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The Black Soldier Fly (*Hermetia illucens*) Larvae Meal Can Fully Replace Fish Meal in Practical Nursery Diets for Post-Larval *Penaeus vannamei* under High-Density Culture

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Simple Summary: In recent years there has been an increased interest in the use of insect meals in aquatic feeds mainly driven by supply and price volatility of conventional protein sources, as well as the need to reduce carbon emissions and promote a circular economy. This study examines how replacing fish meal (FML) with black soldier fly larvae meal (BSFLM) in nursery diets affects the growth and economic performance of post-larval (PL) *Penaeus vannamei*. A control diet was formulated to contain 16.50% FML without BSFLM. Four other diets replaced FML at 25, 50, 75 and 100%. PLs of 2.7 ± 0.2 mg body weight (BW) were stocked in fifty 1.5-m^3 tanks of 1,369 PLs/m^3 and reared for 42 days. Final shrimp survival, daily weight gain, and apparent feed intake were not affected by the replacement of FML. The best results in terms of gained yield and final shrimp BW were achieved when FML was replaced with BSFLM at 50 and 75%. Shrimp fed diets with 0% and 100% FML replacement had higher feed conversion ratios. The study demonstrated that FML can be completely replaced with BSFLM in nursery diets for *P. vannamei*. There was a favorable return on investment (ROI) up to maximum of 75% FML replacement if BSFLM price did not exceed USD 3.00/kg. In conclusion, BSFLM can fully replace FML in nursery diets for *P. vannamei*, but the economic viability of this replacement is dependent on the price of BSFLM and FML.

Abstract: The black soldier larvae meal (BSFLM) has been the most extensively studied insect protein source in shrimp nutrition. However, both the availability and prices of BSFLM are still a constraint for its widespread use as an ingredient in animal feeds. The present study investigated the growth and economic performance of post-larval (PL) *P. vannamei* fed nursery diets with a progressive replacement of fish meal (FML) for BSFLM at 0, 25, 50, 75 and 100%. These replacements corresponded to a dietary inclusion (% of the diet, as-is) of FML and BSFLM of 16.50 and 6.33%, 11.00 and 13.04%, 5.50 and 19.74%, and 0 and 26.46%, respectively. A total of 102,647 shrimp at the age of PL15 with 2.7 ± 0.2 mg body weight (BW) were stocked in fifty 1.5-m^3 tanks under 1,369 PLs/m^3 (2,053 ± 33 PLs/tank) and reared for 42 days. Final shrimp survival (90.5 ± 7.6%), daily weight gain (14.7 ± 1.1 mg/day), and apparent feed intake (0.67 ± 0.03 g of feed per stocked shrimp) were unaffected by dietary treatment. The highest gained yield (791 ± 52 and 776 ± 38 g/m^3) and final BW (621 ± 7.2 and 632 ± 7.2 mg) were attained when FML was replaced for BSFLM at 50 and 75% with the lowest at 0% (726 ± 34 g/m^3 and 598 ± 8.1 mg, respectively). Shrimp fed diets with 0 and 100% replacement of FML exhibited the highest feed conversion ratio (1.25 ± 0.04 and 1.24 ± 0.08) compared to those fed a diet with 50% (1.16 ± 0.06). At a price of USD 2.00/kg, BSFLM demonstrated a favorable ROI (Return of Investment) when compared to FML, irrespective of the replacement level. With 25 and 50% replacement, BSFLM remained cost-competitive up to 3.50 USD/kg. At 75% FML replacement, there were no significant differences in ROI with a price range of 2.00 up to 3.04 USD/kg. At full replacement, ROI dropped significantly at a BSFLM price of 2.50 USD/kg and beyond. It can be concluded that FML can be fully replaced for BSFLM in well-balanced nursery diets for *P. vannamei*. While the full replacement of FML for BSFLM was successfully accomplished, the competitive ROI was sustained only when the price of BSFLM did not exceed 3.04 USD/kg at its dietary highest inclusion of 19.74%. Further research may be necessary to fine-tune cost-effective inclusion levels of BSFLM to optimize the economic outcomes while considering the fluctuating prices of FML.

Keywords: shrimp; nursery diets; black soldier fly; fish meal; replacement

1. Introduction

The use of insect meals in aquatic feeds has gained increased attention in recent years driven by a greater volatility in supply and prices from conventional protein sources (Nunes et al., 2022; Sánchez-Muros et al., 2020) alongside the pressing need to reduce carbon emissions and promote a circular economy (Siva Raman et al., 2022). In comparison to protein sources obtained from fisheries, agriculture, and animal husbandry, the utilization of insects as feed ingredients can comprehensively integrate the advantages linked with sustainability, nutritional value, and functionality (Khalifah et al., 2023; Siva Raman et al., 2022). Insects also have a short life cycle and exhibit high efficiency as food converters demanding fewer resources during their production.

