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Research Article

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Posted Date: November 17th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11369573/v1

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Additional Declarations: Competing interest reported. Ho Gim Chong, Thiago Raggi, and Sofia Morais are employed by Lucta, S.A. (Bellaterra, Spain). The remaining authors state no conflict of interest

THE USE OF FUNCTIONAL PALATABILITY ENHANCER TO MAINTAIN GROWTH AND HEALTH CONDITION OF SHRIMP *Penaeus vannamei* FED WITH LOW FISH MEAL DIET

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Abstract

An 81-day growth study evaluated the supplementing effects of a functional palatability enhancer (FPE) in a low fish meal (FM) diet on growth performance, feed efficiency, nutrient deposition, immune response, total count of bacteria and *Vibrio* sp. in the hepatopancreas and intestine, as well as hepatopancreatic morphology of Pacific white shrimp (*Penaeus vannamei*). Six diets were tested: a control diet formulated with 15% FM and no FPE; a basal diet with 7.5% FM and no FPE; and a basal diet supplemented with graded levels of FPE (0.075%, 0.1%, 0.125%, and 0.15%). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test to determine dose–response relationships. The results show that reducing fish meal leads to a decrease in final mean weight (FMW), thermal growth coefficient (TGC), average daily growth (ADG), survival, protein deposition, total hemocyte count (THC), lysozyme activity (Lys), superoxide dismutase activity (SOD), and histomorphological condition, and an increase in feed conversion ratio (FCR) as well as the number of *Vibrio* sp. in shrimp fed the basal diet compared to the control diet. Adding FPE to the basal diet improved shrimp growth and health condition. The inclusion of 0.125% FPE led to significantly higher biomass, FMW, TGC, ADG, protein deposition, THC, Lys, and SOD than the control and other dietary treatments. FPE also improved hepatopancreatic morphology compared with the basal diet and showed no abnormal changes compared with the control group. Although shrimp fed 0.15% FPE showed better growth performance and health than the control treatment, performance declined compared with 0.125% FPE. These results demonstrate FPE's potential as a functional additive to enhance the attractability of the low-FM diet and that the proper inclusion level of FPE is crucial for optimizing feed attractability, which determines shrimp growth, nutrient deposition, immune function, and health.

Keywords*: Functional palatability enhancer; Fish meal replacement; Pacific white shrimp; Nutrient utilization; Hepatopancreas and intestine histology; Immune response*

1. Introduction

Global aquaculture production, one of the fastest-growing food-producing industries, expanded rapidly from 3.5 million tons in 1970 to about 94 million tons in 2022 (FAO 2024). Meanwhile, total capture fisheries appear to have reached a plateau in recent years (Boyd et al. 2022), and in 2022, for the very first time, aquaculture surpassed the production of capture fisheries (Liu et al. 2025, Tacon et al. 2025). As a result of a growing population, combined with increasing average income that shifts the diets toward more nutritious and higher-quality foods, while wild fish stocks cannot be sustainably exploited above the current level, fish supply from

aquaculture will have to grow accordingly (Tidwell and Allan 2001; Gjedrem et al. 2012). As future expansion will most likely take place in the marine and brackish water environment (Gjedrem et al. 2012), and with species such as shrimp (Mandal and Singh 2025; Neiland et al. 2001), aquaculture production will be constrained by dependence on fish meal (FM) (New and Wijkström 2002, Edwards, Tuan and Allan 2004). Therefore, research in feed formulation and alternative ingredients to replace dietary FM will play an important role in ensuring the sustainability of aquaculture production (Glencross et al. 2007; Boyd et al. 2022; Amaya et al. 2007; Novriadi et al. 2024; Novriadi et al. 2022).

When replacing FM with alternative ingredients such as plant protein sources, it is important to consider dietary changes, such as cholesterol levels, amino acid profile, energy density, and chemical compounds that may affect the palatability and nutritional balance of the resulting feed (Aragão et al. 2022; Hussain et al. 2024; Novriadi et al. 2022). Another key property of fish meal, besides its optimal amino acid profile and digestibility, is its high content of water-soluble chemoattractive substances (Hodar et al. 2020; Li et al. 2022; Barry et al. 2017). Therefore, when fish meal levels are substantially reduced in diets, adding palatability enhancers is essential to maintain the feed's attractiveness to fish and shrimp, ensure good feed intake, and avoid nutrient leaching. This is particularly important for vannamei shrimp, which typically feed slowly and continuously (Darodes de Tailly et al. 2021; Reis et al. 2021) and feed at the bottom of production ponds (Tantikitti 2014, Zhu et al. 2019; Hoffling et al. 2025). With this in mind, we aimed to investigate whether a functional palatability enhancer could reduce FM levels in shrimp feed formulations while maintaining diet attractiveness, thereby improving growth performance, nutrient utilization, and health status in *P. vannamei* cultured in outdoor commercial ponds. The study also examined FPE's effects on total bacterial counts and Vibrio spp., as well as histomorphological conditions in the shrimp intestine and hepatopancreas.

2. Research Methodology

2.1 Ethics statement

This study was conducted in accordance with the ethical and operational research guidelines established by the Center for Research and Community Service, Jakarta Technical University of Fisheries, and the Indonesian Ministry of Marine Affairs and Fisheries for shrimp grow-out production systems. All experimental procedures adhered to the standards specified in Indonesian National Standard (SNI) 7772:2013, which regulates pond-based shrimp grow-out production. Additionally, the research complied with a ministerial decree (PERMEN No.

22/PERMEN-KP/2024), which governs water quality standards and good aquaculture practices to ensure optimal environmental and animal welfare conditions.

2.2 Experimental diets

The tested functional palatability enhancer (FPE) was obtained from Lucta, S.A. (Barcelona, Spain), under the commercial brand name Luctamax® Aqua. It contains a concentrated blend of amino acids, peptides, and nucleotides derived from both synthetic and natural sources, all of non-animal origin. A control diet contained 15% FM, 15% poultry by-product meal (PBM), 25% soybean meal (SBM), and 3.8% corn gluten meal (CGM) as dietary protein sources and contained approximately 38% protein and 6% lipid. A basal diet was formulated with a lower FM level (7.5%) and no FPE supplementation. The four experimental diets matched the basal diet and were supplemented with 0.075%, 0.1%, 0.125%, and 0.15% FPE (**Table 1**). All dry ingredients were ground into a fine powder (<200 µm) using a disk mill (Jinan Shengrun, China). Lipid, micro-ingredients, and distilled water were added to the premixed dry ingredients and mixed until homogeneous. Afterward, the mixture was conditioned in a steam injection conditioner for 12 s before pelleting the diet. Using steam pelleting, the mixture was formed into 1.6-mm-diameter pellets. After drying and cooling, the pellets were sampled for homogeneity and stored in sealed plastic bags until further use at –200 °C. Total dietary fiber and fat were determined gravimetrically, and crude protein was calculated through titrimetric analysis by multiplying the nitrogen (N_2) content by 6.25. All amino acids were determined using Ultra Performance Liquid Chromatography (UPLC) with a Photodiode Array (PDA) detector. The proximate and amino acid profiles of the diet were analyzed at the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia, and are presented in **Table 2**.

2.3 Design of the growth trial and feeding program in the culture tank

The growth trial was conducted at the commercial shrimp farm PT Adi Sarana Permai, located in Patas Village, Gerokgak Sub-district, Buleleng District, Bali, Indonesia, using an outdoor pond. Post-larvae of shrimp *Penaeus vannamei* were obtained from Prima Larvae Bali (Bali, Indonesia) and acclimatized to the culture system for three days. The acclimatized shrimp (initial mean weight: 2.28 ± 0.03 g) were then randomly distributed into thirty-six hapa nets measuring 3 × 2 × 1 m. Each treatment was allocated to six replicate hapa nets containing 300 shrimps per hapa net. Water quality was regularly tested, and regular water exchange was performed. All hapa nets were equipped with air stones to maintain optimum dissolved oxygen conditions during the culture period. Feeding amounts were pre-programmed based on average normal shrimp growth and a feed conversion ratio

(FCR) of 1.5. Shrimp were fed four times daily at 07:00, 11:00, 15:00, and 20:00 h. Daily feed allowances were adjusted based on observed feed consumption, weekly fish counts, and daily mortality.

2.4 Water quality analysis

Parameters of pH, dissolved oxygen (DO), water temperature, total dissolved solids, and salinity were measured four times daily using sensors (Aqua TROLL 500 Multiparameter Sonde instrument), and data were stored in an application (AquaEasy apps, Bosch, Singapore). Meanwhile, ammonia-nitrogen (NH_3-N), nitrite nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and phosphate were measured weekly using spectrophotometry (Table 3).

