stocking densities are adopted during this phase, necessitating high feeding rates, which can reach 35% of the biomass at the beginning of the rearing phase. Furthermore, due to the nutritional requirements of shrimp during the postlarval stage, protein-rich feeds are often used, with up to 50% crude protein (Braga et

Table 5. Growth parameters of *Penaeus vannamei* postlarvae fed with different levels of replacement of commercial feed with fermented soybean meal (FSB). SR: survival rate, IW: initial weight, FW: final weight, DWG: daily weight gain, SGR: specific growth rate, FCR: food conversion rate. The values of the groups in triplicate are presented as mean ± standard deviation; different superscript letters between lines indicate significant differences (Tukey test, $P < 0.05$).

Treatment	SR (%)	IW(g)	FW(g)	Yield (kg m^{-3})	DWG (g d^{-1})	SGR (%)	FCR
FSB0%	79.62 ± 10.46	0.003 ± 0.000	0.16 ± 0.02^{a}	1.13 ± 0.30^{a}	0.007 ± 0.001^{a}	17.29 ± 0.76	0.71 ± 0.27
FSB25%	82.18 ± 8.58	0.002 ± 0.001	0.12 ± 0.02^{ab}	0.72 ± 0.16^{ab}	0.004 ± 0.001^{ab}	16.32 ± 1.08	0.91 ± 0.22
FSB50%	81.28 ± 27.30	0.003 ± 0.002	0.12 ± 0.03^{ab}	0.84 ± 0.16^{ab}	0.005 ± 0.001^{ab}	16.07 ± 1.34	1.36 ± 1.06
FSB75%	81.16 ± 5.32	0.002 ± 0.000	0.09 ± 0.03^{bc}	0.66 ± 0.32^{ab}	0.004 ± 0.001^{bc}	15.65 ± 1.43	1.48 ± 0.17
FSB100%	78.94 ± 2.27	0.002 ± 0.000	0.05 ± 0.02^{c}	0.39 ± 0.21^{b}	0.002 ± 0.001^{c}	13.60 ± 2.15	1.56 ± 0.57

al. 2023), resulting in high levels of nitrogen, which is generally converted into ammonia and nitrite, which harms animal development and can become an environmental problem. (Correia et al. 2014, Schveitzer et al. 2017, Panigrahi et al. 2020). In our study, the water quality parameters remained within the ideal range for the species (Han et al. 2018, Maicá et al. 2018, Valencia-Castañeda et al. 2018, Duan et al. 2019, Ulaje et al. 2020), corroborating the findings of Albuquerque (2019), who did not observe significant differences in water quality when using FRB with BM-PRO® for water quality control and fertilization management of soil ponds during the grow out phase of *P. vannamei* PL fed exclusively on diets composed of fermented vegetable bran. Leite et al. (2020) describes similar positive effects on water quality when using rice byproducts, such as fermented bran. This result is linked to the use of fermented vegetable bran and its positive impact on environmental balance. However, in our study, total ammonia concentrations remained above the ideal range (Cobo et al. 2012, Kathyayani et al. 2019). Despite using FRB with the commercial probiotic BM-PROTM, combined with direct application of the same product and regular partial water changes, it was not possible to eliminate ammonia from the system.