Numerous insects have been examined as potential feed ingredients for aquaculture species (Alfiko et al., 2022). Their nutrient value lies in their adequate levels of digestible protein, amino acids (AAs), lipid, and energy (Alfiko et al., 2022; Khalifah et al., 2023; Siva Raman et al., 2022). A study by Shin & Lee (2021) with juvenile whiteleg shrimp, *Penaeus vannamei*, has shown that the dynastid beetle (*Allomyrina dichotoma*), rice grasshopper (*Oxya chinensis*), black soldier fly (*Hermetia illucens*), whitespotted flower chafer (*Protaetia brevitarsis*), mealworm (*Tenebrio molitor*), two-spotted cricket (*Gryllus bimaculatus*), and silkworm (*Bombyx mori*) contain crude protein (CP) and lipid content varying from 41.7-73.8% and 6.19-30.8%, respectively. Authors have reported high apparent digestibility coefficients for protein (83-89%), lipid (91-98%), energy (84-90%), dry matter (77-81), AAs (76-96%), and fatty acids (89-93%). In shrimp feeds, the black soldier larvae meal (BSFLM) has been the most extensively studied insect protein source. Its reported benefits have included partial replacement of fish meal (Chen et al., 2021; Cummins et al., 2017; Keetanon et al., 2023; Richardson et al., 2021; Wang et al., 2021), improved intestinal health (Chen et al., 2021; He, Zhang, et al., 2022), growth and feed efficiency (Richardson et al., 2021), higher resistance to *Vibrio* sp. infection (Chen et al., 2021; Keetanon et al., 2023; Richardson et al., 2021), and increased levels of digestive protease activity (He, Liu, et al., 2022). However, both the availability and prices of BSFLM are still a constraint for its widespread use as an ingredient in animal feeds (Siva Raman et al., 2022) at times considered uncompetitive (Yildirim-Aksoy et al., 2022). Hence, its current utilization is expected to become more prevalent in specialty diets through the substitution of costly protein ingredients, such as fish meal (FML). The present study aimed at investigating the growth performance of post-larval *P. vannamei* fed nursery diets with a progressive replacement of FML for BSFLM. Additionally, a sensitive analysis over the price of BSFLM was carried out to determine the optimal level of FML replacement.

2. Materials and Methods

2.1. Experimental Design

The study consisted of a 42-day nursery culture carried out to evaluate shrimp growth performance throughout post-larval (PL) and early juvenile stages. Shrimp were fed on diets containing graded levels of a partially-defatted black soldier fly larvae meal (BSFLM). For the experimental phase, shrimp were raised from PL15 to over 600 mg body weight (BW) in 50 outdoor tanks of 1.50 m^3 (1.70 m^2 bottom area x 0.88 m height) under 1,369 PLs/m^3 (2,053 ± 33 PLs/tank). Shrimp were fed five nursery diets with graded levels of BSFLM in replacement for FML at 0, 25, 50, 75 and 100%. One diet without BSFLM containing 22.00% FML (as-is basis) acted as the control. This

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initial set-up allowed 10 replicate tanks per feeding treatment. At harvest, shrimp survival, feed efficiency, and growth performance from each tank were determined.

2.2. Rearing System and Water Preparation

The rearing system adopted in this study consisted of 50 independent outdoor tanks of 1.5 m^3 each equipped with its own water inlet and outlet, and aeration system. Outdoor tanks were round, blue in color, and made from polypropylene. Tanks were sheltered under a roof, but subjected to a natural light cycle (12 h light starting at 05:45 am). The system operated under a minimum water exchange condition, without any water interexchange between rearing tanks over the complete rearing cycle. Weekly water exchange was carried out using sand-filtered seawater mixed with groundwater. Continuous aeration was provided by an air diffusing system made with a 0.5-m aeration tubing (Aero-Tube™, Tekni-Plex Aeration, Austin, Texas, USA) rested near the bottom of each tank, but opposed to the feed delivery point. A 150-kvA (Kilo Volt Amperes or 120 kW) diesel generator was used as a back-up power supply in case of power failure.

