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Partial Replacement of Fish Meal with Protein Hydrolysates in the Diet of *Penaeus vannamei* (Boone, 1934) during the Nursery Phase

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Abstract: The objective was to evaluate the effects of partial replacement of fish meal with protein hydrolysates and a commercial product in the diets of *Penaeus vannamei* post-larvae on zootechnical performance, proximate chemical composition, digestive enzyme activity, and total hemocyte count. The experiment was conducted in a clear water recirculation system comprising 24 experimental units, each with 30 shrimp with an average weight of 0.2 g. The treatments were control, chicken protein hydrolysate (CPH), enzymatic hydrolysate of chicken feathers, Aquabite®, CPH + maltodextrin, and CPH + yeast, with four replicates each. The inclusion level of the different protein sources evaluated was 6%. At the end of the experiment, all shrimp were counted, weighed, and measured to determine the zootechnical performance. The body and feed chemical compositions, as well as the enzymatic activities of the hepatopancreas and the total hemocyte count in the hemolymph, were analyzed. The data obtained were subjected to ANOVA and Tukey's test. There was no statistical difference between the treatments in any of the analyzed parameters. The results showed that all the evaluated protein hydrolysates can be used as partial substitutes for fish meal in *P. vannamei* diets during the nursery phase, maintaining equivalent and adequate digestive enzyme activities, health, growth, and body composition of the shrimp, in addition to being ecologically sustainable ingredients.

Keywords: hydrolysis; peptides; protein; nutrition; Pacific white shrimp; aquaculture

Key Contribution: The protein hydrolysates can be used as partial substitutes for fish meal in *Penaeus vannamei* diets during the nursery phase, maintaining equivalent and adequate digestive enzyme activities, health, growth, and body composition of the shrimp.

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1. Introduction

Aquaculture production is continuously evolving and gaining more significance in global food production. Aquaculture and fishery products remain among the most traded food commodities worldwide, with 225 countries and territories reporting some commercial activity related to fishery and aquaculture products in 2020 [1]. The estimates show

that aquaculture will continue to grow, rising from 154 million tons produced in 2011 to 186 million tons by 2030. It will provide more than 60% of the fish intended for human consumption, resulting in increased demands for feeds and raw materials [2,3]. Sustainable growth in aquaculture will require the development of highly nutritious and functional raw materials to efficiently replace fish meal, which is the most commonly used and valuable protein source due to its high digestibility, well-balanced essential amino acids, and good palatability [3,4].

On the other hand, there is a substantial amount of waste generated in slaughterhouses. Fish and other animal industries should explore new applications for these by-products, considering the environmental problems caused by their improper disposal and the economic importance resulting from converting these low-cost residues into value-added by-products [5]. In this context, the application of chemical, enzymatic, or microbial hydrolysis on animal by-product proteins represents an attractive way to generate quality peptides with nutritional, physiological, or regulatory functions in cattle, poultry, fish, and crustaceans [6].

In aquaculture, one of the main factors that influences the growth of shrimps is the level and quality of the protein and this is related to their capability to digest protein by regulating the synthesis, secretion, and inactivation of digestive enzymes [7]. In the animals' intestines, peptides are hydrolyzed, providing smaller peptides that are absorbed by enterocytes more rapidly than free amino acids, resulting in a more balanced pattern of amino acids in the bloodstream [6]. Some peptides of animal origin also possess antimicrobial, antioxidant, antihypertensive, and immunomodulatory activities [5]. Studies have reported positive results regarding the growth performance and health of shrimp and fish fed with diets containing protein hydrolysates, a promising source of bioactive peptides [4,6,8–11]. The differences in the effects of protein hydrolysates, when used as a substitute for fishmeal, on the growth performance of aquatic animals may be related to the source and processing technology of protein hydrolysates and the molecular weight of peptides and free amino acids that could interact and affect, for example, the protein structure and digestive enzyme recognition sites [12]. Studies show that ingredients composed of protein hydrolysates can be included in lower concentrations in shrimp diets to favor better growth performance and intestinal microbiota [5,9–11].

