ORIGINAL RESEARCH

Effects of a functional palatability enhancer on growth performance, digestive physiology, and immune response in the Pacific white shrimp (*Penaeus vannamei*) fed low-quality fishmeal diets

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ABSTRACT. This study investigated the effects of a functional palatability enhancer on the growth performance, digestive enzyme activity, immune response, gut histology, and thermal shock resistance of the Pacific white shrimp (*Penaeus vannamei*). Shrimp were fed four experimental diets for 56 days: a negative control without the palatability enhancer, two diets supplemented with the additive at 0.08% and 0.2%, and a positive control formulated with higher quality fishmeal. Shrimp fed palatability enhancer supplemented diets exhibited significantly higher final body weight, weekly weight gain, and daily growth coefficient compared to the negative control ($p < 0.05$), with performance comparable to the positive control ($p > 0.05$). No significant differences in feed intake, feed conversion ratio, or survival were observed ($p > 0.05$). The palatability enhancer modulated the activity of digestive enzymes, with increased chymotrypsin activity at 0.08% inclusion and increased trypsin activity at 0.2% inclusion ($p < 0.05$). Total hemocyte count was also elevated in the 0.08% palatability enhancer group ($p < 0.05$), suggesting a potential for enhanced immune function. No significant effects were observed on gut histology or thermal shock resistance ($p > 0.05$). These findings suggest that the palatability enhancer can effectively enhance growth performance in the Pacific white shrimp, potentially by modulating digestive processes and improving nutrient utilization efficiency, offering a potential cost-effective alternative to using higher quality protein sources in shrimp diets.

Key words: Aquaculture nutrition, digestive enzymes, feed additive, shrimp farming.

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Efectos de un potenciador funcional de la palatabilidad sobre el rendimiento del crecimiento, la fisiología digestiva y la respuesta inmune en el camarón blanco del Pacífico (*Penaeus vannamei*) alimentado con dietas de harina de pescado de baja calidad

RESUMEN. Este estudio investigó los efectos de un potenciador de la palatabilidad funcional sobre el rendimiento del crecimiento, la actividad de enzimas digestivas, la respuesta inmunitaria, la histología intestinal y la resistencia al choque térmico del camarón blanco del Pacífico (*Penaeus vannamei*). Los camarones se alimentaron con cuatro dietas experimentales durante 56 días: un control negativo sin el potenciador de la palatabilidad, dos dietas suplementadas con el aditivo al 0,08% y

0,2%, y un control positivo formulado con harina de pescado de mayor calidad. Los camarones alimentados con dietas suplementadas con el potenciador de la palatabilidad mostraron un peso corporal final, una ganancia de peso semanal y un coeficiente de crecimiento diario significativamente mayores en comparación con el control negativo ($p < 0,05$), con un rendimiento comparable al del control positivo ($p > 0,05$). No se observaron diferencias significativas en el consumo de alimento, la tasa de conversión alimenticia ni la supervivencia ($p > 0,05$). El potenciador de la palatabilidad moduló la actividad de las enzimas digestivas con aumento de la actividad de la quimotripsina con una inclusión del 0,08%, y un aumento de la actividad de la tripsina con una inclusión del 0,2% ($p < 0$,05). El recuento total de hemocitos también se elevó en el grupo con una inclusión del 0,08% de potenciador de la palatabilidad ($p < 0,05$), lo que sugiere un potencial para mejorar la función inmunitaria. No se observaron efectos significativos en la histología intestinal ni en la resistencia al choque térmico ($p > 0,05$). Estos hallazgos sugieren que el potenciador de la palatabilidad puede mejorar eficazmente el rendimiento del crecimiento del camarón blanco del Pacífico, posiblemente modulando los procesos digestivos y mejorando la eficiencia de la utilización de nutrientes, ofreciendo una alternativa potencialmente rentable al uso de fuentes de proteína de mayor calidad en las dietas para camarones.

Palabras clave: Nutrición acuícola, enzimas digestivas, aditivo alimentario, cultivo de camarones.

INTRODUCTION

The aquaculture industry has been instrumental in meeting the ever-growing global demand for seafood products. Among the various species of aquatic organisms cultivated, the Pacific white shrimp (*Penaeus vannamei* Boone, 1931) stands as a prominent choice. It is highly valued for its rapid growth and adaptability to varied rearing conditions (Samocha and Prangnell 2019), and it has exceptional market demand, being the most produced aquatic animal species worldwide (FAO 2024). However, achieving optimal growth performance, health, and environmental sustainability in shrimp aquaculture remains a continuous endeavor. A key challenge is the development of cost-competitive and nutritionally balanced diets promoting efficient growth, robust health, and overall well-being in farmed shrimp (Emerenciano et al. 2022).

In recent years, there has been a growing interest in palatability enhancers and their potential to stimulate feeding behavior and attractability of formulated diets. These enhancers often contain a mixture of free amino acids, nucleotides, and flavoring substances known to elicit strong feeding responses (Derby et al. 2016; Zhu et al. 2019; Terrey et al. 2021; He et al. 2022). Palatability enhancers help shrimp aquaculture to stimulate increased food consumption and optimize feeding efficiency, ultimately improving the overall performance and resilience of cultured shrimp (Jannathulla et al. 2021; Nunes et al. 2022). These additives are formulated to enhance the appeal and taste of shrimp diets, thereby reducing the time taken for shrimp to initiate feeding and increasing how fast feed is consumed and/or the overall amount of feed consumed. By focusing on improving consumption-related variables, such as feed intake and feeding rate, palatability enhancers reduce the leaching of feed nutrients into the water and the amount of uneaten feed in ponds, hence contributing significantly to the efficiency and growth of shrimp in aquaculture settings. Moreover, environmental contamination is reduced and healthier environmental conditions are maintained.

Different types of palatability enhancers have gained attention. For instance, krill meal significantly improved feed pellets palatability for Pacific white shrimp (Derby et al. 2016). Authors showed that the enhancement was concentration-dependent, characterized by the prolongation of feeding bouts and increased pellet consumption, without affecting the rate of pellet consumption. Likewise, a study by Terrey et al. (2021) demonstrated that, while the addition of squid meal and krill oil had no significant effect, the inclusion of insect protein hydrolysate significantly improved the palatability of shrimp diets. These authors argue that this was because the

hydrolysate contains high levels of free amino acids. Results indicated that even at 1% inclusion levels, insect protein hydrolysate could enhance the palatability of shrimp diets. This was also argued by Bøgwald et al. (2024) when evaluating the use of a copepod hydrolysate as a feed attractant for shrimp diets. Authors found that the additive significantly improved feed intake, which was explained by the presence of water soluble low molecular weight peptides and known chemoattractive amino acids.