Initially, rearing tanks, and aeration lines, aeration tubes, and feeding trays were cleaned and disinfected prior to water preparation. The internal tank walls underwent cleaning using a high-pressure jet, and the water remains were manually extracted. Subsequently, a descaling agent based on sulfuric acid (dilution at 1 L per 50 L of water) was sprayed on tank walls and bottom, water supply and drainage lines and allowed to rest for 24 h. This was followed by the application of a peracetic acid-based disinfectant (dilution of 1 L per 500 L of water). Finally, tanks were allowed to dry and then filled with sand-filtered brackish water at 15 g/L salinity. This salinity was achieved by mixing groundwater (5 g/L salinity) with seawater (30 g/L salinity). All seawater was previously disinfected with 30 ppm of active sodium hypochlorite.

Water fertilization was carried out using a commercial probiotic (BM-Pro, Biotrends Soluções Tecnológicas Ltda., Eusébio, Brazil) composed of a consortium of microorganisms (*Bacillus* spp., *Lactobacillus* spp. and *Saccharomyces cerevisiae*). A mixture containing 20 g of the probiotic, 3 L of sugar-cane molasses, 5 kg of wheat bran and 1 L of tap water were allowed to ferment in a bucket with aeration over a 24-h period. The mixture was sieved to remove solids and applied to each tank at 50 g/m^3 once daily over a seven-day period. During water preparation, strong aeration was applied in rearing tanks for water mixing.

2.3. BSFLM, Fish Meal, and Diet Formulation, Manufacturing and Chemical Analysis

A commercial partially-defatted BSFLM (*Hermetia illucens*) was obtained from BSF Nutrição e Biotecnologia S.A. – CYS (Piracicaba, Brazil). Fish meal (FML) was produced from the byproducts obtained during processing of farmed Atlantic salmon (Pesquera Pacific-Star, Puerto Montt, Chile). Crude protein (CP) and total lipid content in these meals reached 57.77 and 67.08% and 7.58 and 10.88%, respectively (Table 1). The total amino acid (AA) composition of BSFLM was lower compared to FML. BSFLM also carried lower levels of essential AAs than FML, including methionine (Met, 0.92 versus 1.69%, as-is basis), lysine (Lys, 3.77 vs. 4.47%), and Met plus cysteine (M+C, 1.38 vs. 2.35%, respectively).

Five diets were designed to progressively reduce the inclusion of FML by BSFLM (Table 2). First, a control diet (BSF0%) without any BSFLM was designed to contain 22.00% FML. From this diet, four other diets were formulated to replace FML for BSFLM at 25, 50, 75, and 100%. These replacements corresponded to a dietary inclusion of FML and BSFLM of 16.50 and 6.33% (BSF25%), 11.00 and 13.04% (BSF50%), 5.50 and 19.74% (BSF75%), and 0 and 26.46% (BSF100%), respectively. Soybean meal and wheat gluten meal were used as plant protein sources. Their dietary levels were fixed at 35.00 and 4.00%, respectively. Krill meal and squid meal were included at 2.00% each across all diets to act as feed attractants and stimulants.

Diets were manufactured in an experimental feed mill facility using a laboratory pelleting machine as described by Nunes et al. (2021). First, all dried raw materials were ground to less than 300 microns (mesh #48). Next, ingredients were weighed to a 0.01-g precision in an electronic scale following formula specifications. All micro ingredients (vitamins, minerals, synthetic binder, crystalline AAs) were mixed with a 1-kg sample of all dried macro ingredients in a Y-mixer for 10 min. at 30 RPM. This mix was then combined with all other macro ingredients (dry and liquids) and mixed for 10-min. in a planetary mixer with freshwater until a feed dough was formed. The feed dough was then pressed through a plastic net to obtain small chunks of moist feed for extrusion. For feed cooking and extrusion, a pellet mill was used and adjusted to operate at a maximum temperature of 95°C. The die and knife of the pelleting machine were first adjusted to produce pellets of 2.4 mm in diameter by 5 mm in length. To obtain pellets with a consistent moisture content, feed was dried at 60°C using a convection oven for a maximum period of 3 h. After an initial 30-min. drying, batches of 5 kg of feed were transferred to a pot for steam-cooking during 10 min. under 95°C. Post-cooked pellets were then subjected to final drying in the convection oven until a moisture content between 8 and 10% was reached. Moisture content of pellets was kept as consistent as possible by taking feed samples at 15-min. intervals during drying. Samples were analyzed with a halogen rapid moisture analyzer. After manufacturing, pellets were ground to obtain crumbled particles. These were separated using a mechanical shaker (MA750, Marconi Equipamentos para Laboratórios Ltda., Piracicaba, Brazil) equipped with sieves of 1,000, 850, 600, 425, 300, and 250 microns. For the study, two particle size ranges were used: an equivalent mix of particles of 300 and 425 microns, and an equivalent mix of particles of 600 and 850 microns.