The main commercially important shrimp species is the Pacific white shrimp (*Penaeus vannamei*). Studies of Córdova-Murueta [12] demonstrated that the use of fish protein hydrolysates stimulates the activities of *P. vannamei* proteases (trypsin and chymotrypsin), especially at lower concentrations (below 10%) of hydrolysates. On the other hand, Soares [11] and Shao [9] showed that the activity of amylase, proteases, trypsin, chymotrypsin, and aminopeptidase from *P. vannamei* had no significant differences among treatments with protein hydrolysates of poultry by-products and swine liver (25, 50, 75, and 100% of fish meal replacement) and fish by-products (15% of fish meal replacement), respectively. In *P. vannamei* diets, the optimal inclusion level of chicken by-product protein hydrolysates and pork liver hydrolysates was 4.8%, replacing 24% of the fish meal in the diet [10], and it led to beneficial changes in the shrimp's intestinal microbiota, with a reduction in the abundance of the Vibrionaceae family [11].

Maltodextrin is a polysaccharide that contains an average of five to ten glucose units per molecule and can be produced through enzymatic or acid hydrolysis of starch or a combination of both methods [13]. It can be used as an additive to protect hydrolysates from degradation or a reduction in their functionality due to various reactions, such as oxidation and hydrolysis, among others [14]. On the other hand, yeast has become a new sustainable protein ingredient in aquafeeds due to its promising role in the nutrition and immune stimulation of many species [15]. When combined with hydrolysates, these ingredients can contribute to maintaining the nutritional quality and improving the nutrient supply for the species.

Although there are some studies on the use of hydrolysates in shrimp diets, either isolated or combined with other products, there is still limited information about their

effects on growth and health during different production phases. Therefore, the objective of this study was to evaluate the effects of partial replacement of fish meal with protein hydrolysates, with and without additives (chicken protein hydrolysate (CPH), CPH + yeast, CPH + maltodextrin, enzymatic hydrolysate of feathers, and a commercial product (Aquabite®)) in the diets of *P. vannamei*. The zootechnical performance, proximate chemical composition of the carcass, digestive enzyme activity, and total hemocyte count during the nursery phase were analyzed.

2. Materials and Methods

2.1. Experimental Design and Diet Formulation

The experiment was conducted at the Laboratory of Shrimp Farming in the Nucleus for Research and Development in Sustainable Aquaculture, Federal University of Paraná (UFPR), Maripá—PR, Brazil. Thirty-day-old post-larvae (PL 30) of *P. vannamei* from the speed line strain acquired from Aquatec® (Rio Grande do Norte, Brazil) were used. The experimental period lasted 28 days.

Before the experimental period, the animals were acclimatized in a 1000 L box, with the same conditions as the experimental system, for 14 days, and received commercial food (Guabi®, J 40, Campinas, São Paulo, Brazil).

The experiment was conducted in a clear water recirculation system comprising 24 experimental units (EU), composed of six treatments and four replicates. These units consisted of 100 L circular tanks with a useful volume of 93 L, connected to a 600 L biological filter with a useful volume of 500 L. Each experimental unit was equipped with a hose and a porous stone for constant aeration. The average flow rate of the recirculation system was 1400 L per hour. The system was installed in a room with temperature control and a photoperiod of 12:12 (light:dark). Water salinity was adjusted to 15 g L^{-1} using the commercial sea salt mixture Blue Treasure® Reed Sea Salt (Qingdao Sea Salt Aquarium Technology, Qingdao, China). In each EU, 30 shrimp with an average weight of 0.2 g and average length of 2.7 cm, in the nursery phase, were kept.

Shrimp feeding was conducted six times a day at the following times: 3:00 a.m., 8:30 a.m., 11:00 a.m., 2:00 p.m., 5:00 p.m., and 10:00 p.m. Feeding rates were adjusted considering an average individual growth of 0.15 g per week in the first two weeks and 0.25 g per week in the final two weeks of the experiment. To calculate the feeding rate, an apparent feed conversion rate of 1:1 was considered. The experimental units were siphoned daily and feed consumption was evaluated; shrimp mortality was also considered to adjust the feeding rate.

