fishes

MDPI

Article

Different Protein Hydrolysates Can Be Used in the *Penaeus vannamei* (Boone, 1934) Diet as a Partial Replacement for Fish Meal during the Grow-Out Phase

Celma Negrini [1], Caio Henrique do Nascimento Ferreira [2], Rafael Ortiz Kracizy [3], Rosane Lopes Ferreira [3], Luana Cardoso dos Santos [3], Milena Cia Retcheski [4], Marlise Teresinha Mauerwerk [3], Luisa Helena Cazarolli [4], Wilson Rogério Boscolo [1] and Eduardo Luis Cupertino Ballester [3,*]

[1] Universidade Estadual do Oeste do Paraná, Rua da Faculdade, n° 645, Bloco C, Jardim Santa Maria, Toledo 85903-000, Paraná, Brazil; celma.negrini@hotmail.com (C.N.); wilsonboscolo@hotmail.com (W.R.B.)
[2] Programa de Pós-Graduação em Aquicultura, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal 14884-900, São Paulo, Brazil; caiohenrique1048@gmail.com
[3] Laboratório de Carcinicultura, Programa de Pós-graduação em Aquicultura e Desenvolvimento Sustentável, Núcleo de Pesquisa e Desenvolvimento em Aquicultura Sustentável, Universidade Federal do Paraná, Maripá 85950-000, Paraná, Brazil; kracizy@gmail.com (R.O.K.); rosanelopes1992@gmail.com (R.L.F.); luanaecsantos@gmail.com (L.C.d.S.); marlisemauerwerk1@gmail.com (M.T.M.)
[4] Laboratório de Bioquímica e Genética, Universidade Federal da Fronteira Sul, Laranjeiras do Sul 85319-899, Paraná, Brazil; milenaciar@gmail.com (M.C.R.); luisacazarolli@uffs.edu.br (L.H.C.)
* Correspondence: elcballester@ufpr.br

Abstract: This study evaluated the inclusion of protein hydrolysates and a commercial product as a partial replacement for fish meals in the diet of Pacific white shrimp (*Penaeus vannamei*) during the grow-out phase. A recirculation system with 24 experimental units and a biological filter was used. The experimental design was completely randomized with six treatments: control; chicken protein hydrolysate (CPH); enzymatic hydrolysate of chicken feathers; Aquabite®; CPH + maltodextrin; CPH + yeast; and four replicates. After 50 days, the zootechnical performance and animal welfare parameters, centesimal carcass composition, digestive enzyme activity, and hemocyte count were evaluated. The treatments did not affect the zootechnical performance and total hemocyte count of the animals ($p > 0.05$). The evaluation of the antenna length indicated that all animals were in good health. The antenna length in treatment T5 was significantly greater ($p > 0.05$) than that in T4 and similar to that in treatments T3 and T6, demonstrating a positive influence of dietary protein hydrolysates. Concerning animal welfare, dietary protein hydrolysates influenced the length of the antenna ($p < 0.05$). The antenna length associated with different treatments indicated that the animals were in good welfare conditions in the production environment. With respect to body chemical composition, dry matter, ether extract, and ash were affected by the treatments ($p < 0.05$). Regarding the analysis of digestive enzymes, the treatments influenced the activities of amylase and trypsin ($p < 0.05$). The performance of the animals was satisfactory under all treatments, including enzymatic activity, demonstrating the possibility of using hydrolysates.

Keywords: Pacific white shrimp; nutrition; peptides; aquaculture

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

check for updates

Citation: Negrini, C.; do Nascimento Ferreira, C.H.; Kracizy, R.O.; Ferreira, R.L.; dos Santos, L.C.; Retcheski, M.C.; Mauerwerk, M.T.; Cazarolli, L.H.; Boscolo, W.R.; Cupertino Ballester, E.L. Different Protein Hydrolysates Can Be Used in the *Penaeus vannamei* (Boone, 1934) Diet as a Partial Replacement for Fish Meal during the Grow-Out Phase. *Fishes* **2024**, *9*, 73. https://doi.org/10.3390/fishes9020073

Academic Editor: Fernando Vega-Villasante

Received: 22 December 2023
Revised: 31 January 2024
Accepted: 10 February 2024
Published: 12 February 2024

1. Introduction

The Pacific white shrimp (*Penaeus vannamei*) is the most farmed shrimp species worldwide [1]. To improve the production system efficiency and, consequently, lead to higher

yield volumes, it is necessary to find ingredients that meet the species nutritional requirements, allow high performance, and are supplied sustainably [2].