Finished diets were chemically analyzed ("Official Methods of Analysis of AOAC INTERNATIONAL," 2023). Dry matter (DM) was determined by drying samples in a convection oven for 24 h at 105°C. The Dumas combustion method was applied to analyze CP (AOAC 968.06), while total lipids was determined through acid hydrolysis (AOAC 954.02). Ash content was determined by burning samples in a muffle furnace at 600°C for 2 h (AOAC 942.05) and crude fiber by enzymatic-gravimetric determination (AOAC 992.16). Amino acid (AA) and fatty acid (FA) compositions were determined using high-performance liquid chromatography (Hagen et al., 1993; Road, 1986) and high-resolution gas chromatography (GC) with a flame ionization detection fitted with a capillary GC column, respectively.

Diets reached a mean (± standard deviation, sd) CP and total lipid content of 39.66 ± 0.72 and 8.36 ± 0.30% (% of the diet, as-is), respectively. As the dietary inclusion of BSFLM increased, diets were supplemented with DL-Methionine, L-Lysine, and L-Threonine to reach a corresponding total dietary Met (M+C), Lys, and threonine (Thr) content of 0.92 ± 0.07 (1.45 ± 0.08%, as-is basis), 2.33 ± 0.09, and 1.64 ± 0.10%, respectively (Table 3). The dietary fatty acid profile changed as FML was replaced by BSFLM (Table 4) despite increased inclusion levels of salmon oil. The higher the dietary inclusion of BSFLM, the lower were the levels of omega-3 (n-3), polyunsaturated (PUFA) and highly unsaturated fatty acids (HUFA). However, the levels of eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids were kept within a range of 0.16-0.21 and 0.19-0.31% (% of the diet, as-is), respectively. There was an increasing trend in the dietary levels of saturated fatty acids (SFA) with higher inclusions of BSFLM, from 2.00 (BSF0%) up to 2.62% (BSF100%).

2.4. Shrimp Post-Larvae

The shrimp species used in this trial was the Pacific whiteleg shrimp, *P. vannamei*, purchased as post-larvae (PL) from a commercial hatchery (Aquatec Aquacultura Ltda., Canguaretama, Brazil) distant 469 km from the lab. A total of 132,000 PLs at the age of PL10 (217 PLs per gram) were transported to the lab in twelve 15-L plastic bags (733 PLs/L) individually stored in cardboard boxes lined with Styrofoam. Plastic bags contained seawater at 30 g/L salinity and 25°C temperature, saturated with pure dissolved oxygen.

At arrival, shrimp were acclimated to temperature, pH, and salinity and stocked in six nursery tanks of 3,000 L each. A shrimp sample containing approximately 1,000 animals was collected for RT-PCR (Real Time Polymerase Chain Reaction) to screen for the following viruses, WSS (White Spot Syndrome), Infectious Hypodermal and Hematopoietic Necrosis (IHHN), and Infectious Myonecrosis (IMN). A five-day quarantine period was allowed until diagnostic results were available which indicated that shrimp was free from all selected viruses. For stocking in the rearing tanks, shrimp were counted using a portable smart device for rapid inventory assessment (XperCount2,

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XpertSea, Québec, Canada). PLs were captured with a bag net from the quarantine tanks and transferred to the XperCount2 bucket at total quantity near 1,000 shrimp requiring two individual counting per rearing tank. After each counting, total PL biomass was determined by first removing excess water and then weighing shrimp in bulk using an electronic 0.001-g resolution scale. Subsequently, shrimp were transferred to their respective rearing tank. At stocking, a total of 102,647 PLs at the age of PL15 weighed on average 2.7 ± 0.2 mg BW (body weight). They were stocked under 1,369 PLs/m^3 (2,053 ± 33 PLs/tank).