In addition to improving feed consumption, palatability enhancers may also exhibit functional effects extending to various critical aspects of shrimp nutrition and health. These additives have the potential to influence the digestive enzymes and gut morphology of aquatic animals, promoting increased nutrient absorption and utilization (Zhu et al. 2019; Choi et al. 2023; He et al. 2023). This, in turn, could significantly impact the overall health status of the shrimp, potentially reinforcing their resilience to stressors, such as salinity stress or thermal shock, by enhancing physiological adaptability and strengthening immunological responses.

Most studies have focused on specialty ingredients, commonly originating from the processing of marine or terrestrial animal by-products, as palatability enhancers. These ingredients need to fulfill the double role of supplying nutrients as well as chemoattractants. Another alternative is to employ conventional, readily-available, inexpensive ingredients and supplement diets with low-dose sensory additives to supply the specific chemoattractants eliciting the palatability-related response. In this study we evaluated a sensory additive as a palatability enhancer on the Pacific white shrimp diets in which high quality fishmeal was replaced by a local, more affordable fish meal. We hypothesized that supplementing low-quality fishmeal diets with a palatability enhancer would improve shrimp performance and physiological responses. The objective was to evaluate its effects on growth, digestive enzyme activity, immunological responses, gut histology, nutrient retention, and thermal shock stress resistance.

MATERIALS AND METHODS

The experiment was carried out at the Laboratório de Camarões Marinhos (LCM), part of the Aquaculture Department of the Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil.

Pacific white shrimp (*Penaeus vannamei*) juveniles with an initial weight of 2.91 ± 0.02 (mean ± SD) were used (Aquatec, Rio Grande do Norte, Brazil). Four experimental groups were evaluated during a 56-day essay. Diets were formulated according to the nutritional requirements of the species (NRC 2011). Experimental diets consisted of a negative control diet with no additive, a positive control diet without the additive but made with higher quality fish meal, and two diets containing increasing levels of a patented functional palatability enhancer (Luctamax® FPE AQUA, Lucta S.A., Bellaterra, Barcelona, Spain) - 0.08% and 0.20% (Tables 1 and 2). The product was a concentrated blend of selected amino acids and nucleotides, along with other flavoring substances, derived from both synthetic and natural sources, including yeast extracts and vegetable protein hydrolysates. This palatability enhancer aims to stimulate feed intake and diet attraction in shrimp, particularly in formulations with low palatability.

Sixteen tanks were used (four per treatment) with a useful capacity of 500 L, equipped with constant aeration and water heating. Each tank was stocked with 40 shrimp (80 animals m^{-3}). A clear water culture system was used, with 80% water exchange per day. During the experiment, dissolved oxygen and temperature were monitored twice a day, ammonia, nitrite, pH and salinity once a week (Table 3). Shrimp were fed four times a day (8:00 am, 12:00 pm, 2:00 pm, and 5:00 pm), and the consumption was verified after 90 min to monitor the feed consumption. All shrimp of every tank were sampled and weighed weekly to adjust the feed input.

Table 1. Formulation of experimental diets. NC: negative control.

Ingredient (%)	Positive control	Negative control	NC + 0.08%	NC + 0.2%
Soybean meal[a]	26.38	31.00	31.00	31.00
Wheat flour[a]	18.50	16.00	16.00	16.00
Poultry viscera meal[b]	12.50	15.00	15.00	15.00
Salmon meal[c]	9.50	0.00	0.00	0.00
Fish meal[d]	7.50	12.53	12.53	12.53
Soy lecithin[e]	1.80	1.80	1.80	1.80
Monocalcium phosphate[e]	2.50	2.50	2.50	2.50
Magnesium sulphate[e]	2.00	1.60	1.60	1.60
Sodium chloride[e]	1.50	1.50	1.50	1.50
Potassium chloride[e]	1.50	1.50	1.50	1.50
DL-methionine[e]	0.25	0.50	0.50	0.50
Vitamin premix[f]*	1.00	1.00	1.00	1.00
Mineral premix[f]*	1.50	1.50	1.50	1.50
Vitamin C[g]	0.07	0.07	0.07	0.07
Fish oil[c]	1.50	1.50	1.50	1.50
Soybean oil[a]	1.50	1.50	1.50	1.50
Carboxymethylcellulose[e]	0.50	0.50	0.50	0.50
Kaolin[e]	10.00	10.00	9.92	9.80
Palatability enhancer[h]	0.00	0.00	0.08	0.20
Total	100.00	100.00	100.00	100.00

[a]Cocamar Cooperativa Agroindustrial, Maringá, Brazil.
[b]BRF Ingredients, São Paulo, Brazil.
[c]Tectron Imp e Exp Prod Veterinários LTDA, Toledo, Brazil.
[d]Indústria de Farinha de Peixe Kenya LTDA, Itajaí, Brazil.
[e]Rhoster Industria e Comércio LTDA, Araçoiaba da Serra, Brazil.
[f]In Vivo Nutrição e Saúde Animal LTDA, São Paulo, Brazil.
[g]ROVIMIX STAY-C 35 - DSM Produtos Nutricionais Brasil AS, Botucatu, Brazil.
[h]LUCTA S.A., Barcelona, Spain.
*Vitamin and mineral premix compound by 900 mg kg^{-1} vitamin A. 25 mg kg^{-1} vitamin D. 46.900 mg kg^{-1} vitamin E. 14.000 mg kg^{-1} vitamin K. 20.000 mg kg^{-1} vitamin B2. 40.000 mg kg^{-1} pantothenic acid. 70.000 mg kg^{-1} niacin. 50 mg kg^{-1} vitamin B12. 750 mg kg^{-1} biotin, 3000 mg kg^{-1} folic acid. 30.000 mg kg^{-1} vitamin B1. 33.000 mg kg^{-1} vitamin B6. 20 mg kg^{-1} magnesium, 6.1 mg kg^{-1} potassium. 23.330 mg kg^{-1} copper. 1.000 mg kg^{-1} iodine. 6.500 mg kg^{-1} manganese. 100 g kg^{-1} zinc. 125 mg kg^{-1} selenium.

Table 2. Proximal composition of the experimental diets used in the feeding trial. NC: negative control.

Variable	Positive control	Negative Control	NC + 0.08%	NC + 0.2%
Crude protein[1] (g 100 g^{-1})	34.4	35.9	34.8	35.2
Lipid[2] (g 100 g^{-1})	6.6	6.6	6.1	6.3
Moisture[2] (g 100 g^{-1})	9.8	7.3	9.1	10.5
Ash[2] (g 100 g^{-1})	21.7	23.1	21.8	21.6
Crude fibre[2] (g 100 g^{-1})	2.4	3.2	3.8	3.8

[1]Nitrogen determination by Kjeldahl method (ISO 1871: 2009), followed by multiplication of the values by 6.25.
[2](CBAA, 2017).