Protein is the most important and costly component in feed manufacturing, as it significantly influences animal growth, feed conversion, and carcass composition [3–5]. Its quality is determined by the raw material used [6]. However, fish meals come at a high cost due to these characteristics. To produce high-quality feeds at a lower cost, several experiments have been conducted to replace fish meals with alternative proteins in Pacific white shrimp diet [4,7–11]. Among the alternative proteins available on the market, hydrolyzed proteins offer advantages in terms of physical, chemical, and nutritional property optimization compared to non-hydrolyzed proteins. Protein hydrolysates from animal slaughter by-products have great potential to replace fish meals and can provide high-quality protein sources for the nutrition of aquatic organisms [12]. Hydrolysates contain a high level of crude protein (up to 90%) and are rich in essential amino acids [13], which can increase the attractiveness, palatability, and digestibility of aquafeeds [14].

Protein hydrolysates also act as efficient immunostimulants and antioxidants [15,16]. The replacement of fish meals with protein hydrolysates can lead to satisfactory zootechnical gains in shrimp farming, reducing protein costs and enabling producers to reach new markets that add value to the farmed shrimp [10,11,17]. Additionally, yeasts have been used in the feeding of aquatic organisms as a potential protein ingredient, immunostimulant, and probiotic [18]. When combined with hydrolysates, yeasts can improve growth and health outcomes. Likewise, maltodextrin is a polysaccharide commonly used to protect feed from oxidation, and it offers advantages such as high solubility and rapid dispersion [19]. When combined with hydrolysates, it can provide energy and act as an antioxidant protector.

Therefore, the present research aimed to study the dietary inclusion (6%) of different protein hydrolysates (Chicken Protein Hydrolysate (CPH), CPH + yeast, CPH + maltodextrin, enzymatic hydrolysate of feathers, and a commercial product, Aquabite®, (Adisseo Latin America, São Paulo, Brazil) as partial replacements of fish meals in the Pacific white shrimp (*P. vannamei*) diet during the grow-out phase and evaluate their effect on zootechnical performance, centesimal carcass composition, digestive enzyme activity, and total hemocyte count.

2. Materials and Methods

2.1. Experimental Condition and Diet Formulation

The experiment was conducted at the Laboratory of Shrimp Farming at the Center for Research and Development in Sustainable Aquaculture, Federal University of Paraná (UFPR), in Maripá-PR, Brazil. Juveniles of *P. vannamei* from the speed line strain acquired from Aquatec® were used, and the experimental period lasted 50 days.

A completely randomized experimental design was used, with six treatments and four replicates. The treatments were determined based on the protein source used for partial replacement of fish meals. The substitution level with the different evaluated protein sources was 6% of all components of the diet (Table 1). The diet was formulated using the SuperCrac® software (6.0, Viçosa, MG, Brazil). The treatments were: T1—control (fish meal); T2—chicken protein hydrolysate (CPH); T3—enzymatic hydrolysate of chicken feathers; T4—Aquabite® (based on marine fish peptides); T5—CPH with maltodextrin addition; and T6—CPH with yeast addition.

The experiment was conducted in a clear water recirculation system consisting of 24 experimental units, which were circular tanks with a total volume of 100 L and an effective volume of 93 L, coupled with a biological filter of 600 L with an effective 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 controlled temperature room with a photoperiod of 12:12 (light:dark). The water salinity was adjusted to 15 g L^{-1} using the commercial mixture of Blue Treasure® Reed Sea Salt (Qingdao Sea Salt Aquarium Technology, Qingdao, China). In each experi-

mental unit, five juvenile shrimp with an average weight of 1.35 ± 0.05 g and an average length of 3.22 ± 0.01 cm were kept.

Table 1. Composition of the experimental diet for shrimps during the grow-out phase.