2.5. Feeding and Water Quality

Diets were delivered in rearing tanks daily, including Sundays. Shrimp were fed eight times per day, between 08:00 am and 04:00 pm, exclusively in circular feeding trays (placed at one unit per tank, 19 cm in diameter). Starting on the 12th day of rearing and then on a weekly basis (days 19, 26, 34), shrimp were sampled and weighed using a 0.001-g precision scale. During weighing, shrimp were first collected, blotted dry with absorbent paper, counted individually and then weighed in bulk. Five subsamples of 10 shrimp each per tank were weighed to determine their mean BW which were then returned to their respective tank. Until the next weight check, ration increased assuming this mean daily weight gain for each tank, maintaining a consistent daily drop in survival. Meals were adjusted daily for each rearing tank assuming an estimated daily drop in shrimp survival and an increase in weight gain across all diets (Table 5, Nunes et al., 2021). Dietary particle size changed according to shrimp BW and day of nursery. The particle size range between 300 and 425 microns was delivered from the day of stocking to the 23rd day of nursery (total of 37.7% of all feed delivered); and, particle range between 600 and 850 microns was delivered between the 20th day of nursery until shrimp harvest (total of 62.3% of all feed). A three-day transition was allowed when introducing the second particle size range.

Water quality parameters (*i.e.*, pH, temperature, and salinity) were measured once daily starting at 09:00 am in all rearing tanks. Average pH reached 7.9 ± 0.2 (n = 1,600) with minimum and maximum values ranging from 7.1 and 8.5, respectively. Salinity was kept consistent at 19.2 ± 1.1 g/L (n = 1,600). Temperature was high, with an average of 27.7 ± 0.7°C (n = 1,600). Dissolved oxygen was kept saturated over the complete rearing period. Water alkalinity was adjusted above 160 mg/L of $CaCO_3$ through applications of sodium bicarbonate. Water was exchanged from the tank bottom on a weekly basis starting in the end of the first week after shrimp stocking. A total of 8% of total water volume was drained and replaced by filtered and chemically disinfected seawater. To control nitrogen compounds, a 24-h fermented mix of a commercial probiotic (20 g), sugar-cane molasses (40 mL), and tap water was applied in each tank daily at 10 mL/m^3.

2.6. Shrimp Growth Performance

Shrimp harvest took place after 42 days of rearing. Initially, water from each tank was drained slowly while a batch with 100 shrimp per rearing tank was captured and weighed individually to a 0.001-g precision. In this case, shrimp were blotted dry for individual weighing. Finally, the tank was completely drained and the remainder shrimp captured and weighed in bulk in an electronic scale. Final shrimp survival (%) from each tank was calculated by dividing the total shrimp biomass from each tank by the shrimp mean BW obtained from weighing 100 shrimp individually from the respective tank. The daily weight gain (DWG, mg/day) was determined by the formula: DWG = [(BWf - BWi)/t] × 7, where BWi = wet shrimp BW (mg) at stocking, BWf = final shrimp BW at harvest, and t = number of days in culture. The gain in shrimp yield (YIE, g of gained shrimp biomass/m^3) was determined as YIE = (BIOf - BIOi) ÷ tank volume (m^3), where BIOi = initial shrimp biomass (g) per tank, BIOf = final shrimp biomass (g) per tank, and tank volume = 1.5 m^3. FCR was calculated by dividing the total inputs of feed (g, as-is basis) delivered per tank during the entire rearing period by the total gained shrimp biomass per tank (g, as-is). The apparent feed intake (AFI, g of feed delivered divided by the number of stocked shrimp) was calculated by dividing the total amount of feed delivered (g) by the number of stocked shrimp.

2.7. Price Sensitivity Analysis

The cost of formulation of each individual diet was first calculated by using international FOB (Free on Board) market prices of each ingredient and feed additive. The price of FML (1.86 USD/kg) was based on the Peruvian steam-dried anchovy meal with 67% CP (June 10th, 2023, source: https://hammersmithltd.blogspot.com/). For simulation purposes, a baseline price of 2.00 USD/kg for the BSFLM was considered with gradual 15% increments until 3.50 USD/kg was reached. To carry the price sensitivity analysis for each nursery diet, the following assumptions were made: (1) grower feed price = USD 1.00/kg; (2) feed conversion ratio (FCR) at grow-out = 1.5; (3) final shrimp BW at grow-out = 23.5 g, and; (4) final shrimp survival at grow-out = 75%. These parameters were fixed across all diets. Subsequently, it was assumed that the initial stocked population in grow-out equaled to 1,000,000 shrimp multiplied by the final shrimp survival achieved in each tank during nursery. Therefore, final shrimp production in the grow-out phase was the equivalent to the population available for stocking after nursery phase multiplied by the fixed final BW and final survival in grow-out. The farm gate price for head-on shell-on (HOSO) shrimp was estimated at USD 5.00/kg.