At the end of the experiment, the shrimps were weighed and counted for the determination of the following variables:

$$\text{Final mean weight (g)} = \frac{\text{Fbiom}}{\text{Number of animals}}$$

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}}{\text{Fbiom} - \text{Ibiom}}$$

$$\text{Survival (\%)} = \frac{\text{FNshrimp}}{\text{INshrimp}} \times 100$$

$$\text{WeeklyG (g week}^{-1}) = \frac{(\text{FMweight} - \text{IMweight})}{\text{ExperimentDW}}$$

$$\text{DailyG (\% day}^{-1}) = \frac{\text{FMweight}^{1/3} - \text{IMweight}^{1/3}}{\text{ExperimentDD}} \times 100$$

where Fbiom: Final biomass, Ibiom: Initial biomass, FNshrimp: Final number of shrimp, INshrimp: Initial number of shrimp, WeeklyG: Weekly growth, FMweight: Final mean weight, IMweight: Initial mean weight, ExperimentDW: Experiment duration in weeks, ExperimentDD: Experiment duration in days. Feed intake refers to the total amount of feed supplied per tank. Feed leftovers were not recorded.

The daily growth coefficient was calculated according to (Glencross et al. 2007).

Enzymatic activity

After the feeding trial, the shrimp were sampled and dissected for the collection of hepatopancreas, which were stored in falcon tubes in a freezer at -80 °C. The hepatopancreas were homogenized in 1 mL of Milli-Q water with the help of pestle and mortar, containing only one hepatopancreas per tube and centrifuged at 15,000 × g for thirty minutes at 4 °C. The supernatant was then collected for the analyses, which were performed in triplicate.

Trypsin type activity was measured using N-benzoyl-DL-arginine p-nitroanilide (bz-R-pNA) as a substrate, according to the protocol adapted from Erlanger et al. (1961) and DelMar et al. (1979). This synthetic substrate was originally dissolved in dimethyl sulfoxide and dibasic sodium phosphate buffer 50 mM, pH 7.5 at a concentration of 4 mM. The analyses were made by incubating 50 µL of the sample with 50 µL of the substrate/buffer solution in bain-marie (concentration of the substrate in the reaction medium of 2 mM) for at least four different periods of time at 30 °C. The reactions were

Table 3. Values of water quality variables throughout the 56-day grow-out experiment.

Variable	Overall mean values	One-way ANOVA *p*-value
Dissolved oxygen (mg L^{-1})	5.89 ± 0.46	0.574
Temperature (°C)†	28.3 ± 0.8	< 0.001
TAN (mg L^{-1})	1.27 ± 0.57	0.938
Nitrite-N (mg L^{-1})	0.06 ± 0.07	0.872
Alkalinity (mg $CaCO_3$ L^{-1})	124 ± 5	0.714
pH	8.11 ± 0.05	0.461
Salinity (‰)	32.3 ± 0.8	1.000

Data presented as mean ± standard deviation. Differences considered statistically significant when $p < 0.05$. TAN: Total ammonia nitrogen.

†There was a significant difference by Tukey's test only for the Positive control group (27.9 ± 0.8 °C) compared with the remaining ones (overall mean value without Positive control: 28.3 ± 0.1 °C). Despite the statistical significance, the magnitude of the difference was small, as demonstrated by the measure of effect size η^2 of 0.03, which is equivalent to the R^2 measure commonly used for regression analyses.

stopped with 100 µL of acetic acid 30% (v/v) and then absorbance reads were performed at 410 nm in a microplate reader TECAN (Infinite Pro, California, USA).

Chymotrypsin activity was measured with 4 mM of N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (suc-AAPF-pNA) synthetic substrate that was dissolved in dimethyl sulfoxide and dibasic sodium phosphate buffer at 50 mM, pH 7.5. The analyses were made with 50 µL of the sample with 50 µL of the substrate/buffer in bain-marie (concentration of the substrate in the reaction medium of 2 mM) for at least four different periods of time at 30 °C. The reactions were stopped with 100 µL of acetic acid 30% (v/v), and then absorbance reads were performed at 410 nm in a microplate reader TECAN (Infinite Pro, California, USA).

For both trypsin and chymotrypsin, a standard curve with p-nitroanilide was used as a reference for the activity calculations. A unity of the proteolytic enzymatic activity against substrates derived from p-nitroanilide was defined as the amount of enzyme capable of liberating the equivalent of 1 µmol of p-nitroanilide per minute.

Lipase activity was measured with methylumbelliferone oleate, a synthetic substrate that was dissolved in acetonitrile (ACN) at a concentration of 1 µM. The analyses were made with 50 µL of sample and 50 µL of substrate-buffer (Tris-HCl 100 mM, pH 7.5) with fluorescence reading at an excitation of 355 nm and emission of 460 nm in plate fluorimeter. A standard curve with methylumbelliferone was used as reference for calculation of the activity. Lipolytic activity was calculated according to (Winkler and Stuckmann 1979), with slight modifications. One unit of enzymatic activity (U) was defined as the quantity of enzyme capable of liberating 1 µmol of methylumbelliferone per minute.

For the α-amylase assay, the protocol adapted from (Meyer et al. 1948) was used. First, a buffer with sodium acetate 50 mM, NaCl 20 mM, $CaCl_2$ 2 mM, pH 5.5 and starch 1% (m/v) was made. Twenty-five µL of the samples and 25 µL of starch substrate (m/v) were incubated in the sodium acetate buffer described previously, placed in bain-marie at 30 °C. The reactions were stopped with the addition of 100 µL of DNS (dini-

trosalicylic acid) in at least four-time intervals. The samples were then heated at 100 °C during five minutes and 100 µL of distilled water was added at the end. Later, absorbance reads were made at 550 nm in a microplate reader TECAN (Infinite Pro, California, USA). A standard curve with glucose was used as a reference for the enzymatic activity calculations. It was then considered that one activity unit (U) represents the amount of enzyme that liberates 1 µmol of glucose equivalent per minute.

For the determination of proteins, the method based on the adsorption of the reagent Coomassie Brilliant Blue G-250, proposed by (Bradford 1976), was employed using serum cattle albumin as a standard.

Histology

For histological analysis, five shrimp per experimental unit were collected, dissected on the second abdominal segment to remove cuts of the midgut, and fixed in Davidson solution for 24 h. After this period, the samples were transferred to alcohol 70° GL where they remained immersed. The samples were then transferred to the AQUOS laboratory of the Universidade Federal de Santa Catarina (UFSC), in Florianópolis, Santa Catarina, Brazil, for the analyses.

At the AQUOS laboratory, the samples were stored in histocassettes, followed by the standard histological procedure of dehydration, diaphanization, paraffinization and inclusion in paraffin blocks. After this, the blocks were cut with the help of a manual microtome (3 to 5 µm), the slices were collected in slides and dyed with the H&E method. After the coloration, the slides were mounted in Entellan® medium.