In intensive systems, ammonia is produced through animal excretion and the mineralization of feces and uneaten food (Lin & Chen 2001). In high concentrations, ammonia can impair shrimp growth, alter the frequency of ecdysis, and affect the physiological state of the gills and hepatopancreas, as well as the osmoregulatory capacity (Wu et al. 2023). However, our study did not demonstrate a harmful effect on post-larvae, as the FSB25% and FSB50% treatments yielded the best growth responses, despite ammonia concentrations exceeding 2 mg L^{-1} throughout the trial, compared to the FSB100% treatment. These results could likely be enhanced if ammonia levels throughout the trial were kept below 1 mg L^{-1}. The addition of FRB is a strategy to aid in controlling nitrogen (Emerenciano et al. 2013, Martins et al. 2020). These findings support those of Maicá (2015), who observed no negative effects on the SGR, food consumption, and weight gain of *P. vannamei* at ammonia concentrations up to 4 mg L^{-1}. The maximum safe concentration for rearing *P. vannamei* has been established at 3.95 mg L^{-1} of total ammonia (Lin & Chen 2001), which accounts for the lack of negative effects on shrimp growth at the concentrations observed in our study. Leite et al. 2020, when evaluating the effect of fertilizers formulated with different compositions of bran and other fermented vegetable byproducts on the rearing parameters of *P. vannamei* juveniles, reported average total ammonia nitrogen levels of 0.06 ± 0.03 mg L^{-1}. The same author, while experimenting with different proportions of commercial feed replaced by fermented vegetable bran in the diet of Pacific white shrimp juveniles, reported average total ammonia levels ranging from 0.03 ± 0.02 at the start of the experiment to 0.14 ± 0.07 mg L^{-1} at its conclusion. Sharawy et al. (2016) observed ammonia concentrations between 0.08 and 0.12 ± 0.26 mg L^{-1} in their study on the partial and complete replacement of fishmeal with FSB by *Saccharomyces cerevisiae* in diets for PL of the penaeid shrimp *Fenneropenaeus indicus*.

The absence of feed led to a lower average concentration of total ammonia in FSB100%. However, the postlarva fed 100% FSB had their growth limited by the absence of nutritionally complete food, demonstrating that a feeding program based solely on

FSB supply is insufficient to sustain shrimp growth at this stage. However, fermentation, as a method to enhance the nutritional value of vegetable meals, has been widely described in the scientific literature (Siddik et al. 2024). This process is capable of bringing improvements in the centesimal composition of these vegetable inputs, improving both the nutrients proportion and bioavailability, in addition to treating antinutritional factors such as trypsin inhibitors, phytate saponin, tannin and glycine (Wang et al. 2016, Jannathulla et al. 2018, Romano et al. 2018, Albuquerque 2019). The fermentative action can increase the protein and concentrations of both essential and non-essential amino acids in vegetable brans (Hassaan et al. 2015, Sharawy et al. 2016, Razak et al. 2017, Ribeiro 2018). The fermentation process of vegetable brands, such as soybeans, wheat, and rice, with the probiotic BM-PRO™ improved both protein, lipid, and total carbohydrate levels in the plant material used in *P. vannamei* growout (Ribeiro 2018, Albuquerque 2019). The increase in protein content was also observed in the fermentation of rice bran used as fertilizer during the breeding of *Clarias gariepinus*, resulting in subsequent benefits to the centesimal muscle composition, including increased protein and lipid levels (Romano et al. 2018). Studies have demonstrated the various health benefits of feeding aquatic animals diets formulated based on vegetable bran fermented with probiotic bacteria (Ray et al. 2010, Jamali et al. 2015). Reports indicate the positive influence of fermented vegetable bran on the intestinal bacterial community, highlighting an increase in lactic acid bacteria, which play a crucial role in regulating the intestine and inhibiting pathogens in the gastrointestinal tract of aquatic organisms (Harzallah & Belhadj 2013, Catalán et al. 2018). Soybean bran fermented by the bacterium *Bacillus subtilis* E20 was capable of producing antimicrobial peptides with the ability to combat pathogenic bacteria, such as species from the genus *Vibrio* (Cheng et al. 2017, Cheng & Chen 2020). The use of plant bran in water fertilization can benefit aquatic organisms by maintaining water quality and promoting the growth of zooplankton, which serve as a food source, similar to the aquamimicry technique (Khanjani et al. 2022). Fermented bran can potentially serve as a direct food source for zooplankton, inducing blooms of live food in nurseries and rearing tanks (Vilani et al. 2016, Dauda et al. 2017, Kumar et al. 2017, Romano et al. 2018).