Biometrics were performed at the beginning of the experiment and subsequently every seven days. All experimental units were siphoned daily to prevent the accumulation of feed residues, feces, and debris. The experimental design was completely randomized, with six treatments and four replicates, and the treatments were determined according to the protein source used for the partial replacement of fish meal. The inclusion level of the different, evaluated protein sources was 6% [13], in the replacement of fish meal (Table 1). The treatments used were T1: control (fish meal); T2: chicken protein hydrolysate (CPH), composed of chicken viscera, chicken offal, chicken meat, an antifoaming agent, an antifungal, and an antioxidant; T3: enzymatic hydrolysate of chicken feathers, composed of chicken feathers, an antifoaming agent, an antifungal, and an antioxidant (BRF®, Toledo, Paraná, Brazil); T4: Aquabite® (Belgium), a palatability and attractiveness enhancer for fish and shrimp based on marine fish peptides; T5: CPH with added maltodextrin; and T6: CPH with added yeast.

The diets were formulated using the software SuperCrac 6.1® (Viçosa, Minas Gerais, Brazil) and are described in Table 1. For the production of experimental diets, the ingredients were individually ground in a knife mill with a 0.5 mm sieve [16], mixed according to their formulation, pelleted, and stored under refrigeration at 4 °C [17].

Table 1. Composition of the experimental diets for shrimp in the nursery phase.

Ingredients (g kg^{-1})	Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Soybean meal	350.0	350.0	350.0	350.0	350.0	350.0
Wheat flour	129.9	136.8	136.7	135.2	125.8	124.0
Wheat bran	50.0	50.0	50.0	50.0	50.0	50.0
Poultry viscera flour	150.0	146.2	137.8	150.0	160.3	162.7
Fish meal	247.6	165.0	165.0	165.0	165.0	165.0
Chicken protein hydrolysate (CPH)	0.0	60.0	0.0	0.0	0.0	0.0
CPH + maltodextrin	0.0	0.0	0.0	0.0	60.0	0.0
CPH + yeast	0.0	0.0	0.0	0.0	0.0	60.0
Feather hydrolysate BRF®	0.0	0.0	60.0	0.0	0.0	0.0
Aquabite®	0.0	0.0	0.0	60.0	0.0	0.0
Antifungal	1.0	1.0	1.0	1.0	1.0	1.0
Antioxidant	0.2	0.2	0.2	0.2	0.2	0.2
Limestone	0.0	4.4	4.0	5.1	4.0	4.0
Binder	5.0	5.0	5.0	5.0	5.0	5.0
Methionine	2.1	2.4	2.9	2.0	2.3	2.4
Dicalcium phosphate	0.0	10.7	12.8	11.2	9.3	9.0
Lysine	11.1	0.4	3.2	0.0	1.3	1.2
Soy lecithin	20.0	20.0	20.0	20.0	20.0	20.0
Fish oil	29.2	32.7	36.0	30.2	30.8	30.6
Vitamin and mineral supplement [1]	8.0	8.0	8.0	8.0	8.0	8.0
Common salt	5.7	7.2	7.3	7.1	7.0	6.9
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Bromatological composition [2]						
Dry matter (%)	93.61	94.06	93.76	93.82	93.61	92.39
Crude protein (%)	43.02	42.70	42.53	42.83	42.73	42.27
Ethereal extract (%)	7.24	8.04	6.75	7.74	7.80	7.06
Mineral matter (%)	11.49	11.31	10.90	11.18	10.86	10.77
Crude energy (kcal kg^{-1})	4400.00	4420.00	4370.00	4410.00	4330.00	4390.00

[1] Vitamin and mineral supplement with guaranteed levels per kg of product: vit. A—1.000.000 IU; vit. D3—500,000 IU; vit. E—20,000 mg; vit. K3—500 mg; vit. B1—1900 mg; vit. B2—2000 mg; vit. B6—2400 mg; vit. B12—3500 mg; folic acid—200 mg; calcium pantothenate—4000 mg; vit. C—25 g; biotin—40 mg; niacin—5000 mg; Fe—12.5 g; Cu—2000 mg; Mn—7500 mg; Zn—25 g; I—200 mg; Se—70 mg. [2] Bromatological composition of experimental feeds analyzed in the laboratory, based on natural matter.

2.2. Water Quality Monitoring

The variables of temperature, dissolved oxygen, and pH were monitored daily using a multiparameter equipment (Hanna HI98196, Limena, Italy). Salinity was determined weekly using a manual refractometer (Biobrix model 211, Brazil). Alkalinity and hardness were measured every two weeks, and total ammonia and nitrite concentrations were determined three times a week [18].