Feed production cost (USD) was determined by multiplying the feed price (USD/kg) by the FCR and the total shrimp production in both nursery and grow-out phases. Feed accounts for 40% of the total shrimp production costs (USD/kg) in grow-out. Thus, the remainder production costs were attributed to other variables (PLs, amendments, sediment removal, electricity, fuel, labor) and fixed costs (Engle et al., 2017). The gross revenue (USD/kg) was determined by multiplying the farm gate shrimp price (USD/kg) with the gained shrimp yield (kg) from each tank. The gross profit (USD) was given as the gross revenue subtracted by the total production cost. The return on investment (ROI, %) was calculated by subtracting the gross revenue by the total production cost and then dividing the result by the total production cost multiplied by 100.

2.8. Statistical Analysis

The effect of FML replacement over water quality, shrimp performance, and ROI was analyzed through one-way ANOVA. When significant differences were detected, they were compared two-by-two with the Duncan's test. The significant level of 5% was set in all statistical analyses. The statistical package IBM® SPSS® Statistics 23.0 (SPSS Inc., Chicago, Illinois, USA) was used.

3. Results

Final shrimp survival was high and unaffected by dietary treatment ($P > 0.05$, Table 6). From a total of 50 rearing tanks used in the study, two were excluded from statistical analyses due to out-of-range survival (below 50%). The average final survival of the remaining tanks reached 90.5 ± 7.6%. Similarly, DWG, and AFI (Apparent Feed Intake), reached an average of 14.7 ± 1.1 mg/day, and 0.67 ± 0.03 g of feed per stocked shrimp, respectively. These parameters were not statistically affected by dietary treatment ($P > 0.05$).

However, the replacement of FML for BSFLM had a significant impact on both gained yield and FCR ($P < 0.05$). The highest gained yield was attained when FML was replaced for BSFLM at 50% and 75% levels (diets BSF50% and BSF75%). Conversely, the lowest gained yield was observed when FML was included at its highest dietary inclusion, *i.e.*, 22.00%, without any BSFLM (diet BSF0%). Notably, fully replacing FML for BSFLM did not adversely affect the gained yield ($P > 0.05$). There were no significant differences in gained yield between shrimp fed diets BSF0% and BSF100%. Shrimp fed these two diets exhibited the highest FCRs (1.25 ± 0.04 and 1.24 ± 0.08) compared to those fed diet BSF50% (1.16 ± 0.06). No differences in FCR were found among the remaining diets or between diet BSF100% ($P < 0.05$).

Throughout the nursery period, shrimp demonstrated a progressive growth (Fig. 1). Statistical differences in shrimp BW among dietary treatments were observed a week prior to harvest, on the 34th day of nursery ($P < 0.05$). By this stage, shrimp had surpassed 430 mg in BW. Shrimp fed diets BSF75% and BSF100% displayed the highest BWs in comparison to those fed remaining diets. At harvest, the lowest BW was recorded for shrimp fed the BSF0% diet (598 ± 8.1 mg). In contrast, shrimp fed diets containing BSFLM, regardless of the replacement level, achieved higher BWs ranging from 621 ± 7.2 mg (BSF50%) to 632 ± 7.2 mg (BSF75%).

The Return on Investment (ROI) was influenced by the various price scenarios for BSFLM and the dietary inclusion adopted. At a price of USD 2.00/kg, BSFLM demonstrated a favorable ROI when compared to FML, irrespective of the chosen replacement level and dietary inclusion. However, as the price of BSFLM increased to 2.50, 3.00, and 3.50 USD/kg, ROI progressively deteriorated with higher levels of FML replacement. With 25 and 50% replacement, BSFLM remained cost-competitive at all simulated prices. At 75% FML replacement, there was a decline in ROI with higher BSFLM prices. However, there were no statistical differences in ROI with a price range of 2.00 up to 3.04 USD/kg. At full replacement, ROI dropped significantly at a BSFLM price of 2.50 USD/kg and beyond. In general, there was a significant drop in ROI when replacement levels of 75 and 100% were adopted, corresponding to dietary inclusions of BSFLM at 19.74% (BSF75%) and 26.46% (BSF100%).