At the end, the slides were analyzed in a differential interference contrast microscope Axio Imager A.2 (DIC) (Zeiss, Gottingen, Germany) and the following measurements were made: mean length, mean width, total area and number of gut folds, according to (Schleder et al. 2018).

Shrimp nitrogen and phosphorus content

Whole shrimp samples (≈ 90 g) from each replicate were collected at the end of the experiment for the determination of nitrogen by the Kjeldahl method (ISO 1871: 2009) and phosphorus (CBAA 2017) contents.

Immunological analysis

Hemolymph from five shrimp was collected from the ventral sinus to form a pool per experimental unit with the help of 1 mL sterile syringes with a 21G needle cooled at 4 °C. From the hemolymph collected, 10 µL were fixed in modified Alsever anticoagulant solution (MAS) (27 mM sodium citrate, 9 mM EDTA, 115 mM glucose, 336 mM NaCl, pH 7.2) with 4% formaldehyde for total hemocyte count (THC). The remaining hemolymph was coagulated at 4 °C and centrifuged at 10,000 × g for 10 min to obtain serum, which was aliquoted and stored at -20 °C for analysis of phenoloxidase activity, serum protein concentration and serum agglutinating activity, following the methodology described in detail in (Bolívar-Ramírez et al. 2022).

Thermal shock resistance

After eight weeks, 10 shrimp per tank were transported in plastic containers of 20 L, containing water at 28 °C and transferred simultaneously to 60 L containers containing cold saltwater at 10.9 °C, which was established according to a previous test, and kept for one hour. The temperature of the cold water was constantly monitored. Afterwards, the animals were transported back to containers with water at 28 °C. Survival was monitored for 48 h.

Statistical analysis

Data was submitted to Shapiro-Wilk and Levene tests to evaluate normality and homoscedasticity, respectively. When the prerequisites were

met, data was submitted to one-way Analysis of Variance (ANOVA), followed by Fisher's Least Significant Difference (LSD) test to differentiate the means. When the normality assumption was not met, non-parametric Kruskall-Wallis test was applied, followed by Dwass-Steel-Critchlow-Fligner pairwise comparisons. Thermal-shock data was analyzed by Kaplan-Meier test. In all cases, differences were considered significant when p-value < 0.05. The size effect was reported as partial η^2 for ANOVA models and ε^2 for Kruskal-Wallis tests (supplementary material, Table S1). The statistical analyses were performed with Jamovi software (The Jamovi Project 2022).

RESULTS

Shrimp final body weight, weekly weight gain, and daily growth coefficient were significantly different between treatments ($p < 0.05$) (Table 4). The same pattern was found in all three cases, with shrimp fed diets with the functional palatability enhancer exhibiting higher values when compared to the negative control, whilst shrimp from the positive control group showed no difference from the remaining groups. There were no significant differences in total feed intake, feed conversion ratio and survival ($p > 0.05$) (Table 4), whose overall mean values were 963.23 ± 18.52 g, 1.55 ± 0.05 and 96.4 ± 4.3%, respectively.

Enzymatic activity

Significant differences were found in shrimp hepatopancreas amylase, chymotrypsin, and trypsin activities ($p < 0.05$) (Figure 1). Amylase activity was higher in the negative control group than in the positive control and negative control + 0.08% additive inclusion, whereas the enzymatic activity in the negative control + 0.2% did not differ from the other groups. Chymotrypsin activity was higher in the negative control + 0.08% compared with the negative control group, while positive and negative controls + 0.2% did not differ from the remaining treatments. Trypsin activity in the negative control + 0.2% was higher than in the negative control, but values of the positive and negative controls + 0.08% did not differ from the

Table 4. Performance variables of Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative and positive control, containing lower and higher quality fish meal, respectively) for 56 days in a clear water system. NC: negative control.

	Treatment				
Variable	Positive control	Negative control	NC + 0.08%	NC + 0.20%	p-value
Final body weight (g)	$19.51^{ab} \pm 0.47$	$18.87^{a} \pm 0.30$	$19.84^{b} \pm 0.26$	$19.98^{b} \pm 0.71$	0.026
Total feed intake (g)	973.89 ± 19.88	966.49 ± 13.18	968.15 ± 19.89	944.40 ± 8.94	0.103
FCR	1.54 ± 0.07	1.58 ± 0.03	1.55 ± 0.05	1.52 ± 0.04	0.346
Weekly weight gain (g week^{-1})	$2.08^{ab} \pm 0.06$	$2.00^{a} \pm 0.04$	$2.11^{b} \pm 0.03$	$2.14^{b} \pm 0.09$	0.028
Daily growth coefficient (% day^{-1})	$2.26^{ab} \pm 0.04$	$2.21^{a} \pm 0.03$	$2.28^{b} \pm 0.02$	$2.30^{b} \pm 0.06$	0.034
Survival (%)	97.5 ± 3.5	98.1 ± 1.3	95.0 ± 4.1	95.0 ± 7.0	0.661

Data presented as mean ± standard deviation. Fisher's one-way Analysis of Variance (ANOVA) p-value. Different letters across rows indicate significant differences by Fisher's Least Significant Difference (LSD) test. Differences were considered significant when $p < 0.05$. n = 4 tanks per treatment.