2.5 Growth sampling and feed cost gain analysis

At the end of the experiment, all shrimp in each hapa net were counted and weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR), and thermal unit growth coefficient (TGC) as follows:

$$\text{PWG} = \frac{(\text{average individual final weight} - \text{average individual initial weight})}{(\text{average individual initial weight})} \times 100$$

$$\text{FCR} = \frac{\text{feed given (g)}}{\text{alive weigh gain (g)}}$$

$$\text{SR} = \frac{\text{final number of shrimp}}{\text{initial number of shrimp}} \times 100$$

$$\text{TGC} = \frac{\text{FBW}^{1/3} - \text{IBW}^{1/3}}{\sum \text{TD}} \times 100$$

Where FBW is the final body weight of shrimp, IBW is the initial body weight of shrimp, T is the water temperature (0C), and D is the number of trial days (81 days).

Meanwhile, the feed cost per weight gain was calculated as follows:

FCG = FCR × Feed cost/Kg

2.6 Feed attractability assay

A simple behavioral assay was performed on one net per treatment and carried out randomly on DOC 30 to assess feed attractability. For each treatment, 50 g of experimental feed was gently introduced into a designated feeding area at the center of the net pen. Shrimp behavior was continuously recorded using an underwater GoPro camera positioned to provide a clear view of the feeding zone while minimizing disturbance. Feed attractability was quantified by latency-to-contact time, defined as the time from feed introduction to the

first physical contact between 10 shrimps and the feed pellet. To minimize observer bias, all video analyses were performed by a single trained observer blinded to treatment allocation.

2.7 Whole-body composition analysis

On the termination day, we randomly sampled eighteen shrimp from each dietary treatment (or three shrimp per culture tank) and stored them at -80 °C for body composition analysis. Prior to proximate, energy, and amino acid analyses, dried whole fish were rigorously blended and chopped in a mixer according to methods described by Helrich (1990). All parameters were analyzed at the Saraswanti Indo Genetech Laboratory (Bogor, West Java, Indonesia)

2.8 Total bacteria and Vibrio

Tryptic soy agar (TSA, Difco) was used to count total bacteria, and thiosulphate-citrate-bile salts-sucrose (TCBS, Difco) was used to selectively enumerate *Vibrio* spp. Sampling was restricted to three randomly selected hapa nets per dietary treatment to minimize handling stress and disturbance to culture shrimp while maintaining representative data collection and providing sufficient replication for statistical analysis. Briefly, one milligram of hepatopancreas and intestine was serially diluted in autoclaved (121 °C for 15 min) seawater to 10^{-4}. Portions (0.1 mL) of each dilution were streaked on the media in duplicate and incubated for 24 h at 35 °C. Bacteria and *Vibrio* spp. counts were analyzed regularly on days 0, 45, and 81 of the culture periods.

2.9 Total haemocyte count

At the end of the growth trial, we sampled hemolymph from three intermolt shrimp per hapa net (18 shrimp per treatment) and determined the total hemocyte count. Hemolymph (100 µL) of individual shrimp was withdrawn from the pleopod base of the second abdominal segment with a sterile 1-mL syringe (25 G × 13 mm needle). Before hemolymph extraction, the syringe was loaded with a precooled (4°C) solution (10% EDTA Na2) used as an anticoagulant. The hemolymph with the anticoagulant solution was diluted in 150 µL of formaldehyde (4%), and then 20 µL was placed on a hemocytometer (Neubauer) to determine the total hemocyte count (THC) using an optical microscope (Olympus, DP72) following methods described by (del Carmen Flores-Miranda et al. 2011)

2.10 Lysozyme and superoxide dismutase activity of shrimp hepatopancreas

Lysozyme activity was measured using a lysozyme detection kit (Sigma-Aldrich, Cat. no. LY0100) according to the manufacturer's instructions. Lysozyme activity was determined by the lysis of *Micrococcus lysodeikticus* cells. The reactions were conducted at 25°C, and absorbance at 450 nm was measured on a UV-visible spectrophotometer (Perkin Elmer, Lambda XLS, USA).

$$\text{Lysozyme activity } \left(\frac{\text{Units}}{\text{mL}}\right) = \frac{(\Delta \text{A450}/\min Test - \Delta \text{A450}/\min Blank)\ (df)}{(0.001)(0.03)}$$

df = dilution factor

0.001 = ΔA_{450} as per unit definition

0.03 = volume (in mL) of enzyme solution (Seong et al. 2018)

Superoxide dismutase (SOD) activity was measured following the method described by Parrilla-Taylor and Zenteno-Savín (2011), which employs a xanthine/xanthine oxidase system to generate superoxide radicals, with nitro blue tetrazolium (NBT) serving as a detector. Samples were diluted (1:20) in potassium phosphate buffer (0.1 M, pH 7.5, containing 60 mM EDTA). The reaction mixture consisted of sodium carbonate working solution (50 mM) supplemented with 0.1 mM xanthine, 0.025 mM NBT, and 0.1 mM EDTA, along with xanthine oxidase (0.1 μM mL^{-1} in 2 M ammonium sulfate) and either sample or blank (potassium phosphate buffer). The rate of change in absorbance at 560 nm was monitored and expressed as absorbance increase per minute. Enzymatic activity was expressed in U mg^{-1} of protein. One unit of SOD activity is defined as the amount of enzyme needed to inhibit the reaction of superoxide radicals with NBT by 50%.

2.11 Histomorphological condition of hepatopancreas and intestine of shrimp

Shrimp samples for the histopathological studies of hepatopancreas and intestine were selected randomly. A section of approximately 0.5 cm was immediately preserved in Davidson's at room temperature and then transferred to 70% ethanol solution (VWR, Radnor, PA, USA) until processed by standard histological analysis procedures (Hasson et al. 1995). Samples were embedded in paraffin wax and cut into 4-μm-thick sections by a manual rotary microtome (RM2235, Leica, Germany). The sections were stained with hematoxylin and eosin (Merck, Darmstadt, Germany) and examined using a light microscope (Merck, Darmstadt, Germany). For interpretation, a double-blinded evaluation with a grading scale of 1 to 5 was used, where 1 was considered the normal condition and higher scores indicated increasing histopathological alteration relative to the normal

condition. Images were acquired using a digital imaging microscope (Olympus BX41, Olympus Optical Co., Ltd., Tokyo, Japan).

2.12 Statistical analysis

Growth parameters, total bacteria and *Vibrio* spp. counts, total hemocyte counts, lysozyme activity, and superoxide dismutase activity were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among treatments, followed by Tukey's multiple comparison tests to determine the differences between treatment means. Score data on shrimp hepatopancreas and intestine histomorphological condition were treated as categorical data, tested for normality and homoscedasticity, and subsequently analyzed using a linear regression model. All statistical analyses were conducted using the SAS system (V9.4. SAS Institute, Cary, NC, USA).

3. Results

3.1 Diet composition

All experimental feeds had comparable protein levels (38.29–38.68%) and similar total calories (348.10–359.69 kcal per 100 g) (Table 2). For total fat, the control diet with 15% FM had 6.29% fat, while the basal diet formulated with 7.5% FM supplemented with graded levels of FPE had 5.22–5.60%. Overall, crude fiber levels were comparable among dietary treatments.

3.2 Water quality analysis

During the 81-day culture period, the daily water quality measurements presented in Table 3, including dissolved oxygen, temperature, salinity, and pH, were within the range of 7.195 ± 1.808 mg L^{-1}; 30.569 ± 0.997 °C; 25.461 ± 0.912 ‰; and 8.848 ± 0.159, respectively. Meanwhile, weekly analysis of total dissolved solids, ammonia-nitrogen (NH3-N), nitrite-nitrogen (NO2-N), nitrate-nitrogen (NO3-N), and phosphate (PO4-N) ranged from 25.504 ± 0.819; 0.011 ± 0.008; 0.024 ± 0.008; 3.118 ± 0.977, and 0.013 ± 0.005 mg L^{-1}, respectively. Salinity and pH did not change significantly, and all water parameters remained within the acceptable range to support P. vannamei growth.

3.3 Shrimp growth performance

Reducing FM inclusion from 15% to 7.5% in the feed formulation negatively affected final biomass, final mean weight (FMW), percentage weight gain (PWG), thermal growth coefficient (TCG), and average daily growth (ADG) of shrimp ($p=<0.0001$) (Table 4). However, adding a functional palatability enhancer (FPE) to the low FM diet (basal) improved shrimp growth performance ($p=<0.0001$), with the 0.075% and 0.1% groups showing growth similar to shrimp fed the control (15% FM) diet. Including higher levels of 0.125% and 0.15% FPE in the low FM diet also resulted in better FMW, PWG, FCR, TGC, and ADG than the control diet. However, numerically, 0.125% FPE showed better growth performance than 0.15% FPE. Feed conversion ratio (FCR) followed the same pattern as growth: it was significantly higher in the basal group, similarly reduced in the control, 0.075%, and 0.1% groups, and lowest in shrimp fed the 0.125% and 0.15% diets. Survival was slightly, but significantly, reduced in shrimp fed the basal diet (87.07%) and was similar (90.30-92.78%) in the other groups.

3.4 Feed Attractability response

Shrimp offered the PE-supplemented diets exhibited markedly faster feeding responses: latency, or time to approach the feed, decreased by up to 168% compared with the basal feed and by 155% relative to the control. The dose-response followed the growth performance results, with a dose-related reduction in latency-to-contact time up to 0.125%, and no further improvement from 0.125% to 0.15% FPE (Figure 1).