The mean values of the monitored water quality parameters were as follows: temperature, 27.6 ± 1.21 °C; pH, 7.67 ± 0.15; dissolved oxygen, 6.78 ± 0.41 mg L^{-1}; salinity, 15.00 ± 0.00 g L^{-1}; alkalinity, 127.8 ± 21.1 mg L^{-1} $CaCO_3$; hardness, 2043.03 ± 57.40 mg L^{-1} $CaCO_3$; total ammonia, 0.02 ± 0.02 mg L^{-1}; and nitrite, 0.08 ± 0.09 mg L^{-1}. All water quality parameters remained within the recommended range for *P. vannamei* production [19–22].

2.3. Zootechnical Performance and Animal Welfare Assessment

At the end of the experiment, all shrimp were counted, weighed, and measured (total length) to determine the percentage of survival (final number of shrimp/initial number of shrimp × 100), biomass gain ((final number of shrimp × final average weight) − (initial number of shrimp × initial average weight)), apparent feed conversion (amount of feed supplied/biomass gain), and specific growth rate {[log_e(Final Weight) − log_e(Initial Weight)]/period} × 100. To assess animal welfare, the total length of the antenna was measured [23].

2.4. Centesimal Chemical Composition of Feeds and Shrimp

Diet samples and five animals from each experimental unit were randomly collected for the evaluation of the proximate chemical composition, including crude protein, crude energy, ether extract, and mineral matter, following the methodologies described in AOAC [24]. The moisture content was determined by drying the pre-weighed samples in porcelain cups at 105 °C for 12 h and ash content was determined by incinerating the dried samples at 600 °C for 3 h. The protein content was determined using the Kjeldahl method, the ether extract was obtained by petroleum ether extraction in an ANKOM® XT10 extractor, and the energy was determined using the IKA® C5000 control equipment. All analyses were performed in triplicate at the Laboratory of Animal Nutrition and Feeding (LANA), Federal University of Paraná—Palotina Campus, Brazil.

2.5. Enzymatic Activity Assessment

At the end of the experiment, three animals per replicate were randomly selected and used to evaluate the activity of enzymes related to the shrimp's digestive system. The hepatopancreas samples were homogenized using a tissue homogenizer in a PBS solution (pH 7.2) and centrifuged at 5000 rpm for 10 min, and the supernatants were separated for the analyses. The digestive enzymatic activities of amylase (U/L/mg protein), cellulase (nmol/min/mg protein), maltase (µmol/min/mg protein), saccharase (µmol/min/mg protein), lipase (U/L/mg protein), and trypsin (µmol/min/mg protein) were determined.

Maltase and saccharase activities were determined using the methodology described in [25]. To determine the enzyme activity, glucose dosing was performed at the end of the incubation period using a colorimetric commercial kit (Gold Analisa® Belo Horizonte, Minas Gerais, Brazil), following the manufacturer's recommendations. The results were expressed as µmol/min/mg protein.

The activities of amylase and lipase were determined using a commercial colorimetric kit, following the manufacturer's recommendations (GoldAnalisa® Belo Horizonte, Minas Gerais, Brazil), and the results were expressed as U/L/mg protein. Trypsin activity was determined according to the method of Hummel [26]. To determine the activity of this enzyme, the substrate α-ρ-toluenesulphonyl-L-arginine methyl ester hydrochloride (TAME) was used. The molar extinction coefficient used for the enzyme activity calculation was 540 M, considering the product formation, and the result was expressed as µmol/min/mg of protein. Cellulase activity was determined according to the methodology of Niiyama and Toyohara [27]. The amount of reducing sugar formed was measured by the nitroblue tetrazolium (NBT) method at 660 nm. D-glucose was used as the standard for the calibration curve and the results were expressed as nmol/min/mg protein.

2.6. Total Hemocyte Count

At the end of the experiment, ten shrimp per treatment were anesthetized by thermonarcosis [28] and the hemolymph was collected using a 1 mL syringe and a 13 × 0.45 mm needle from the second central segment (somite). The collected hemolymph was placed in a 2 mL microtube containing the modified isotonic anticoagulant Alsever's solution (composed of glucose, sodium citrate, sodium chloride, and deionized water) at approximately 4° C in a 1:9 ratio (hemolymph:anticoagulant) [29]. The total hemocyte count (THC) was determined in a Neubauer chamber and calculated by the equation:

$$\text{THC mL}^{-1} = \frac{N^{\circ}\ TCC}{N^{\circ}\ QC} \times FD \times 10{,}000 \quad (1)$$

where THC mL^{-1}: total hemocyte count per milliliter; N° TCC: total number of cells counted; N° QC: number of quadrants counted; and FD: dilution factor.