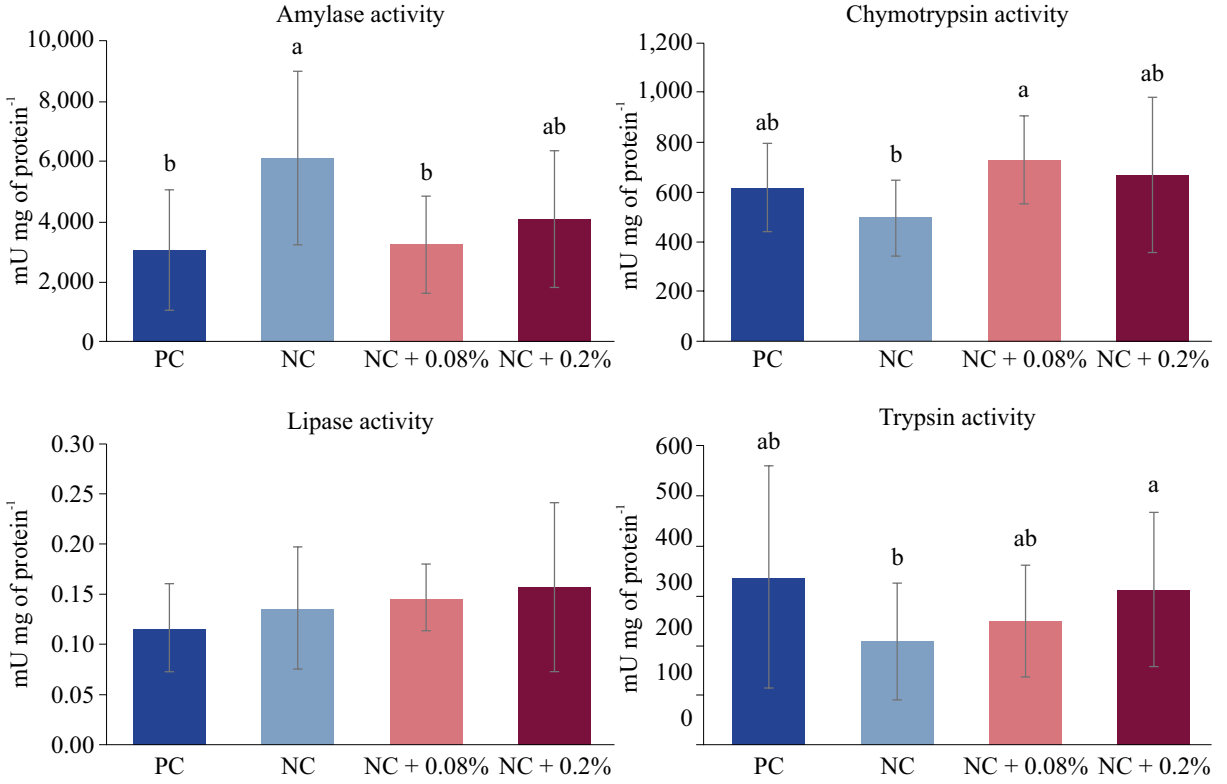

Figure 1. Digestive enzyme activities in the hepatopancreas of the Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative (NC) and positive control (PC), containing lower and higher quality fish meal, respectively) for 56 days in a clear water system. Data presented as mean ± standard deviation. Kruskal-Wallis *p*-value for amylase (< 0.001), chymotrypsin (0.001), lipase (0.070) and trypsin (0.032), followed by Dwass-Steel-Critchlow-Fligner pairwise comparison. Means were considered significant when $p < 0.05$. n = 24 shrimp per treatment (6 shrimp per tank).

other groups. Lipase activity was not significantly different between treatments ($p > 0.05$) (Figure 1), with the overall mean value being 0.14 ± 0.06 mU mg of protein^{-1}.

Histology

During the histological analysis, only slides with suitable tissue were measured. No necrosis or infiltrates were found. The shrimp gut histomorphometry variables fold width, length, area and number of folds did not differ between treatments ($p > 0.05$) (Table 5). The overall mean values were 27.6 ± 7.5 µm width, 31.6 ± 17.5 µm length, 5808.4 ± 1701.6 µm^2 area and 71.4 ± 27.8 folds.

Shrimp nitrogen and phosphorus content

No significant differences were found in shrimp nitrogen and phosphorus content ($p > 0.05$) (Table 6). The overall mean values were 2.7 ± 0.32% of wet weight for nitrogen and 0.3 ± 0.02% of wet weight for phosphorus.

Immunological analysis

Total hemocyte count (THC) in shrimp hemolymph differed between treatments ($p < 0.05$) (Table 7), with the highest values found in the positive control and negative control + 0.08% compared with the negative control. The negative control +

Table 5. Gut histomorphometry of Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative and positive control, containing lower and higher quality fish meal, respectively) for 56 days in a clear water system. NC: negative control.

	Treatment				
Variable	Positive control	Negative control	NC + 0.08%	NC + 0.20%	p-value
Width (μm)	25.7 ± 5.8	29.7 ± 8.4	24.1 ± 8.8	30.4 ± 6.4	0.371
Length (μm)	36.5 ± 17.9	30.3 ± 20.7	22.0 ± 6.6	37.7 ± 20.7	0.188†
Area (μm^2)	5,879.6 ± 1,578.9	6,811.6 ± 1,906.0	4,830.4 ± 2,068.8	5,992.6 ± 1,019.7	0.253
Number of folds	65.8 ± 36.7	78.4 ± 26.6	74.1 ± 28.8	68.1 ± 26.2	0.888

Data presented as mean ± standard deviation. Fisher's one-way Analysis of Variance (ANOVA) *p*-value.
†Welch's ANOVA *p*-value. n = 5 shrimp per treatment.

0.2% group was intermediate and did not differ from the other ones. Serum protein, phenoloxidase enzyme activity and agglutination titer did not differ between treatments ($p > 0.05$) (Table 7).

Thermal shock resistance

The Kaplan-Meier survival curves for treatments following the thermal shock were analyzed. According to the log-rank test, there were no significant differences in survival between treatment groups ($p > 0.05$) (Figure 2). The overall final mean survival was 47.2 ± 20.0%.

DISCUSSION

The aquaculture industry strives to provide high-quality feeds while balancing nutritional needs with economic considerations and ingredient availability. However, cheaper and more accessible ingredients often have lower biological value, poorer attractability, and greater quality fluctuations compared to traditional fishmeal. Therefore, interest in different types of feed additives, such as antioxidants, probiotics, organic acids and attractants as a means to fill gaps in the feed formulations due to the use of lower quality ingredients is growing (Nunes et al. 2022; Chen et al. 2024). Among these, functional feed additives, in particular palatability enhancers, can allow for not only improvements in feed intake and attraction but also potentially enhance nutrient utilization and overall animal resilience (Zhu et al. 2019; Li et al. 2025).

Inclusion of functional palatability enhancer improved shrimp final body weight, weekly weight gain, and daily growth coefficient at both 0.08% and 0.2% *versus* the negative control, with performance comparable to the positive control. This indicates the additive helped maintain growth when a high-quality fish meal was replaced by a local source. However, growth improved without changes in feed intake or FCR, indicating mechanisms other than intake stimulation. Prior work with this additive showed higher intake with growth (Zhu et al. 2019). Other enhancers raised intake without growth (Walsh et al. 2022), showed no effects (He et al. 2023; Qu et al. 2024), or matched our pattern of higher growth without intake changes (Silva-Neto et al. 2012). Intake was similar in negative and positive controls, suggesting fish meal replacement did not depress palatability. Effects on feed intake were not significant under the conditions evaluated, despite the fact that the aforementioned studies reported varying intake responses to palatability enhancers.

Table 6. Whole body nitrogen (N) and phosphorus (P) contents of Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative and positive control, containing lower and higher quality fish meal, respectively) for 56 days in a clear water system. NC: negative control.