3.5 Body composition analysis

Data on the body composition of protein, fat, amino acids, and other nutrients is summarized in Table 5. After 81 days of feeding, shrimp fed 0.125% FPE had the highest protein deposition, followed by 0.075% and 0.15% FPE, and the control diet; shrimp receiving the basal diet had the lowest protein deposition. Amino acid levels in shrimp also supported these differences in protein deposition between treatments. Moisture ranged from 66.90–74.13%, and the highest fat deposition occurred in shrimp fed the basal low FM diet, followed by the 0.075% FPE diet; 0.1% to 0.15% FPE supplementation reduced fat levels to values closer to the control.

3.5 Total bacteria and *Vibrio* spp. in the hepatopancreas and intestine of shrimp

Figures 2 and 3 present data on total bacteria and total Vibrio spp. in the hepatopancreas and intestine of P. vannamei, respectively, at three time points: Day of culture (DOC) 0 (beginning of the culture period), DOC 45 (mid-point), and DOC 81 (end-point). In the hepatopancreas, total bacteria were similar across treatments at

the beginning of the culture period, but by DOC 45 and DOC 81, total bacteria were higher in shrimp fed the basal diet and were significantly reduced by FPE inclusion, especially at a dose ≥0.1%. Similarly, total *Vibrio* spp. were higher in shrimp fed the basal diet, and FPE inclusion led to a significant reduction ($p<0.05$). Total bacteria and Vibrio spp. counts in the intestine followed the same trend as in the hepatopancreas, with significantly higher levels in shrimp fed the basal diet and a significant reduction in shrimp fed the FPE-supplemented feeds, especially at 81 days of the culture period.

3.6 Total hemocyte count

Figure 4 shows that shrimp fed 0.1% FPE had the highest total hemocyte count (THC), at 9.92±1.01 × 106 cells mL^{-1}, followed by the 0.125% and 0.15% FPE treatments, at 7.11±0.71 and 5.08±0.67 × 106 cells mL^{-1}, respectively. The control and 0.075% FPE treatments had the same THC level, and the lowest THC value was obtained in shrimp fed the basal feed, with only 1.57±0.43 × 10^6 cells mL^{-1}.

3.7 Lysozyme activity in shrimp hepatopancreas

Based on the data in Figure 5, shrimp fed 0.1%, 0.125%, and 0.15% FPE had comparable lysozyme activity of 2.62±0.01, 2.63±1.01, and 2.63±0.03 U mL^{-1}, respectively, and were significantly higher than the other treatments ($P<0.0001$). The control and 0.075% FPE treatments had the same intermediate lysozyme activity. Meanwhile, the basal diet treatment showed the lowest lysozyme activity (2.42±0.62 U mL^{-1}; $p<0.0001$).

3.8 Superoxide dismutase activity in shrimp hepatopancreas

Figure 6 presents superoxide dismutase (SOD) activity in the hepatopancreas of Pacific white shrimp, Penaeus vannamei (units SOD mg^{-1} protein). Shrimp fed 0.1%, 0.125%, and 0.15% FPE had comparable and significantly higher SOD values compared to other treatments ($P<0.0001$). The control and 0.075% FPE treatments did not differ significantly, and both had significantly higher SOD activity than shrimp fed the basal diet, which had the lowest level (19,672±1.24 U mg^{-1} of protein; $P<0.0001$).

3.9 Histomorphological condition of hepatopancreas and intestine of shrimp

Figures 7 and 8 show representative histomorphological images of the shrimp hepatopancreas and intestine at the end of the growth trial. Histological examination of transverse sections of hepatopancreas tubules revealed distinct cell types, including B cells, characterized by their blister-like, vacuolated morphology, and R

cells, identified as resorptive cells. These tubules were separated from each other by the basal lamina (BL) and connective tissue (con). Shrimp fed diets containing 0.1% FPE and 0.125% FPE showed a clear increase in B- and R-cell numbers in the hepatopancreas compared with shrimp receiving the basal diet or 0.075% FPE supplementation. In contrast, shrimp fed the 0.150% FPE diet showed a different pattern of B- and R-cell abundance than the 0.1% and 0.125% groups, although the available histological images did not allow a conclusive determination of the direction of this change. In the intestine, shrimp fed the 0.1% FPE diet had taller villi than those fed the basal diet or supplemented with 0.075%, 0.125%, or 0.150% FPE. Histomorphological measurements further indicated that shrimp fed the control, basal, and 0.075% FPE diets had similar epithelial height (EH) and wall thickness (WT). The foregut of shrimp fed 0.1% FPE showed the highest EH and WT, whereas both parameters declined modestly in shrimp receiving 0.125% and 0.15% FPE diets.

4. Discussion

Shrimp production currently faces significant farm-gate price fluctuations, influencing farming decisions and profitability (Bhargavi et al. 2025; Tian et al. 2024). Feed is typically the largest operational cost in aquaculture, commonly representing 50–70% of total production costs, depending on the cultured species and production system (Gong et al. 2019; Llagostera et al. 2019). Therefore, one way to improve financial viability is to intervene in feed production by replacing volatile and expensive raw materials, particularly fish meal (FM), with alternatives such as soybean meal (SBM), corn meal, and other plant-protein sources (Novriadi et al. 2024; Chen et al. 2024). However, reducing FM will result in the loss of several essential nutrients and reduced palatability and attractiveness of the feed for shrimp (Hoffling et al. 2025; Moss et al. 2024). This presents a challenge, given that shrimp are slow, continuous, and selective feeders (Ullman et al. 2019), increasing the risk of nutrient leaching before feed can be optimally consumed (Bardera et al. 2019; Riisgård et al. 2015)thereby impacting shrimp growth, feed conversion ratio (FCR), and health status (Hoffling et al. 2025; Tantikitti 2014). This study corroborates these negative impacts by showing that, even though the feed was formulated to maintain the same protein content, total calories, methionine, and lysine levels, reducing FM from 15% to 7.5% negatively affected shrimp growth performance after 81 days of culture. Possible reasons for the decline in shrimp growth and feed efficiency may be related to the presence of anti-nutritional factors in high inclusion levels of SBM in the basal diet compared to the control, which reduce protein, lipid, and energy digestibility (Van den Ingh et al. 1996; Yue et al. 2012) and reduced feed palatability, which results in lower nutrient intake and, consequently, slower growth. (Hoffling et al. 2025)

However, adding FPE to the basal diet improved shrimp growth rate and feed efficiency, outperforming the control group. Table 4 shows that supplementing the basal diet with 0.075% FPE resulted in shrimp growth performance similar to the control diet in final biomass, FMW, PWG, TGC, and ADG. Moreover, shrimp fed 0.075% FPE showed feed utilization efficiency and survival rates comparable to the control diet. Adding FPE at higher levels (0.1% to 0.15% FP) significantly improved shrimp growth compared with the basal diet, and at 0.125% and 0.15% FPE, growth was even higher than the control. However, the peak effect on growth, survival, and biomass occurred at 0.125% FPE and did not improve further at 0.15% FPE. Previous studies with different dietary formulations, including higher FM levels, and in shrimp exposed to a sub-optimal temperature, similarly showed significant improvements in *P. vannamei* growth at 0.1% inclusion compared to a basal diet containing no FPE, with no further growth enhancement observed at 0.15% (Zhu et al. 2019) or 0.2% (Hoffling et al. 2025). Therefore, the growth-promoting effect of FPE appears to follow a non-linear, saturating response, increasing up to an inclusion level of 0.125% in the present study and remaining unchanged at higher doses. One plausible reason for this improvement can be seen in the analysis of latency-to-contact time, where the administration of FPE, containing a mixture of free amino acids, peptides, and nucleotides of synthetic (nature-identical) and natural (non-animal hydrolysate) origin, accelerates feed recognition, thus optimizing nutrient utilization to support shrimp growth (Carr and Derby 1986; Lee and Meyers 1996). Marine crustaceans are highly sensitive to chemical signals, especially water-soluble molecules such as amino acids and nucleotides (Derby and Sorensen 2008; Derby et al. 2002); at an appropriate concentration, the FPE used in this study further accelerates feed consumption. Because nucleotide and amino acid utilization depends on dose and metabolism, the reduced growth observed at high doses might be due to the inappropriate dose used in this commercial production study (Hossain et al. 2020; Wu et al. 2023, Swanepoel et al. 2023).