4. Discussion

Shrimp performance in the present study was consistent with other work carried out under similar rearing conditions. Nunes et al. (2021) reared post-larval *P. vannamei* of 3.6-2.5 mg BW in an outdoor and indoor tank system under 2,371-2,504 PLs/m^3, respectively. After 52-41 days of nursery, shrimp reached an average of 84.0-91.8% final survival, 1,568-1,611 g/m^3 gained yield, 14.2-14.2 mg daily growth, and 1.70-0.89 FCR, respectively. In their work, shrimp final BW varied according to the diet, from 683 to 775 mg and from 567 to 629 mg in the outdoor and indoor tanks, respectively. Our results demonstrated that it is possible to fully replace FML for BSFLM in nursery diets for post-larval *P. vannamei* with no detriment to shrimp performance. The highest dietary inclusion level obtained for BSFLM was 26.46% (% of the diet, as-is) which is equivalent to a full replacement of FML. This contrasts with other studies which have only reported partial replacement of FML for BSFLM. For example, Chen et al. (2021) evaluated the replacement of brown FML (68.21% CP and 9.00% lipid) for BSFLM at 10, 20, and 30%. This corresponded to a dietary inclusion of FML and BSFLM of 25.0 and 0%, 22.5 and 4.75%, 20.0 and 9.5%, and 17.5 and 14.25%, respectively. Diets were formulated to contain 42.30 ± 0.64% CP and 7.31 ± 0.34% lipid. Juvenile *P. vannamei* (0.88 g initial BW) were reared for seven weeks in 12 tanks of 300 L at 40 shrimp/tank. They reported a significant drop in final shrimp BW (from 7.76 ± 0.09 to 7.06 ± 0.16 g) and weight gain (776.4 ± 10.3 to 698.4 ± 19.0%) when shrimp were fed diets with 0 and 14.25% BSFLM, respectively. At 30% FML replacement, they reported intestinal cell apoptosis and degeneration. Unlike the research conducted by Chen et al. (2021), our study did not identify any adverse impact on shrimp performance that could indicate potential harm to shrimp health. This could be due to the nutrient profile of the BSFLM which may alter depending on the type of residues they were raised on.

In our study, the favorable outcomes regarding the replacement of FML were probably influenced by an appropriate supplementation with crystalline AAs, a sufficient provision of n-3 fatty acids, and the inclusion of feed attractants in all diets. The importance of the dietary supplementation of these nutrients and feed attractants when FML is challenged has been demonstrated in several other studies with penaeid shrimp (Nunes et al., 2014, 2019; Nunes & Masagounder, 2023; Sá et al., 2013; Sánchez-Muros et al., 2020; Xie et al., 2018). As BSFLM inherently possesses lower levels of these nutrients compared to FML (Mohan et al., 2022), achieving full FML replacement necessitates the formulation of a well-balanced diet (Nunes et al., 2022; Sánchez-Muros et al., 2020). Cummins et al. (2017) conducted a dose-response study with juvenile *P. vannamei*, replacing menhaden meal (FML) with 66.02% CP for BSFLM with 52.03% CP. They formulated six different diets, with protein replacement of FML ranging from zero to 100%. While maintaining isonitrogenous diets, there was a progressive drop in dietary levels of Lys and Met as FML was replaced, from 2.74% to 2.40% and from 0.79% to 0.53%, respectively. The authors achieved a maximum replacement of FML for BSFLM at only 20%, equivalent to a dietary inclusion of 10.00% FML and 21.20% BSFLM. The authors acknowledged that the decrease in shrimp performance observed as BSFLM content increased was likely a result of rising deficiencies in essential AAs and an increasing imbalance between essential

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and nonessential AAs. Thus, ensuring these key nutrients are appropriately supplemented in the diet appears as a key element to achieve full FML replacement for BSFLM.

Similarly, Wang et al. (2021) evaluated the replacement of FML for BSFLM in diets for juvenile *P. vannamei* (1.1 g initial BW) over a 56-day culture period. The authors dropped the dietary inclusion of FML from 25.0% to zero by progressively increasing the dietary levels of a defatted BSFLM up to 31.32%. Their results demonstrated that up to 60% of FML could be replaced by BSFLM without any adverse effects to shrimp performance. In their work, while diets were properly supplemented with Met and Lys, fish oil levels reduced with higher inclusions of BSFLM. This caused a significant reduction in the dietary levels of EPA and DHA as higher inclusions of BSFLM were adopted. In another study, He, Liu, et al. (2022) have found that up to 50% of a commercial shrimp diet (42% CP and 11% moisture) for juvenile *P. vannamei* (0.22 g initial BW) could be successfully replaced by fresh BSFL (17.8% CP and 69.3% moisture) without negative effects on growth performance, digestive and antioxidant enzyme activity, and intestinal histology. In summary, complete replacement of FML for BSFLM is possible if diets are properly balanced for key nutrients, such as essential AAs and n-3 fatty acids.