	Treatment				
Variable	Positive control	Negative control	NC + 0.08%	NC + 0.20%	p-value
Nitrogen content (% of wet weight)	2.63 ± 0.24	2.73 ± 0.36	2.62 ± 0.51	2.82 ± 0.09	0.565
Phosphorus content (% of wet weight)	0.29 ± 0.02	0.31 ± 0.01	0.30 ± 0.01	0.30 ± 0.03	0.237

Data presented as mean ± standard deviation. Welch's one-way Analysis of Variance (ANOVA) *p*-value. n = 4 tanks per treatment (composite sample: 100 g shrimp per tank).

Table 7. Immunological variables of Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative and positive control, containing lower and higher quality fish meal, respectively) for 56 days in a clear water system. NC: negative control.

	Treatment				
Variable	Positive control	Negative control	NC + 0.08%	NC + 0.20%	p-value
THC ($\times 10^6$ cells mL^{-1})	$39.3^{a} \pm 8.3$	$19.4^{b} \pm 8.5$	$44.5^{a} \pm 14.0$	$28.7^{ab} \pm 14.5$	0.044
Total protein (mg mL^{-1})	221.7 ± 57.5	206.7 ± 13.9	236.0 ± 70.0	207.7 ± 48.3	0.837
PO activity (U min^{-1} mg^{-1} protein)	13.0 ± 3.4	12.8 ± 3.3	11.5 ± 2.0	14.2 ± 2.7	0.627
Agglutination titer ($\log_2 \times + 1$)	9.54 ± 1.01	10.25 ± 0.96	10.25 ± 0.50	9.75 ± 0.96	0.588

THC: total hemocyte count. PO: phenoloxidase. Data presented as mean ± standard deviation. Fisher's one-way Analysis of Variance (ANOVA) *p*-value. Different letters across rows indicate significant differences by Fisher's Least Significant Difference (LSD) test. Differences were considered significant when $p < 0.05$. n = 4 pools per treatment. Hemolymph pooled from 5 shrimp per tank.

Functional palatability enhancers may also regulate digestion and nutrient use. The tested product contains bioactive compounds (e.g. amino acids, nucleotides) that can stimulate chemoreceptors and support enzyme synthesis, enabling modulation of digestive enzymes (Hossain et al. 2020, 2024). Enhanced protease activity has been a recurring outcome when this same additive (Zhu et al. 2019) and other enhancers (He et al. 2022) were used in Pacific white shrimp diets, with similar findings also occurring with other crustaceans fed diets with palatability enhancers, such as Chinese mitten crab (*Eriocheir sinensis*) (Li et al. 2025). Consistent with this aforementioned literature, the additive in our study modulated digestive enzyme activity. Amylase was lower in supplemented diets than in the negative control, suggesting carbohydrate-digestion compensation in unsupplemented shrimp. Proteases increased selectively (i.e. chymotrypsin at 0.08% and trypsin at 0.2%) in accordance with

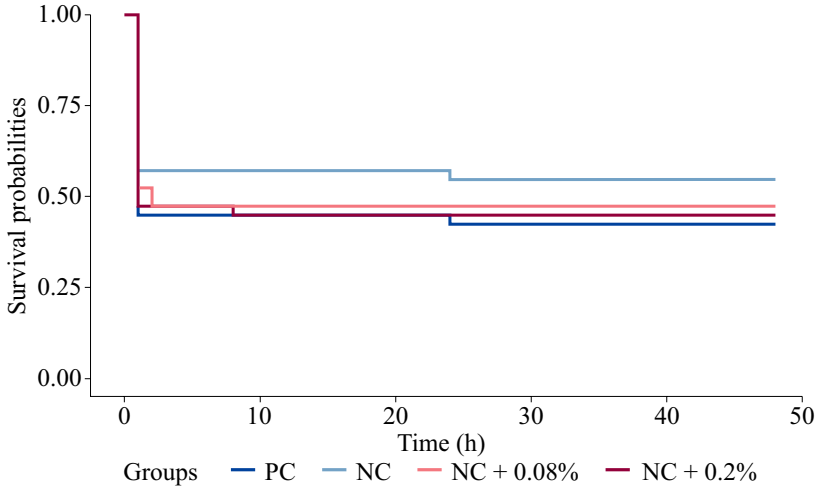

Figure 2. Kaplan-Meier survival curves of the Pacific white shrimp (*Penaeus vannamei*) fed diets containing a functional palatability enhancer (at 0.08% and 0.2% inclusion) and two control diets without the additive (negative (NC) and positive control (PC), containing lower and higher quality fish meal, respectively) for 56 days in a clear water system and submitted to a thermal shock (10.9 °C) for one hour. Log-rank test *p*-value = 0.710. n = 4 tanks per treatment (10 shrimp per tank).

previous results (Zhu et al. 2019), suggesting that protein digestion may have been stimulated, which aligns with the growth response observed. Lipase was unchanged, indicating no effect on lipid digestion. For enzymes showing effects, activities in supplemented groups were comparable to the positive control, indicating mitigation of drawbacks from the lower-quality protein source.

A better protein digestion, resulting in a higher amino acid availability, could potentially have a positive influence in the histological structure of shrimp digestive tract. Hence, it is not surprising that Zhu et al. (2019) observed a higher height of intestinal mucosal folds in its previous study of the functional palatability enhancer. Also, other studies with different palatability enhancers positively affected muscle thickness and mucosal fold width (He et al. 2023) or only muscle width (Qu et al. 2024). These changes, which effectively increased the surface area for nutrient absorption, may be associated with an improved weight gain and survival, as observed in the first two mentioned studies, but not in the latter. In the present study, however, no significant effects of the palatability enhancer on gut histology were found. Still, the absence of necrosis or infiltrates in all treatments is a positive finding, consistent with the observation of similar, and high, survival rates across all treatments.

The lack of significant differences in shrimp nitrogen and phosphorus content between the treatment groups suggests that the functional palatability enhancer, at the inclusion levels tested, did not significantly affect the retention or accumulation of these nutrients in shrimp tissues. Similar results were also observed by Zhu et al. (2019) and He et al. (2023), who found no significant differences in shrimp body protein were observed after feeding diets with the herein tested proprietary palatability enhancer and betaine, respectively, despite improved shrimp growth performance. In addition, Walsh et al. (2022) assessed shrimp body protein and phosphorus contents and found no significant differences after feeding animals with diets containing the attractants krill meal, squid meal and fish hydrolysate in soy-optimized diets, despite higher feed intake but no improved growth performance.

One of the previous trials performed with the same palatability enhancer tested herein observed a significantly higher survival after an acute salinity

shock of shrimp fed the supplemented feed, without significantly affecting growth (Tabbara et al. 2024). In view of those results, we further investigated some immunological parameters to assess the health status of shrimp fed the different diets. The higher THC observed in shrimp fed the diet supplemented with 0.08% of feed additive compared to the negative control indicates a potential for enhanced immune function. Hemocytes play an important role in the innate immune system of shrimp, being involved in phagocytosis, encapsulation, and the release of antimicrobial compounds (Ng et al. 2013; Yang et al. 2021). The positive control also had a significantly higher THC in hemolymph than the negative control, and a similar level as the 0.08% supplemented group. We interpret the THC increase as compatible with immune activation rather than stress-induced elevation, given unchanged survival and histology. However, without hemocyte subtype counts or stress biomarkers, a stress contribution cannot be excluded. Therefore, it might be concluded that the additive can similarly contribute to the shrimp's health status as a diet made with higher quality protein sources. This could be due to improved nutrient absorption and utilization, resulting in improved overall health and immune status.