Dietary FPE inclusion also affected nutrient deposition in shrimp, enhancing whole-body protein deposition compared with the basal diet. Meanwhile, FPE addition to the basal diet resulted in lower total fat, comparable to the control diet. Faster or more efficient feed recognition (allowing less time for soluble protein leaching) could enhance nutrient deposition by increasing the supply of digestible amino acids and energy available for tissue accretion in shrimp (Bureau et al. 2000; Simon et al. 2021). This may explain why the low FM inclusion level in this study resulted in lower protein deposition than the control, and why the FPE-supplemented treatments showed higher protein deposition. Moreover, a previous study found that adding FPE to the diet increased protease activity in shrimp hepatopancreas (Zhu et al. 2019), which could also partly explain higher protein body deposition. Notably, shrimp fed the basal diet had considerably higher total body fat than the control,

363 despite being fed a diet with 0.95% less total fat. Abd El-Naby et al. (2024) reported that adding 9.4%, 14.1%,
364 and 18.8% fermented soybean meal (48% protein) to partially replace FM (72% protein) in P. *vannamei* feed also
365 increased total lipid deposition after a 12-week culture period. Chen et al. (2017) also found that *P. vannamei* fed
366 diets with increasing levels of soy-protein concentrate to partially replace FM had a higher crude lipid fraction in
367 the whole body than the control treatment. Based on this, we speculate that the elevated body lipid content
368 observed in shrimp fed the reduced FM basal diet might be related to the quality of soybean meal used in the diet
369 formulation. However, adding FPE partially reversed the basal diet's negative effect. Shrimp fed FPE-
370 supplemented diets, especially at ≥0.1%, achieved a total lipid content closer to that of shrimp receiving 15% FM.
371 In this study, total bacteria and *Vibrio* spp. were comparable across all treatments at the start of the culture
372 period. However, by DOC 45 and at the end of the trial (DOC 81), total bacteria and *Vibrio* spp. growth in both
373 the hepatopancreas and intestine was highest in shrimp that received the basal diet. Both external and internal
374 factors influence the composition of the intestinal microbiota in *P. vannamei* (Li et al. 2018, Suo et al. 2017).
375 Dietary components and culture environment are exogenous factors, while developmental stage and health status
376 are the main endogenous factors affecting the intestinal microbiota in shrimp (Li et al. 2018). *Vibrio* spp. are
377 naturally found in marine and estuarine waters and are considered opportunistic pathogens. (Hoffling et al. 2025).
378 In the current study, at salinity levels reflecting natural conditions, *Vibrio* spp. decreased in the FPE treatment
379 compared with the control, resulting in higher shrimp survival. This suggests that adding FPE might selectively
380 prevent pathogen growth in a low-FM diet by providing substrate that induces heterotrophic bacterial growth.
381 This line of reasoning will require further experimental evidence by observing the mechanisms, as *Vibrio* spp.
382 still have good growth at several sampling points in the FPE treatment.
383 Nutrients can strongly affect aquatic animals' immune systems (Trichet 2010; Barreto et al. 2023),
384 including hemocytes that perform a variety of physiological and pathogen defense functions, including
385 coagulation, phagocytosis, recognition of foreign materials, carbohydrate transport, and encapsulation (Hose et
386 al. 1987), as well as lysozyme activity (Novriadi et al. 2021; Hu et al. 2022). In this study, shrimp fed the basal
387 diet had the lowest total hemocyte count (THC), while those fed 0.1%, 0.125%, and 0.15% FPE showed higher
388 THC than the control and 0.075% FPE treatments. Poor nutrition can cause low hemocyte counts (hemocyte
389 depletion) (Novriadi et al. 2021). Thus, the results showed that FPE supplementation could promote hemocyte
390 proliferation in hematopoietic tissue and increase hemocyte mobilization in shrimp. Lysozyme activity was
391 reduced in the basal diet compared with the control and then recovered or improved in a saturating dose-response
392 manner with FPE supplementation, indicating that dietary FPE could improve the innate immune response of

Pacific white shrimp (*Penaeus vannamei*) juveniles. This aligns with research by Yuangsoi and Poonnual (2025), which found that adding tuna hydrolysate concentrate as a marine palatability enhancer increased shrimp lysozyme activity.

Superoxidase dismutase (SOD) is one of the most important antioxidant enzymes in the cellular defense system against oxidative stress, and its activity can also be considered a parameter of immune response (Feng et al. 2019; Campa-Córdova, et al. 2002a,b). In our research, reducing FM from 15% in the control to 7.5% in the basal diet reduced SOD activity, and adding FPE significantly stimulated this enzyme's activity in Pacific white shrimp (*P. vannamei*). Interestingly, the dose-response pattern matched that in growth, with increasing effects up to 0.125% and no further enhancement at 0.15%. Higher feed intake ultimately ensures adequate intake of energy, protein, vitamins (especially vitamins C and E), and trace minerals (Zn, Cu, and Mn). As these nutrients act as cofactors or co-enzymes for antioxidant enzymes, including SOD, the enhanced and/or faster feed intake likely promoted by FPE (thereby reducing the leaching of water-soluble nutrients) may have contributed to the observed increase in SOD activity by improving the availability of nutrients required for the synthesis and proper functioning of the antioxidant defense system.

Histological evaluation of *P. vannamei* revealed distinct responses in the hepatopancreas and intestine to dietary FPE supplementation. In shrimp fed the control, basal, and 0.075% FPE diets, hepatopancreas tubules were predominantly composed of B-cells (vacuolated) and R-cells (resorptive). Under normal conditions, F-, B-, and R-cells are expected to be balanced, reflecting simultaneous digestive activity and nutrient absorption or storage (Lalitha et al. 2022). The observed predominance of B- and R-cells therefore suggests either intensive feeding or a stress-related response, characterized by stimulated digestion but limited nutrient storage. Notably, shrimp fed 0.1% and 0.125% FPE diets showed a marked increase in B- and R-cell abundance, whereas those fed 0.15% FPE showed comparatively fewer of these cell types. Physiologically, elevated B-cell numbers indicate enhanced digestive function, while increased R-cell numbers reflect improved nutrient absorption and storage capacity (Andayani and Astutik 2026; Ko et al. 2025). Parallel histomorphological analysis of the intestine demonstrated similar epithelial height (EH) and wall thickness (WT) in shrimp fed with the control, basal, and 0.075% FPE diets. The foregut of shrimp fed 0.1% FPE showed the highest EH and WT, while both parameters declined in the 0.125% and 0.15% FPE groups. Tall epithelial cells (B, R, and F types) indicate active metabolism, with fully extended organelles supporting secretion (B-cells) and absorption or storage (R-cells). Elevated EH is typically associated with healthy, well-fed shrimp, in which hepatopancreas tubules engage in both digestion and

nutrient storage (Mogalekar et al. 2025). Similarly, increased WT reflects tall epithelial cells and a robust basement membrane, indicating strong tissue integrity.

5. Conclusion

A substantial reduction in FM inclusion from 15% to 7.5% compromised growth performance, protein deposition, and immune competence in Pacific white shrimp, *Penaeus vannamei*, underscoring the limitations of a low FM-based diet. This study shows that supplementing a low FM diet with FPE effectively mitigates these negative effects under practical outdoor culture conditions. Adding FPE not only restored growth but also consistently improved growth performance, microbial balance, and health indicators at higher inclusion levels (0.1–0.15%), while partially preserving hepatopancreatic and intestinal histomorphology. Although feed intake was not directly measured, these benefits may have been mediated, at least in part, by enhanced feed attractiveness and palatability, leading to greater or faster feed consumption and, consequently, improved availability of dietary nutrients. Notably, the 0.125% inclusion level emerged as the optimal dose, providing a robust balance between growth and health protection. These findings highlight FPE as a viable nutritional solution to support the development of economical feeds by enabling significant FM reduction without compromising shrimp productivity and health

Data availability statement

Data will be made available upon request.

Acknowledgments

The authors would like to thank the research staff of PT Adi Sarana Permai for their valuable assistance with sample collection and support throughout the production process. The authors also express their sincere gratitude to all individuals who devoted their time and expertise to critically reviewing this manuscript and providing constructive feedback. The mention of any trademark, trade name, or proprietary product in this publication is solely for the purpose of providing specific information and does not constitute an endorsement or recommendation by the Jakarta Technical University of Fisheries (Politeknik Ahli Usaha Perikanan) or the National Research and Innovation Agency. Such mention does not imply approval of the product to the exclusion of other products that may be equally suitable.

Funding

This work was partly supported by Lucta, S.A

Conflict of interest disclosure

Ho Gim Chong, Thiago Raggi, and Sofia Morais are employed by Lucta, S.A. (Bellaterra, Spain). The remaining authors state no conflict of interest

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Figure 1. Attractability response (latency-to-contact time) for 10 shrimp to physically contact the feed were measured and recorded as response to the feed (second).