In our study, we have observed a growth response with higher dietary inclusion levels of BSFLM, even in diets deprived of FML. In terms of protein digestibility, both BSFLM and FML exhibit similar characteristics. Apparent protein digestibility coefficient for BSFLM has been reported at 85.1 ± 5.58% (Shin & Lee, 2021) in comparison to 78.9% (Vieira et al., 2022) and 85.8% (Galkanda-Arachchige et al., 2020) for the salmon meal (FML) used in the present study. Our diets were formulated to contain nearly the same AA content, but an increase was found in the total dietary Met (Met plus cysteine) content with a progressive replacement of FML. Total dietary levels increased from 0.85% (1.39%) in the CTL diet to a high of 1.03% (1.56%) in the diet with 100% replacement of FML. On the other hand, the CTL diet contained slightly higher levels of n-3 fatty acids and HUFA than the diets containing BSFLM. Hence, the growth enhancement effect recorded in shrimp fed BSFLM may not have been associated with the dietary nutrient levels which were likely above shrimp requirements.

A similar enhancement in shrimp growth performance with the dietary inclusion of BSFLM has been reported by other authors. Richardson et al. (2021) investigated the use of BSFLM for post-larval *P. vannamei* (PL41, 0.1 g initial BW). Four diets were formulated to contain a high quality FML (70% CP) at 15.00, 10.50, 7.50, and 4.50% with BSFLM at 0, 4.50, 7.50, and 10.50% (% of the diet, as-is), respectively. Shrimp were raised for 28 days in 12 plastic tanks of 290 L under 100 animals/tank (20 g/L water salinity). Authors reported that all diets with BSFLM improved weight gain, FCR and specific growth rate (SGR) compared to the diet with the highest FML level. Additionally, they reported that SGR was significantly improved from the dietary inclusion of 4.5%, whilst FCR was significantly improved at 7.5%. Shin and Lee (2021) also identified BSFLM as one of the top two options for improving the growth performance of juvenile *P. vannamei* (0.17 and 11.1 g initial and final BW respectively) among seven insect meals. Shrimp were fed 38% CP diets for 65 days in which tuna meal was reduced from 27.0 to 17.0% and the insect meal included at 10.0%.

We have found that the ROI for farming shrimp until 23.5 g can be impacted by the price and dietary inclusion of BSFLM in nursery diets. As the price of BSFLM rises along with its inclusion level, there is a progressive decrease in ROI. Nonetheless, a noticeable reduction in ROI occurred only when replacing more than 75% of FML, but this occurred when the price of BSFLM surpassed 3.04 USD/kg. At 100% FML replacement, a price of 2.65 USD/kg for the BSFLM was still advantageous. It is important to note that these economic simulations focused solely on the impact of shrimp survival and FCR during the nursery phase in response to dietary treatment. Shrimp BW at the end of the nursery phase also plays a crucial role in subsequent shrimp performance. For example, initiating the grow-out phase with larger shrimp can lead to shorter production cycles, early harvest, reduced operating costs, and increased annual yield (Jory-, 2020). In our simulations, the operating costs associated with the use of nursery diets accounted for a small portion of the total feed costs to produce a 23.5-gram shrimp, ranging from 3.8 to 4.5% depending on the diet formulation. This is attributed to the minimal feed quantities used in the early stages of shrimp culture. The nursery phase allows for a greater control of feed inputs due to more compact farming areas resulting in lower FCRs compared to grow-out.

5. Conclusions

Based on the findings from the present study, it can be concluded that FML can be fully replaced for BSFLM in well-balanced nursery diets for *P. vannamei* (between 2.7 and 600 mg). This corresponds to a dietary inclusion level of 26.46% BSFLM (% of the diet, as-is). While the full replacement of FML for BSFLM was successfully accomplished, it should be noted that the competitive return on investment (ROI) was sustained only when the maximum price of BSFLM did not exceed 3.04 USD/kg. This price level corresponds to a maximum dietary inclusion of 19.74% or a 75% replacement of FML. Further research may be necessary to fine-tune cost-effective inclusion levels of BSFLM to optimize the economic outcomes while considering the fluctuating prices of FML.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, since shrimp do not fall under phylum Chordata and subphylum Vertebrata which require ethical approval for research activities according to the Brazilian Federal Law No. 11794 of 8 October 2008 (http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2008/lei/l11794.htm) and Brazilian Federal Decree No. 6899 of 15 July 2009 (http://www.planalto.gov.br/ccivil_03/_ato2007-2010/2009/decreto/d6899.htm). All rearing procedures were performed in compliance with relevant local laws and institutional guidelines, including those related to animal welfare. All applicable international, national, and institutional guidelines for the care and use of animals were followed by the authors.

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