However, contrary to what was observed in response to the salinity stress challenge by Tabbara et al. (2024), the functional palatability enhancer, at the inclusion levels tested, did not significantly affect the survival of the Pacific white shrimp after exposure to thermal shock in the present study. These results imply that the palatability enhancer, while being beneficial for growth and potentially improving immune function, did not confer a significant advantage in terms of resilience to acute temperature stress. Other palatability enhancers, such as DMPT and squid paste, did not improve stress resistance to hypoxia (Zhou et al. 2023). Nevertheless, it is possible that stress resistance might depend on the type of stressor, or the intensity of the stress applied. For instance, in the earlier study by (Tabbara et al. 2024), average survival of the control group following the salinity stress challenge was 67.3%, compared to 47.2% in the present temperature shock test, suggesting a more intense challenge in the present study.

CONCLUSIONS

This study demonstrates the beneficial effects of a functional palatability enhancer on the Pacific white shrimp diet. Enhancement in growth performance and potential modulation of digestive enzymes and immune status were observed, with no negative effects found in shrimp gut histology or differences in total feed intake. Similar results between the animals fed the supplemented diets and the positive control in terms of final body weight, weekly weight gain, daily growth coefficient, digestive enzyme activity and total hemocyte count suggest that the feed additive might offer an effective solution to use lower quality protein sources in shrimp diets while maintaining animal performance and health status. Overall, the results highlight the potential of the functional palatability enhancer to improve the efficiency and sustainability of shrimp aquaculture.

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Data statement

Data to support the findings of this study can be requested from the corresponding author upon reasonable demand.

Ethics statement

Not applicable-study not involving humans or vertebrate animals, according to the Brazilian law (Federal Law No 11.794, October 8th 2008, known as 'Lei Arouca').

Declaration of interest

Relationships: Sofia Morais and Thiago Raggi report a relationship with Lucta SA that include: employment. No further interests to declare.

Author contributions

Mateus Aranha Martins: formal analysis; investigation; writing original draft and review and editing; visualization. Jaqueline da Rosa Coelho: formal analysis; investigation; writing original draft. Cristina Rios: formal analysis; investigation; writing original draft. Carlos Peres Silva: investigation; resources; supervision. Tamiris Henrique Ferreira: formal analysis; investigation; writing original draft. Sofia Morais: conceptualization; methodology; formal analysis; resources; writing review and editing. Thiago Raggi: conceptualization; methodology; formal analysis; resources; writing review and editing. Walter Quadros Seiffert: resources; supervision. Felipe Boéchat Vieira: conceptualization; methodology; resources; writing review and editing; supervision; project administration; funding acquisition. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Bøgwald I, Herrig S, Pedersen AM, Wubshet SG, Eilertsen K-E. 2024. Effect of *Calanus finmarchicus* hydrolysate inclusion on diet attractiveness for whiteleg shrimp (*Litopenaeus vannamei*). Fishes. 9 (4): 134. DOI: https://doi.org/10.3390/fishes9040134

Bolívar-Ramírez NC, Mallmann AS, Schleder DD, Machado C, Seiffert WQ, do Nascimento Vieira F. 2022. Tannins as a food additive in pacific white shrimp diet. Aquaculture. 556: 738232. DOI: https://doi.org/10.1016/j.aquaculture.2022.738232

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72 (1): 248-254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

[CBAA] Compêndio Brasileiro de Alimentação Animal. 2017. São Paulo: Sindirações/ANFAL.

Chen Y, Mitra A, Rahimnejad S, Chi S, Kumar V, Tan B, Niu J, Xie S. 2024. Retrospect of fish meal substitution in Pacific white shrimp (*Litopenaeus vannamei*) feed: alternatives, limitations and future prospects. Rev Aquacult. 16 (1): 382-409. DOI: https://doi.org/10.1111/raq.12843

Choi W, Moniruzzaman M, Hamidoghli A, Bae J, Lee Seunghyung, Lee Seunghan, Min T, Bai SC. 2023. Effect of four functional feed additives on growth, serum biochemistry, antioxidant capacity, gene expressions, histomorphology, digestive enzyme activities and disease resistance in juvenile olive flounder, *Paralichthys olivaceus*. Antioxidants. 12 (8): 1494. DOI: https://doi.org/10.3390/antiox12081494

DelMar EG, Largman C, Brodrick JW, Geokas MC. 1979. A sensitive new substrate for chymotrypsin. Anal Biochem. 99 (2): 316-320. DOI: https://doi.org/10.1016/S0003-2697(79)80013-5

Derby CD, Elsayed FH, Williams SA, González C, Choe M, Bharadwaj AS, Chamberlain GW. 2016. Krill meal enhances performance of feed pellets through concentration-dependent prolongation of consumption by Pacific white shrimp, *Litopenaeus vannamei.* Aquaculture. 458: 13-20. DOI: https://doi.org/10.1016/j.aquaculture.2016.02.028

Emerenciano MGC, Rombenso AN, Vieira F DN, Martins MA, Coman GJ, Truong HH,

Noble TH, Simon CJ. 2022. Intensification of penaeid shrimp culture: an applied review of advances in production systems, nutrition and breeding. Animals. 12 (3): 236. DOI: https://doi.org/10.3390/ani12030236

Erlanger BF, Kokowsky N, Cohen W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch Biochem Biophys. 95 (2): 271-278. DOI: https://doi.org/10.1016/0003-9861(61)90145-X

[FAO] Food and Agriculture Organization of the United Nations. 2024. The state of world fisheries and aquaculture (SOFIA) 2024. Rome: FAO. DOI: https://doi.org/10.4060/cd0683en

Glencross BD, Booth M, Allan GL. 2007. A feed is only as good as its ingredients - a review of ingredient evaluation strategies for aquaculture feeds. Aquacult Nutr. 13 (1): 17-34. DOI: https://doi.org/10.1111/j.1365-2095.2007.00450.x

He G, Chen X, Zeng Q, Zhu W, Chen Z, Tan B, Xie S. 2022. Effects of compound feed attractants on growth performance, feed utilization, intestinal histology, protein synthesis, and immune response of white shrimp (*Litopenaeus Vannamei*). Animals. 12 (19): 2550. DOI: https://doi.org/10.3390/ani12192550