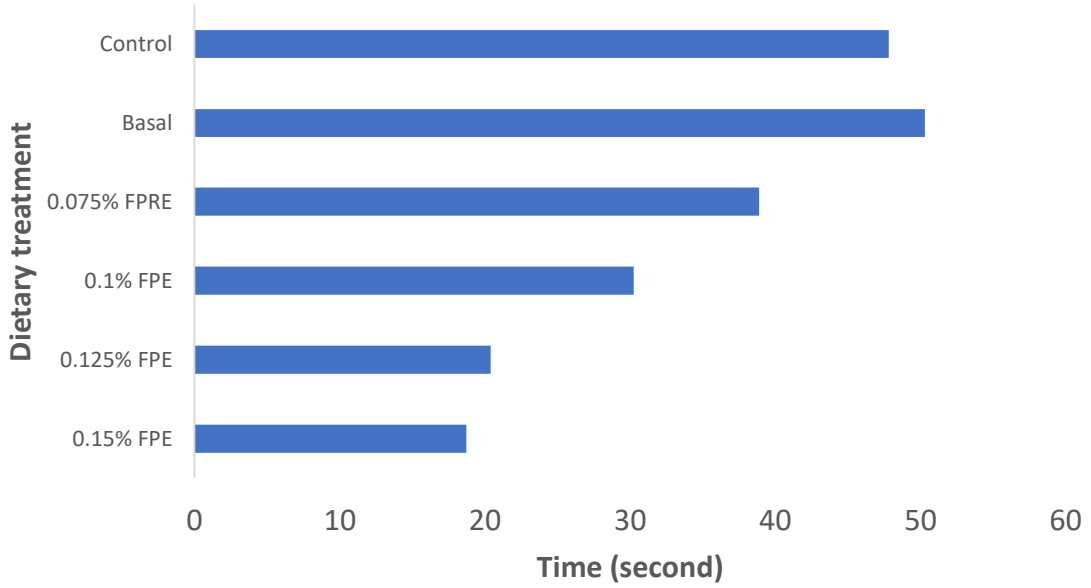


Figure 2. Total bacteria (A) and total *Vibrio* spp. (B) in the hepatopancreas of shrimp, on day of culture (DOC) of 0, 45 and 81. Values represent the mean ± standard deviation of three replicates per dietary treatment.

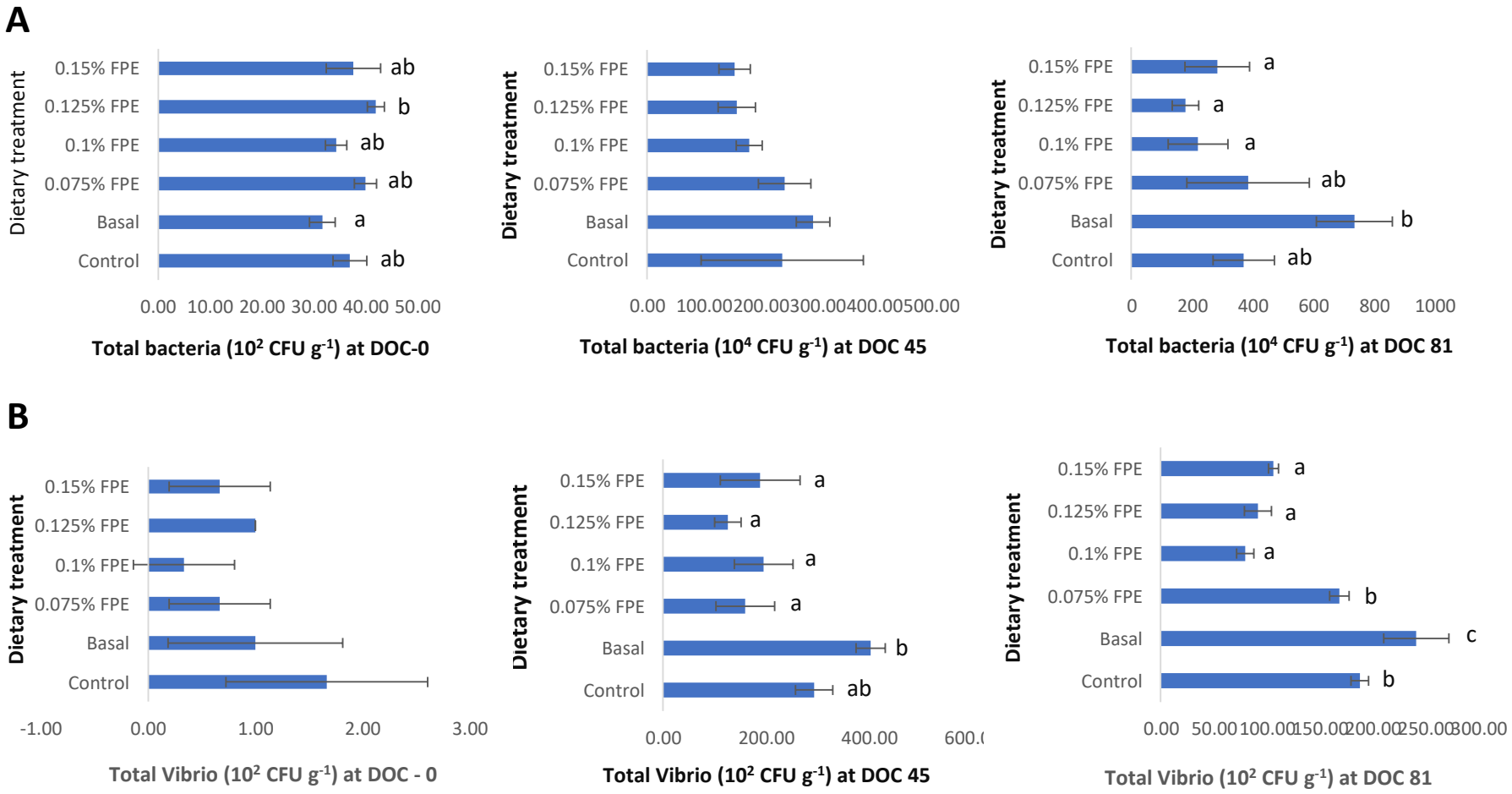


Figure 3. Total bacteria (A) and total *Vibrio* spp. (B) in the intestine of shrimp, on day of culture (DOC) of 0, 45 and 81. Values represent the mean ± standard deviation of three replicates per dietary treatment (p<0,05).

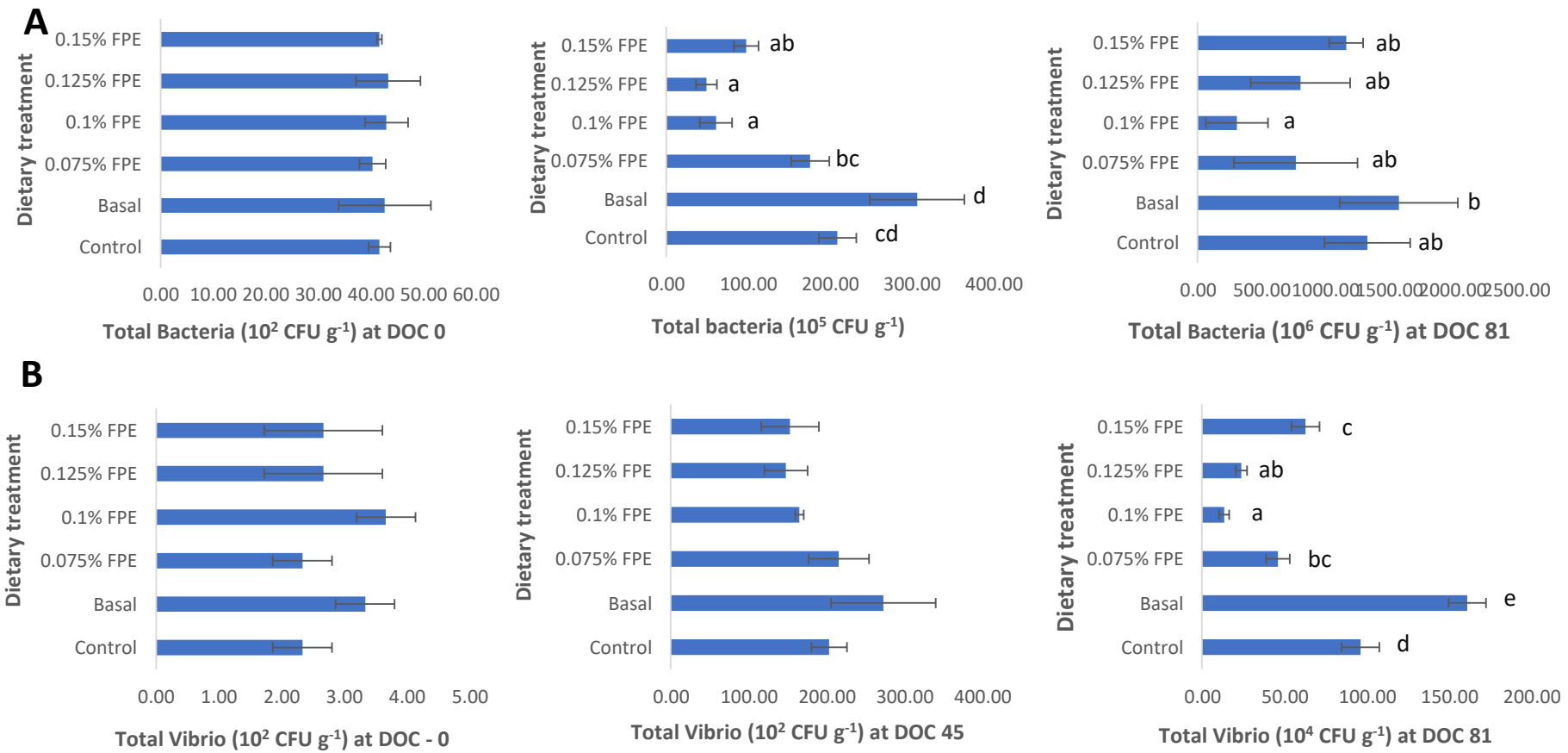


Figure 4. Total hemocyte count (THC) in the hemolymph of Pacific white shrimp *Penaeus Vannamei* (10^6 cell mL^{-1}) fed a 15% fishmeal control diet, a 7.5% fishmeal basal diet, or the basal diets further supplemented with 0.075%, 0.1%, 0.125% or 0.15% of a functional palatability enhancer (FPE), at the end of the growth trial. Values represent the mean ± standard deviation of eighteen replicates per dietary treatment. Columns with different superscript letters are significantly different (P<0.0001).