Ingredients (g kg^{-1})	Treatments					
	T1	T2	T3	T4	T5	T6
Soybean meal	400.00	400.00	400.00	374.20	400.00	400.00
Wheat flour	138.70	143.20	143.10	154.20	132.10	130.60
Wheat bran	100.00	100.00	100.00	100.00	100.00	100.00
Poultry viscera flour	125.00	126.30	118.10	147.30	140.50	142.70
Fish meal	137.90	50.00	50.00	50.00	50.00	50.00
Chicken Protein Hydrolysate (CPH)	0.00	60.00	0.00	0.00	0.00	0.00
CPH + maltodextrin	0.00	0.00	0.00	0.00	60.00	0.00
CPH + yeast	0.00	0.00	0.00	0.00	0.00	60.00
Feather hydrolysate BRF®	0.00	0.00	60.00	0.00	0.00	0.00
Aquabite®	0.00	0.00	0.00	60.00	0.00	0.00
Antifungal	1.00	1.00	1.00	1.00	1.00	1.00
Antioxidant	0.20	0.20	0.20	0.20	0.20	0.20
Limestone	13.90	20.40	19.90	20.40	19.90	19.90
Binder	5.00	5.00	5.00	5.00	5.00	5.00
Methionine	2.70	3.00	3.70	3.00	2.90	2.90
Dicalcium phosphate	0.00	8.40	10.50	7.30	6.90	6.70
Lysine	0.90	0.20	3.00	0.00	1.20	1.00
Soy lecithin	20.00	20.00	20.00	20.00	20.00	20.00
Fish oil	38.90	42.30	45.40	37.60	40.40	40.00
Vitamin and mineral supplement [1]	8.00	8.00	8.00	8.00	8.00	8.00
Common salt	7.80	9.20	9.30	9.00	9.10	9.10
Magnesium sulfate	0.00	2.80	2.80	2.80	2.80	2.80
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Bromatological Composition [2]						
Dry matter (%)	91.16	90.44	93.21	91.71	93.76	90.18
Crude protein (%)	38.35	38.13	39.01	38.62	39.52	38.21
Ethereal extract (%)	4.34	5.08	4.22	4.37	4.93	6.83
Ash (%)	12.83	9.15	9.96	9.42	9.84	9.14
Crude energy (kcal kg^{-1})	4400.00	4440.00	4380.00	4470.00	4360.00	4360.00

[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—1.900 mg; vit. B2—2.000 mg; vit. B6—2.400 mg; vit. B12—3.500 mg; folic acid—200 mg; calcium pantothenate—4.000 mg; vit. C—25 g; biotin—40 mg; niacin—5.000 mg; Fe—12.5 g; Cu—2.000 mg; Mn—7.500 mg; Zn—25 g; I—200 mg; Se—70 mg. [2] Bromatological composition of experimental feed analyzed based on natural matter, in triplicate per sample.

Feeding was provided six times a day (3:00 a.m., 08:30 a.m., 11:00 a.m., 2:00 p.m., 5:00 p.m., and 10:00 p.m.). The feeding rate was adjusted to achieve a minimum weekly growth of one gram and a maximum feed conversion rate of 1.5:1 (feed provided:shrimp growth in grams). Biometrics (weight) were performed at the beginning of the experiment and subsequently every seven days to adjust the feeding rate. Daily siphoning of all experimental units was performed to prevent accumulation of leftover feed, feces, and debris.

2.2. Water Quality Monitoring

Daily monitoring of water quality variables such as temperature, dissolved oxygen, and pH was conducted using multiparameter equipment, Hanna HI98196. Salinity was determined weekly using a manual refractometer (Biobrix model 211, São Paulo, Brazil). Alkalinity and hardness were measured biweekly, and the concentrations of total ammonia and nitrite were determined three times a week [20].

The average values of the monitored water quality parameters were: temperature of 28.68 ± 1.46 °C; pH of 7.48 ± 0.17; dissolved oxygen of 4.99 ± 0.95 mg L^{-1}; salinity of 15.00 ± 0.00 g L^{-1}; alkalinity of 93.00 ± 9.2 mg L^{-1} $CaCO_3$; hardness of 1929.3 ± 71.4 mg L^{-1} $CaCO_3$; total ammonia of 0.02 ± 0.03 mg L^{-1}; and nitrite of 0.03 ± 0.02 mg L^{-1}. All water quality parameters remained within the recommended range for the production of *P. vannamei* [21–24].

2.3. Zootechnical Performance and Animal Welfare Assessment

At the end of the experiment, all shrimps were counted, weighed, and measured (total length) to determine the survival rate (final number of shrimps/initial number of shrimps x 100), biomass gain (final number of shrimps × final average weight) − (initial number of shrimps × initial average weight), apparent feed conversion (amount of feed provided/biomass gain), and specific growth rate {[log_e(final weight) − log_e(initial weight)]/period} × 100. For the assessment of animal welfare, the total antenna length was measured [25].