The densities of total zooplankton and copepods decreased with the increased inclusion of FSB to the detriment of commercial feed, demonstrating a possible compensation mechanism. High rates of commercial feed replacement with fermented grain-based pellets lead to greater consumption of natural food, which is due to the inability of fermented bran to meet all the nutritional requirements of farmed shrimp (Sharawy et al. 2016, Leite et al. 2020). In addition to food management, stocking density can have an effect on the zooplankton abundance during the nursery phase of *P. vannamei*. There is a tendency for zooplankton abundance to decrease as higher postlarvae stocking densities are adopted (Santos 2018). The presence of natural food (zooplankton) reduces the demand for artificial feeding, thereby decreasing production costs and promoting animal health. Rotifers have interesting nutritional characteristics; their dry biomass can contain 25 to 63% protein and 6 to 36% lipids, and these proteins have high digestibility, ranging from 84 to 95% (Watanabe & Kiron 1994, Maia et al. 2003, Demir & Dicken 2011, Jeeja et al. 2011).

Furthermore, rotifers are tolerant to a wide range of salinity levels, have a low mortality rate and rapid reproduction, which makes them an adequate complementary food source for marine shrimp (Lubzens 1987, 2001, Demir & Diken 2011, Jeeja et al. 2011, Rahman et al. 2018, Das et al. 2021). Copepods, in turn, are rich in protein, fatty acids, antioxidant pigments, vitamins, and LC-PUFA, including eicosapentaenoic, docosahexaenoic, and arachidonic acids, which are essential for growth and development (Drillet et al. 2006, Khanjani et al. 2022). It is common for rotifers, nematodes, and copepods to be the predominant taxa in zooplankton communities found in aquaculture (Marinho et al. 2014, Santos 2018, Santos et al. 2020). In our study, copepods predominated, especially in the FSB0% and FSB25% treatments, indicating that the gradual increase in the replacement of artificial food with FSB favored the selective consumption of natural food. Copepods are nutritionally richer than rotifers, which could explain the negative pressure exerted on FSB75% and FSB100%, probably to compensate for the absence of more complete nutritional sources, such as commercial feed. In general, *P. vannamei* PL showed greater weight gain when rotifers, copepods, and *Artemia* nauplii were added to the culture during the nursery (Andrade et al. 2021, Abbaszadeh et al. 2022). Treatments with lower FSB replacement showed higher densities of copepods and rotifers. The use of organic carbon sources such as FRB as a fertilization strategy in *P. vannamei* culture is capable of modulating the zooplankton community. Although it stimulates the development of phyla such as Rotifera and Copepoda, protozoa are its primary

beneficiaries, becoming the most abundant group (Leite et al. 2020, Xavier et al. 2022, Pimentel et al. 2023, 2025b).

Additionally, plant-based meals have a lower capacity to meet the nutritional requirements of farmed shrimp (Sharawy et al. 2016, Leite et al. 2022). As a result, the animals rely more on natural food as a nutritional supplement, highlighting the importance of a healthy planktonic community to promote better development of the cultured animals under conditions of feed replacement or restriction of balanced feed use (Van et al. 2017, Chakravarty et al. 2018, Andrade et al. 2021, Khanjani et al. 2023, Gonçalves-Júnior et al. 2025). Our findings show that the development of the zooplankton community, following fertilization with rice bran, supports the growth of postlarvae while allowing for the replacement of up to 50% of the feed with FSB. Our results demonstrate that it is possible to replace commercial feed with FSB by up to 50% during the rearing of *P. vannamei* PL.

Credit the author's contribution

I.G.R.F. Gomes: conceptualization, methodology, formal analysis, investigation, and writing – original draft; L.K. Oliveira: formal analysis, review, and editing; J.R.O. César: review and editing; E.L.C. Ballester: formal analysis, review and editing; F.H.F. Costa: funding acquisition, project administration, supervision, review, and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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