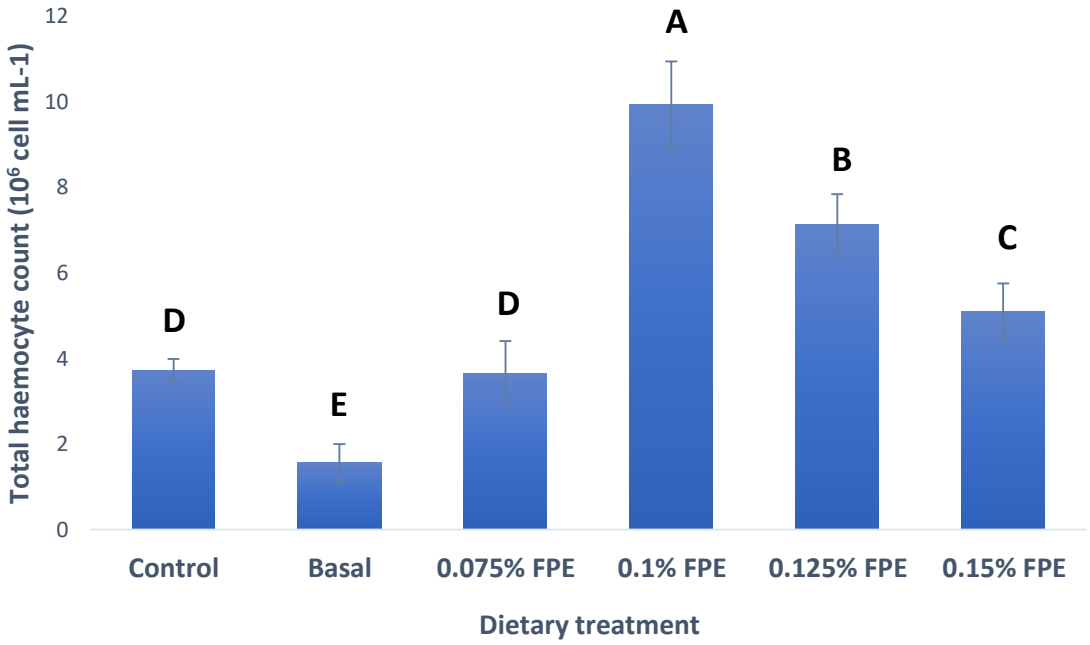


Figure 5. Lysozyme activity in the hepatopancreas of Pacific white shrimp *Penaeus Vannamei* (U mL^{-1}) fed a 15% fishmeal control diet, a 7.5% fishmeal basal diet, or the basal diets further supplemented with 0.075%, 0.1%, 0.125% or 0.15% of a functional palatability enhancer (FPE), at the end of the growth trial. Values represent the mean ± standard deviation of eighteen replicates per dietary treatment. Columns with different superscript letters are significantly different (P<0.0001)

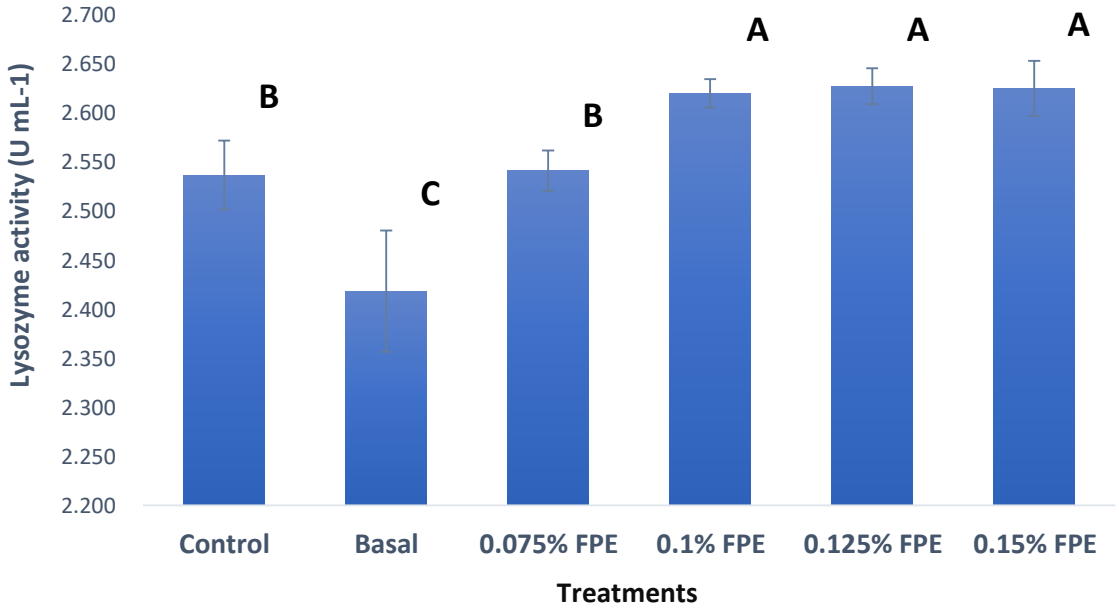


Figure 6. Superoxide dismutase activity (SOD) in the hepatopancreas of Pacific white shrimp *Penaeus Vannamei* (units SOD mg-^{1} of protein) fed a 15% fishmeal control diet, a 7.5% fishmeal basal diet, or the basal diets further supplemented with 0.075%, 0.1%, 0.125% or 0.15% of a functional palatability enhancer (FPE), at the end of the growth trial. Values represent the mean ± standard deviation of eighteen replicates per dietary treatment. Columns with different superscript letters are significantly different (P<0.0001).

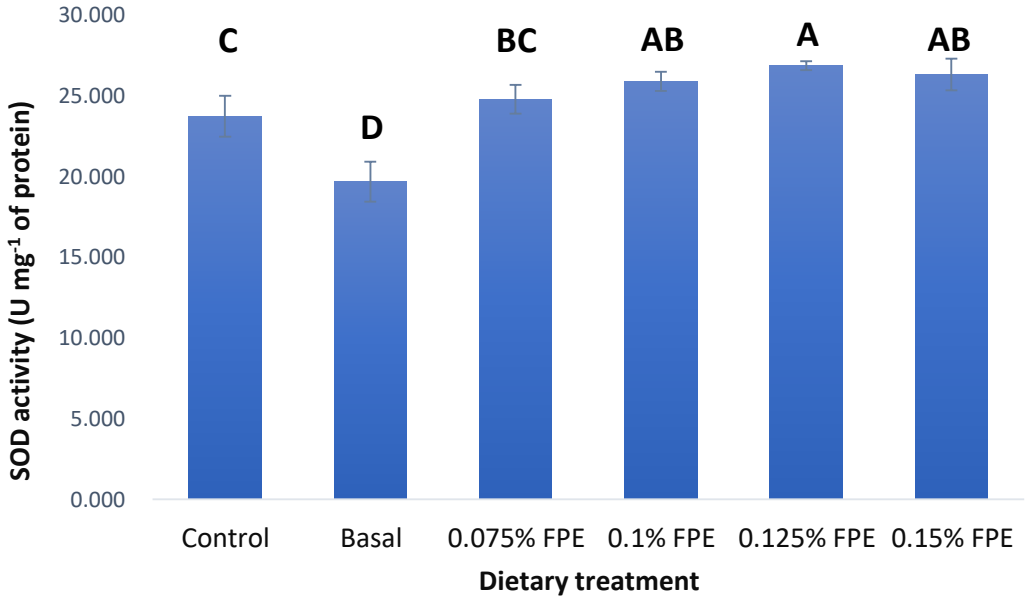


Figure 7. Representative histomorphological images of hematoxylin and eosin-stained sections of hepatopancreas of shrimp *P. vannamei* fed a 15% fishmeal control diet (1), a 7.5% fishmeal basal diet (2), or the basal diets further supplemented with 0.075% (3), 0.1% (4), 0.125% (5) or 0.15% (6) of a functional palatability enhancer (FPE), at the end of the growth trial (n=6 per treatment).