He G-L, Shi M-L, Liu Y-C, Chen L-T, Chen X, Zhu W-B, Chen Z-B, Tan B-P, Xie S-W. 2023. Effects of dietary betaine supplementation on growth performance, feed intake, intestinal histology, lipid metabolism, and immune response of black tiger shrimp (*Penaeus monodon*) fed diets containing two levels of raw feed attractants. Aquacult Int. 32: 653-673. [accessed 2023 Oct 3]. DOI: https://doi.org/10.1007/s10499-023-01180-x

Hossain MdS, Koshio S, Kestemont P. 2020. Recent advances of nucleotide nutrition research in aquaculture: a review. Rev Aquacult. 12 (2): 1028-1053. DOI: https://doi.org/10.1111/raq.12370

Hossain MdS, Small BC, Kumar V, Hardy R. 2024. Utilization of functional feed additives to produce cost-effective, ecofriendly aquafeeds high in plant-based ingredients. Rev Aquacult. 16 (1): 121-153. DOI: https://doi.org/10.1111/raq.12824

[ISO] International Organization for Standardization 2009. ISO 1871:2009 - Food and feed products. General guidelines for the determination of nitrogen by the Kjeldahl method.

Jannathulla R, Sravanthi O, Khan HI, Moomeen HS, Gomathi A, Dayal JS. 2021. Chemoattractants: their essentiality and efficacy in shrimp aquaculture. Indian J Fish. 68 (1): 151-159. [accessed 2024 Sep 5]. DOI: https://doi.org/10.21077/ijf.2021.68.1.95994-20

Li W, Li E, Wang S, Liu J, Wang M, Wang X, Qin C, Qin J, Chen L. 2025. Comparative effects of four feed attractants on growth, appetite, digestion and absorption in juvenile Chinese mitten crab (*Eriocheir sinensis*). Aquaculture. 594: 741441. DOI: https://doi.org/10.1016/j.aquaculture.2024.741441

Meyer KH, Noelting G, Bernfeld P. 1948. Recherches sur l'amidon XXXVII. Déermination du poids moléculaire de polysaccharides naturels par dosage colorimétrique. Helv Chim Acta. 31 (1): 103-105. DOI: https://doi.org/10.1002/hlca.19480310122

Ng TH, Chang S-H, Wu M-H, Wang H-C. 2013. Shrimp hemocytes release extracellular traps that kill bacteria. Dev Comp Immunol. 41 (4): 644-651. DOI: https://doi.org/10.1016/j.dci.2013.06.014

[NRC] National Research Council. 2011. Nutrient requirements of fish and shrimp. Washington: The National Academic Press. DOI: https://doi.org/10.17226/13039

Nunes AJP, Dalen LL, Leonardi G, Burri L. 2022. Developing sustainable, cost-effective and high-performance shrimp feed formulations containing low fish meal levels. Aquacult Rep. 27: 101422. DOI: https://doi.org/10.1016/j.aqrep.2022.101422

Qu K, He G, Shi M, Chen X, Zhu W, Chen Z, Tan B, Xie S. 2024. Effects of compound feed

attractants on the growth rate, feed consumption, intestinal histology, protein synthesis, and immune response of black tiger shrimp (*Penaeus monodon*). Anim Feed Sci Technol. 311: 115952. DOI: https://doi.org/10.1016/j.anifeedsci.2024.115952

Samocha TM, Prangnell DI. 2019. Water quality management. In: Samocha TM, editor. Sustainable biofloc systems for marine shrimp. Elsevier. p. 133-151. DOI: https://doi.org/10.1016/B978-0-12-818040-2.00007-1

Schleder DD, Peruch LGB, Poli MA, Ferreira TH, Silva CP, Andreatta ER, Hayashi L, do Nascimento Vieira F. 2018. Effect of brown seaweeds on Pacific white shrimp growth performance, gut morphology, digestive enzymes activity and resistance to white spot virus. Aquaculture. 495: 359-365. DOI: https://doi.org/10.1016/j.aquaculture.2018.06.020

Silva-Neto JF, Nunes AJP, Sabry-Neto H, Sá MVC. 2012. Spirulina meal has acted as a strong feeding attractant for *Litopenaeus vannamei* at a very low dietary inclusion level. Aquacult Res. 43 (3): 430-437. DOI: https://doi.org/10.1111/j.1365-2109.2011.02846.x

Tabbara M, Strebel L, Peixoto S, Soares R, Morais S, Davis DA. 2024. Use of passive acoustic monitoring to evaluate the effects of a feed effector on feeding behavior, growth performance, and salinity stress tolerance of *Litopenaeus vannamei*. Aquaculture. 582: 740499. DOI: https://doi.org/10.1016/j.aquaculture.2023.740499

Terrey D, James J, Tankovski I, Dalim M, van Spankeren M, Chakraborty A, Schmitt E, Paul A. 2021. Palatability enhancement potential of *Hermetia illucens* larvae protein hydrolysate in *Litopenaeus vannamei* diets. Molecules. 26 (6): 1582. DOI: https://doi.org/10.3390/molecules26061582

The Jamovi Project. 2022. Jamovi [computer software]. https://www.jamovi.org.

Walsh S, Nguyen K, Strebel L, Rhodes M, Davis DA. 2022. Utilising feed effectors and automated feeders for semi-intensive pacific white shrimp (*Litopenaeus vannamei*) production. Aquacult Fish Fish. 2 (6): 540-551. DOI: https://doi.org/10.1002/aff2.83

Winkler UK, Stuckmann M. 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J Bacteriol. 138 (3): 663-670. DOI: https://doi.org/10.1128/jb.138.3.663-670.1979

Yang W, Tran NT, Zhu C-H, Yao D-F, Aweya JJ, Gong Y, Ma H-Y, Zhang Y-L, Li G-L, Li S-K. 2021. Immune priming in shellfish: a review and an updating mechanistic insight focused on cellular and humoral responses. Aquaculture. 530: 735831. DOI: https://doi.org/10.1016/j.aquaculture.2020.735831

Zhou Z, Wang L, Dai M, Gao Q, Wang P, Zhao L, Li Y, Xi R, Pan M, Ma Q, et al. 2023. Three types of enteromorpha prolifera bio-products based on different processing procedures as feed additives in the diets of pacific white shrimp (*Litopenaeus vannamei*). Fishes. 8 (12): 587. DOI: https://doi.org/10.3390/fishes8120587

Zhu T, Morais S, Luo J, Jin M, Lu Y, Le Y, Zhou Q. 2019. Functional palatability enhancer improved growth, intestinal morphology, and hepatopancreas protease activity, replacing squid paste in white shrimp, *Litopenaeus vannamei*, diets. J World Aquacult Soc. 50 (6): 1064-1077. DOI: https://doi.org/10.1111/jwas.12615