2.7. Statistical Analysis

The collected data were tested using one-way analysis of variance (ANOVA) after checking for normality and homoscedasticity assumptions, and the mean values of each

treatment were compared using Tukey's test. All results were tested at the 5% significance level ($\alpha = 0.05$) and the statistical package used was R Studio 2023.09.1 [30].

3. Results

3.1. Zootechnical Performance and Animal Welfare Assessment

No significant differences were observed in any of the zootechnical performance parameters evaluated ($p > 0.05$) (Table 2).

Table 2. Mean ± standard deviation of zootechnical performance parameters of shrimp (*Penaeus vannamei* (Boone, 1934)) fed during the nursery phase with diets containing different sources of protein hydrolysates in partial substitution of fishmeal.

Treatment *	Final Weight (g)	Total Length (cm)	Antenna Length (cm)	Final Biomass (g)	Biomass Gain (g)	Survival (%)	Feed Conversion	Specific Growth Rate
T1	1.48 ± 0.75	4.10 ± 1.09	6.93 ± 1.77	41.32 ± 3.13	35.32 ± 3.13	91.00 ± 2.00	0.93 ± 0.06	9.27 ± 0.46
T2	1.46 ± 0.80	3.86 ± 1.12	6.74 ± 2.03	39.38 ± 2.20	33.38 ± 2.20	89.00 ± 6.00	0.98 ± 0.05	9.50 ± 0.30
T3	1.45 ± 0.67	3.92 ± 0.92	6.68 ± 2.11	46.90 ± 17.33	40.90 ± 17.33	93.00 ± 7.00	0.89 ± 0.27	9.50 ± 0.32
T4	1.39 ± 0.67	4.03 ± 1.01	7.11 ± 2.04	40.94 ± 6.25	34.94 ± 6.25	90.00 ± 6.00	0.95 ± 0.14	9.66 ± 0.49
T5	1.64 ± 0.72	4.18 ± 0.94	7.18 ± 1.90	44.70 ± 3.03	38.70 ± 3.03	91.00 ± 7.00	0.86 ± 0.06	10.02 ± 0.29
T6	1.49 ± 0.76	4.02 ± 1.06	6.77 ± 1.07	39.88 ± 5.94	33.88 ± 5.94	90.00 ± 5.00	0.98 ± 0.14	9.53 ± 0.62

* T1: control; T2: chicken protein hydrolysate (CPH); T3: enzymatic hydrolysate of chicken feathers; T4: Aquabite®; T5: CPH with added maltodextrin; T6: CPH with added yeast.

3.2. Centesimal Chemical Composition of Feeds and Shrimp

There was no statistical difference between treatments in any of the centesimal chemical composition parameters evaluated ($p > 0.05$) (Table 3).

Table 3. Mean of centesimal chemical composition parameters of shrimp carcass, on a natural matter basis of *Penaeus vannamei* (Boone, 1934) during the nursery phase.

Treatment *	Dry Matter (%)	Crude Protein (%)	Ethereal Extract (%)	Ash (%)	Crude Energy (kcal kg^{-1})
T1	21.48 ± 0.85	15.85 ± 0.15	1.34 ± 1.03	3.04 ± 0.20	1060 ± 12.34
T2	21.84 ± 0.94	16.07 ± 0.27	1.45 ± 1.02	2.90 ± 0.17	1110 ± 20.34
T3	22.15 ± 1.02	16.35 ± 0.23	1.87 ± 1.29	2.84 ± 0.12	1120 ± 22.54
T4	21.81 ± 0.45	15.92 ± 0.17	1.65 ± 0.10	2.85 ± 0.13	1110 ± 18.25
T5	21.47 ± 0.54	15.84 ± 0.19	1.33 ± 0.26	2.85 ± 0.10	1100 ± 13.21
T6	21.45 ± 0.48	15.60 ± 0.23	1.18 ± 0.34	2.98 ± 0.18	1070 ± 10.23

* T1: control; T2: chicken protein hydrolysate (CPH); T3: enzymatic hydrolysate of chicken feathers; T4: Aquabite®; T5: CPH with added maltodextrin; T6: CPH with added yeast.