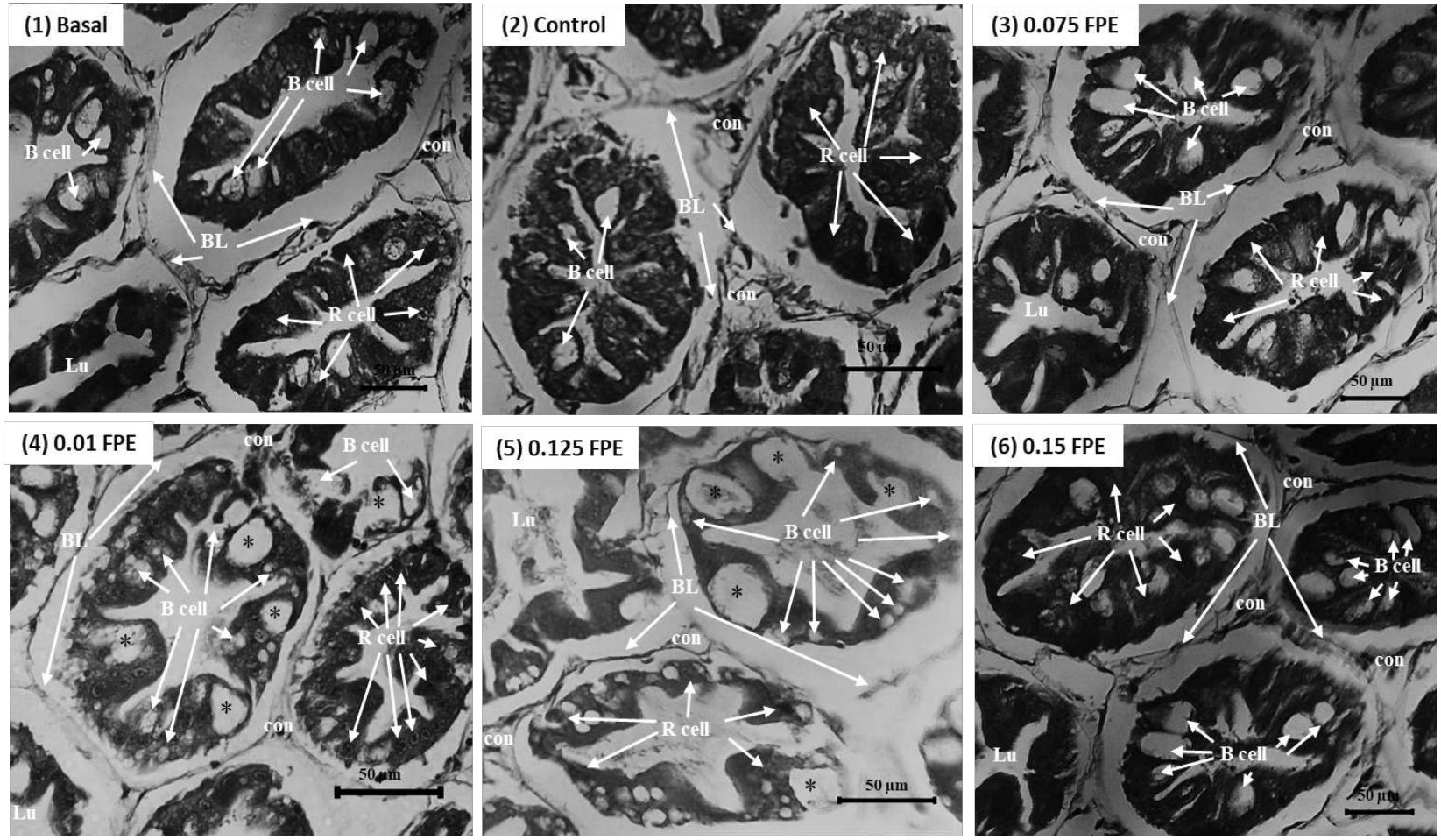


Figure 8. Representative histomorphological images of hematoxylin and eosin-stained sections of the intestine of shrimp *P. vannamei* fed a 15% fishmeal control diet (1), a 7.5% fishmeal basal diet (2), or the basal diets further supplemented with 0.075% (3), 0.1% (4), 0.125% (5) or 0.15% (6) of a functional palatability enhancer (FPE), at the end of the growth trial (n=6 per treatment).

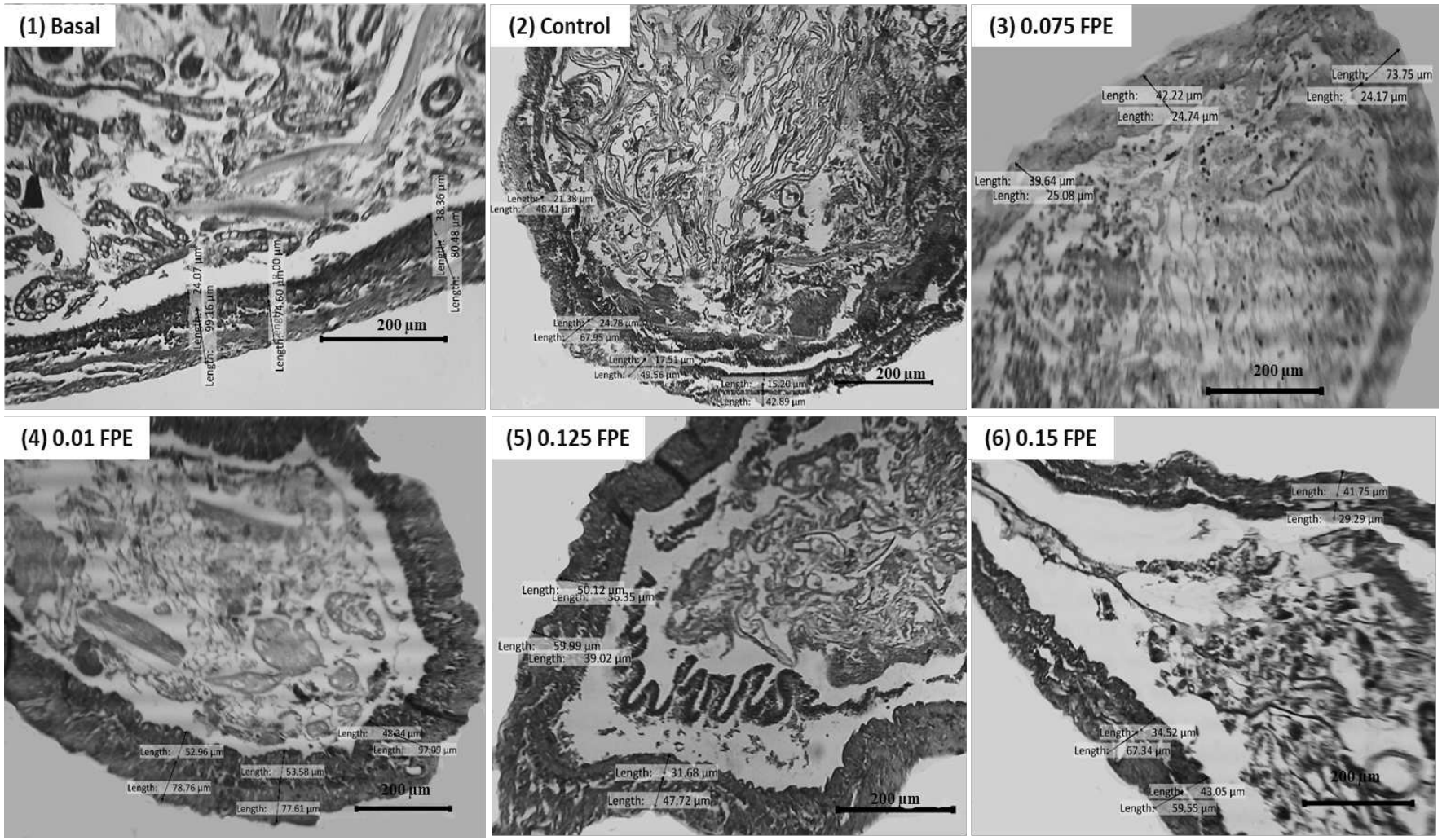


Table 1. Composition (*% as is*) of control, basal and experimental diets consisting of several inclusion levels of functional palatability enhancer (FPE, Luctamax® Aqua, Lucta S.A., Barcelona, Spain), which were fed to *P. vannamei* for 81 days**.**

Diet name	**Control**	**Basal**	**0.075% FPE**	**0.1% FPE**	**0.125% FPE**	**0.15% FPE**
Fishmeal[1]	15.00	7.50	7.50	7.50	7.50	7.50
Poultry by-product meal 1	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal[1]	25.00	34.90	34.90	34.90	34.90	34.90
Corn Gluten meal[1]	3.80	3.80	3.80	3.80	3.80	3.80
DDGS [1]	8.00	8.00	8.00	8.00	8.00	8.00
Fish oil[1]	1.00	1.00	1.00	1.00	1.00	1.00
Lecithin (soy)[1]	1.50	1.50	1.50	1.50	1.50	1.50
Corn Starch[1]	10.60	7.81	7.74	7.71	7.69	7.66
Cholesterol[2]	0.00	0.01	0.01	0.01	0.01	0.01
Wheat flour[3]	17.00	17.00	17.00	17.00	17.00	17.00
Mineral premix[4]	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[2]	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin C[2]	0.10	0.10	0.10	0.10	0.10	0.10
Monocalcium phosphate[2]	2.00	2.00	2.00	2.00	2.00	2.00
Lysine[2]	0.00	0.20	0.20	0.20	0.20	0.20
Methionine[2]	0.00	0.18	0.18	0.18	0.18	0.18
Functional Palatability enhancer[6]	0.000	0.000	0.075	0.100	0.125	0.150
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1] PT FKS Multi Agro, Tbk. Jakarta, Indonesia

[2] PT Fenanza Putra Perkasa, South Jakarta, Jakarta, Indonesia

[3] PT Pundi Kencana, Cilegon, Banten, Indonesia

[4] Trace mineral premix (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.550; ferrous sulfate, 2.000; magnesium sulfate anhydrous, 13.862; manganese sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; alpha-cellulose, 69.664.