3.3. Enzymatic Activity Assessment

There was no statistical difference between treatments in the activity of the evaluated digestive enzymes ($p > 0.05$) (Table 4).

Table 4. Mean and standard deviation of the enzymatic activity of the evaluated digestive enzymes of *Penaeus vannamei* (Boone, 1934) during the nursery phase.

Treatment *	Amylase (U/L/mg Protein)	Cellulase (nmol/min/mg Protein)	Lipase (U/L/mg Protein)	Maltase (µmol/min/mg Protein)	Sucrase (µmol/min/mg Protein)	Trypsin (µmol/min/mg Protein)
T1	13.71 ± 6.73	0.16 ± 0.06	33.73 ± 5.72	4.75 ± 1.52	1.08 ± 0.29	0.11 ± 0.10
T2	16.14 ± 5.85	0.15 ± 0.04	29.42 ± 6.31	3.72 ± 0.90	0.82 ± 0.13	0.10 ± 0.02
T3	21.25 ± 2.84	0.16 ± 0.06	38.07 ± 9.77	4.99 ± 2.90	1.00 ± 0.23	0.13 ± 0.13
T4	17.14 ± 5.51	0.14 ± 0.03	33.30 ± 8.47	2.99 ± 0.73	0.86 ± 0.25	0.10 ± 0.11
T5	16.81 ± 8.34	0.19 ± 0.08	41.09 ± 21.69	3.84 ± 1.77	0.95 ± 0.33	0.07 ± 0.05
T6	8.78 ± 9.64	0.19 ± 0.08	34.20 ± 18.21	3.38 ± 1.23	1.39 ± 0.85	0.05 ± 0.06

* T1: control; T2: chicken protein hydrolysate (CPH); T3: enzymatic hydrolysate of chicken feathers; T4: Aquabite®; T5: CPH with added maltodextrin; T6: CPH with added yeast.

3.4. Total Hemocyte Count

There was no statistical difference between treatments in the total hemocyte count ($p > 0.05$) (Table 5).

Table 5. Total hemocyte count of *Penaeus vannamei* in the nursery phase.

Treatment *	Total Hemolytic Cells
T1	$7 \times 10^6 \pm 5 \times 10^5$
T2	$5 \times 10^6 \pm 3 \times 10^5$
T3	$5 \times 10^6 \pm 4 \times 10^5$
T4	$5 \times 10^6 \pm 3 \times 10^5$
T5	$8 \times 10^6 \pm 4 \times 10^5$
T6	$7 \times 10^6 \pm 4 \times 10^5$

* T1: control; T2: chicken protein hydrolysate (CPH); T3: enzymatic hydrolysate of chicken feathers; T4: Aquabite®; T5: CPH with added maltodextrin; T6: CPH with added yeast.

4. Discussion

Protein hydrolysates have been widely reported as a protein source in shrimp feeds. Research with *P. vannamei* indicates that partial substitution of fishmeal with protein hydrolysates leads to comparable health and performance results [9,10,31]. The best growth results are obtained with low inclusion levels, and as the inclusion level increases, growth decreases. The reduced growth of shrimp fed with diets containing high levels of hydrolysates may be related to high concentrations of free amino acids and peptides in protein hydrolysates that can be rapidly absorbed and metabolized rather than being used for protein synthesis and growth [32].

Similar to this study, protein hydrolysates can be included up to 6% in *P. vannamei* diets, partially replacing fishmeal [13,31]. Moreover, considering the zootechnical performance variables and the absence of statistical differences between the tested treatments and the control, all protein hydrolysates tested in this study can be included (at 6%) as a partial substitution of fishmeal in the diets of *P. vannamei* post-larvae. These results reinforce that diets with hydrolysates are nutritionally balanced for the Pacific white shrimp. This ensures less dependence on fishmeal [11], the main protein ingredient and the higher cost component in shrimp feeds [4]. Hydrolysates are environmentally and economically sustainable. They are a renewable resource and replace noble ingredients such as fish meal, and their processing technology uses less energy compared to conventional flour processes, as they use less steam and lower temperatures.

Among the main chemosensory organs of shrimp are the antennules, antennas, mouthparts, and legs [33]. Broken and shortened antennas are the first signs of compromised shrimp health [34]. In the present study, no tested ingredient interfered with antenna length. The antenna length is related to animal welfare because, considering their fragility in shrimp, their integrity indicates that the animal is in good condition in the production environment and adequately performing its functions.

The nutritional quality of the food is a factor that not only influences the zootechnical performance of shrimp but also reflects in the centesimal body composition or flesh. The enzymatic activity present in the digestive tract of these crustaceans can be influenced by the nutritional composition of the ingredients used in the diets and nutrient assimilation by the organism [35]. The tested protein hydrolysates and the commercial product did not alter the centesimal body composition of *P. vannamei* in the nursery phase, keeping it equivalent to the shrimp that had only fishmeal in their diet. This demonstrates that the evaluated products met the nutritional requirements of *P. vannamei* and could be used without affecting the quality and composition of the shrimp.

The ability of an organism to digest food particles depends on the presence and appropriate concentration of digestive enzymes, as well as their activity [36–38]. The study of the hepatopancreas stands out mainly due to its function in the synthesis and secretion of digestive enzymes and nutrient absorption from the diet [38]. The hepatopancreas

was used in this study for the extraction of digestive enzymes. The activities of digestive enzymes in shrimp fed with diets containing protein hydrolysates remained the same as in the control treatment containing fishmeal. This leads to the inference that the shrimp possessed the presence and adequate quantities for the digestion of biomolecules from both protein sources and their additives.

Among the proteolytic enzymes in shrimp, trypsin is the most important, as it hydrolyzes 50–60% of the consumed protein, catalyzing the separation of peptide bonds on the carboxyl side of lysine and arginine amino acids [39]. As widely known, protein sources are the most expensive ingredients in the diet, and thus, in this study, the maintenance of trypsin's hydrolysis activity, regardless of the protein source used, indicates greater utilization and consequent animal growth.

After proteins, carbohydrates constitute the most abundant group of biomolecules in the diet. The activities of carbohydrases (amylase, cellulase, maltase, sucrase) indicate the species' capacity to adapt to different diets and benefit from various types of carbohydrates [36], such as the additives included in the hydrolysate (maltodextrin). Changes in the expression of several genes in the hepatopancreas and muscle tissues of *P. vannamei* have been observed when promoting a change in diets from an animal protein source to a plant protein source and vice versa, suggesting that these changes may be related to metabolic and physiological adjustments to assimilate the type of food provided [40]. However, it is believed that the ability to digest different foods is related to the enzymatic profile of a given species [41].

Lipase is responsible for the hydrolysis of glycerol esters of long-chain fatty acids [41]. Although there were no statistical differences in the chemical composition of the ether extract in the tested diets, the use of different hydrolysates did not affect the enzymatic activity of the shrimp. Even though there was no chemical difference between the ingredients tested, they could have triggered different enzymatic responses.

Hemocytes are part of the cellular defense system of *P. vannamei*, responsible for phagocytosis, nodulation, and encapsulation, and can be used as a quantitative parameter to measure the response to stress in shrimp. An increase in total hemocytes indicates an improvement in the organism's health, as it will form phagocytic cells that play a role in the defense against microbial infections. The supply of dietary immunostimulants is intended to activate the non-specific immune system of cells, such as hemocytes, in invertebrates [42]. In the present study, the hemocyte count was not altered, thus indicating that the tested hydrolysates did not have an immunostimulatory effect.

Finally, different protein hydrolysates can be potentially included to partially replace fishmeal protein in the shrimp feed industry [9,10,31,43], with or without additives, maintaining performance, health, and body composition.

5. Conclusions

All the tested hydrolysates, with or without additives, as well as the commercial product, can be used to partially replace fishmeal in the diets of *Penaeus vannamei* in the nursery phase, maintaining equivalent and good growth performance, welfare, carcass chemical composition, and digestive enzyme activity. The recommended inclusion level in the diets of *P. vannamei* in the nursery phase for any of the tested ingredients is 6%. Additionally, this study provides important information for the formulation of nutritionally balanced diets with reduced dependence on fishmeal for the Pacific white shrimp.

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