[5] Vitamin premix (g/kg premix): thiamin·HCL, 4.95; riboflavin, 3.83; pyridoxine·HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D3 (1,000,000 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 856.81

[6] Lucta S.A., Barcelona, Spain

Table 2. Proximate and amino acid (AA) composition (*% as is*, *dry matter basis*) of diets utilized in the trial

No	Parameter	Unit	Control	Basal	0.075% FPE	0.1% FPE	0.125% FPE	0.15% FPE
1	Protein Content	%	38.45	38.49	38.29	38.54	38.68	38.65
2	Total Calories	Kcal 100 g^{-1}	348.55	348.10	355.00	359.69	359.68	359.42
3	Calorie From Fat	Kcal 100 g^{-1}	56.61	47.42	46.94	48.15	47.12	48.86
4	Total Lipid	%	6.29	5.34	5.22	5.35	5.24	5.60
5	Moisture Content	%	11.35	10.04	9.28	9.07	9.30	10.11
6	Crude Fiber	%	1.71	1.88	2.08	1.94	1.82	1.91
7	Carbohydrate	%	36.36	39.71	39.73	39.85	39.92	39.93
8	Ash Content	%	9.38	8.52	8.49	8.50	8.58	8.29
9	L-Alanine	%	1.97	2.19	2.31	2.17	2.23	2.19
10	L-Arginine	%	2.32	2.18	2.13	2.29	2.32	2.65
11	L-Aspartic Acid	%	2.27	2.67	2.72	2.37	2.75	2.64
12	Glycine	%	2.74	2.63	2.61	2.63	2.68	2.66
13	L-Glutamic Acid	%	5.30	4.92	4.82	4.80	4.88	4.85
14	L-Histidine	%	1.12	0.93	0.93	0.98	0.97	1.02
15	L-Isoleucine	%	1.63	1.68	1.73	1.74	1.76	1.74
16	L-Cystine	%	2.26	1.68	1.67	1.66	1.69	1.71
17	L-Leucine	%	2.68	2.61	3.12	2.63	2.92	2.96
18	L-Lysine	%	1.97	2.04	1.95	1.98	2.10	2.10
19	L-Methionine	%	0.62	0.63	0.64	0.64	0.67	0.67
20	L-Tryptophan	%	0.14	0.15	0.17	0.21	0.13	0.14
21	L-Valine	%	2.07	2.05	2.08	2.08	2.11	2.09
22	L-Phenylalanine	%	1.88	1.87	1.83	1.88	1.83	2.11
23	L-Proline	%	2.44	2.56	2.47	2.57	2.31	2.49
24	L-Serine	%	0.85	1.51	1.53	1.54	1.44	1.42
25	L-Threonine	%	1.03	1.12	1.09	1.09	1.17	1.19
26	L-Tyrosine	%	0.61	0.59	0.50	0.51	0.75	1.33

* Analysis conducted by the Saraswanti Indo Genetech Laboratory, Bogor, West Java, Indonesia. Website www.siglaboratory.com

Table 3. Water quality during the 81 days feeding trial in hapa nets installed within a commercial pond. Data is presented as mean± standard deviation (range).

Parameters	Unit	Analysis results
Dissolved oxygen	mg L^{-1}	7.195 ± 1.808
Temperature	^{0} C	30.569 ± 0.997
Salinity	‰	25.461 ± 0.912
pH		8.848 ± 0.159
Total dissolved solid	mg L^{-1}	25.504 ± 0.819
Ammonia-nitrogen (NH_3-N)	mg L^{-1}	0.011 ± 0.008
Nitrite-nitrogen (NO_2-N)	mg L^{-1}	0.024 ± 0.008
Nitrate-nitrogen (NO_3-N)	mg L^{-1}	3.118 ± 0.977
Phosphate (PO_4-N)	mg L^{-1}	0.013 ± 0.005

Table 4. Performance of pacific white shrimp *Penaeus vannamei* (2.28 ± 0.03 g initial mean weight) fed experimental diets for 81 d in an out-door ponds. Values represent the mean of six replicates. Results in the same column with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by Tukey's multiple comparison test.

Diet code	**Final Biomass (g)**	**Final Mean Weight (g)**	**Survival (%)**	**PWG[1] (%)**	**FCR[2]**	**TGC[3]**	**ADG[4]**
Control	5991.00^{c}	22.12^{c}	90.30^{a}	865.14^{c}	1.2433^{b}	0.6005^{c}	0.2417^{b}
Basal	5286.33^{d}	20.24^{d}	87.07^{b}	791.37^{d}	1.3740^{a}	0.5692^{d}	0.2183^{c}
0.075% FPE	6024.17^{c}	22.14^{c}	90.70^{a}	876.84^{bc}	1.2419^{b}	0.6029^{c}	0.2423^{b}
0.1% FPE	6130.50^{bc}	22.43^{bc}	91.11^{a}	882.74^{abc}	1.2267^{bc}	0.6066^{bc}	0.2457^{a}
0.125% FPE	6352.83^{a}	22.82^{a}	92.78^{a}	904.81^{a}	1.2009^{c}	0.6141^{a}	0.2506^{a}
0.15% FPE	6252.33^{ab}	22.78^{ab}	91.49^{a}	903.25^{ab}	1.2033^{c}	0.6134^{ab}	0.2501^{a}
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
PSE	39.7789	0.0950	0.6676	6.9455	0.0073	0.0018	0.0017

Note: [1] PWG = Percentage weight gain; [2] FCR= Feed conversion ratio; [3] TGC = Thermal growth coefficient; [4] ADG=Average daily growth in gram per day, [5] PSE = Pooled standard error

Table 5. Nutritional profile[1] of shrimp at the end of the growth trial

No	Parameter	Unit	Control	Basal	0.075% FPE	0.1% FPE	0.125% FPE	0.15% FPE
1	Protein Content	%	20.83	20.28	21.20	20.78	21.30	21.10
2	Total Calories	Kcal/100 g	127.07	101.35	105.24	102.72	100.19	106.75
3	Calorie From Fat	Kcal/100 g	2.43	8.19	6.48	2.88	3.51	3.87
4	Total Fat	%	0.27	0.91	0.72	0.32	0.39	0.43
5	Moisture Content	%	66.90	74.13	73.02	73.14	73.87	72.21
6	Crude Fiber	%	0.32	0.45	<0.02	0.88	0.47	0.14
7	Carbohydrate	%	10.33	3.01	3.49	4.18	2.87	4.62
8	Ash Content	%	1.67	1.67	1.57	1.58	1.57	1.64
9	L-Alanine	%	1.07	1.11	1.20	1.14	1.13	1.13
10	L-Arginine	%	1.65	1.61	1.68	1.60	1.68	1.65
11	L-Aspartic Acid	%	1.49	1.43	1.71	1.69	1.58	1.61
12	Glycine	%	1.34	1.28	1.36	1.25	1.55	1.23
13	L-Glutamic Acid	%	2.67	2.61	3.08	2.99	3.04	2.96
14	L-Histidine	%	0.30	0.28	0.27	0.29	0.26	0.32
15	L-Isoleucine	%	0.72	0.71	0.74	0.73	0.78	0.79
16	L-Cystine	%	0.45	0.29	0.43	0.42	0.45	0.47
17	L-Leucine	%	1.19	1.17	1.24	1.23	1.33	1.37
18	L-Lysine	%	1.24	1.14	1.27	1.22	1.25	1.28
19	L-Methionine	%	0.44	0.33	0.46	0.12	0.12	0.46
20	L-Tryptophan	%	0.15	0.16	0.15	0.12	0.01	0.18
21	L-Valine	%	0.56	0.56	0.58	0.59	0.57	0.64
22	L-Phenylalanine	%	0.87	0.88	0.88	0.87	0.95	1.00
23	L-Proline	%	0.99	1.09	1.27	1.11	1.11	1.18
24	L-Serine	%	0.66	0.64	0.70	0.63	0.64	0.70
25	L-Threonine	%	0.69	0.68	0.71	0.68	0.71	0.74
26	L-Tyrosine	%	0.42	0.41	0.41	0.42	0.44	0.53

[1]Analysed at Saraswanti Indo Genetech laboratory (Bogor, West Java, Indonesia)