2.4. Centesimal Chemical Composition of Feeds and Shrimp

Diet samples and the tails of eight animals from each treatment were randomly collected for the evaluation of the centesimal body chemical composition, including crude protein, crude energy, ether extract, and ash, following the methodologies described in AOAC [26]. The moisture content was determined by drying the pre-weighed samples in porcelain cups at 105 °C for 12 hours. Ash content was determined by incinerating the dried samples at 600 °C for 3 hours. Protein content was determined using the Kjeldahl method [26], ether extract by petroleum ether extraction in an ANKOM® XT10 extractor, and energy content using an IKA® model C 5000 control. All analyses were performed in triplicates at the Laboratory of Animal Feeding and Nutrition—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 collected and their hepatopancreas sampled to evaluate the activity of enzymes related to the shrimp digestive system. The hepatopancreas samples were homogenized with PBS solution (pH 7.2) using a tissue homogenizer, centrifuged at 5000 rpm for 10 minutes, and the supernatants were separated for the determinations. The concentrations of amylase (U/L/mg protein), cellulase (nmol/min/mg protein), maltase (µmol/min/mg protein) [27], sucrase (µmol/min/mg protein) [27], lipase (U/L/mg protein), and trypsin (µmol/min/mg protein) [28] were determined.

Maltase and saccharase activities were determined [27]. To determine the enzyme activity, glucose dosage 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 per the manufacturer's recommendations. Trypsin activity was determined according to the method by Hummel [28]. 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 expressed in µmol/min/mg of protein. Cellulase activity was determined according to the methodology by Niiyama and Toyohara [29]. The amount of reducing sugar formed was measured using the nitroblue tetrazolium (NBT) method at 660 nm. D-glucose was used as the standard for the calibration curve, and results were expressed as nmol/min/mg protein.

2.6. Total Hemocyte Count

At the end of the experiment, eight shrimps per treatment were desensitized by thermonecrosis [30]. The hemolymph was collected, and the remaining animal body was used for proximate composition analysis. The hemolymph was collected using a 1 mL syringe and a 13 × 0.45 mm needle from the second central segment (somite), and stored in a 2 mL microtube containing cold, modified Alsever anticoagulant (composed of glucose, sodium citrate, sodium chloride, and deionized water) in a 1:9 ratio (hemolymph:anticoagulant) [31]. The total hemocyte count (THC) was attained using a Neubauer chamber and calculated using the equation: $\text{THC mL}^{-1} = \frac{N^{\circ}\ TCC}{N^{\circ}\ QC} \times FD \times 10,000$

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

2.7. Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) after verifying the assumptions of normality and homoscedasticity, and the mean values from each treatment were compared using Tukey's test. All results were tested at a significance level of 5% ($\alpha = 0.05$) and the statistical package used was R Studio 2023.09.1 [32].

3. Results

3.1. Zootechnical Performance and Animal Welfare Assessment

No significant differences were observed among the treatments regarding the zootechnical performance parameters of the shrimps ($p < 0.05$). However, significant differences were found with respect to the antenna length of the shrimps, indicating variations in animal welfare among the treatments ($p = 0.04$) (Table 2). The antenna length of the shrimps from treatment T5 was significantly greater than that from T4, while it was similar to that recorded under treatments T3 and T6. There was no significant difference among treatments T1, T2, T3, T4, and T6.

Table 2. Mean ± standard deviation of zootechnical performance parameters of shrimps that were fed diets containing different sources of protein hydrolysates as partial replacements for fish meals during the grow-out phase.

Treatment *	Final Weight (g)	Total Length (cm)	Antenna Length (cm)	Final Biomass (g)	Biomass Gain (g)	Survival (%)	Feed Conversion	Specific Growth Rate
T1	11.62 ± 2.40	9.86 ± 1.00	12.00 ± 3.40 ^{a}	47.60 ± 8.70	40.83 ± 10.20	90.00 ± 12.00	1.31 ± 0.31	4.18 ± 0.14
T2	10.61 ± 1.34	9.75 ± 0.50	12.10 ± 4.20 ^{a}	48.21 ± 11.50	41.44 ± 12.40	93.00 ± 10.00	1.23 ± 0.35	4.10 ± 0.13
T3	11.21 ± 1.85	9.71 ± 0.90	13.10 ± 2.60 ^{ab}	50.67 ± 12.00	43.90 ± 13.20	93.00 ± 14.00	1.14 ± 0.40	4.17 ± 0.05
T4	9.70 ± 1.98	9.52 ± 1.00	12.30 ± 2.20 ^{a}	45.30 ± 9.90	38.53 ± 11.00	90.00 ± 12.00	1.40 ± 0.56	3.98 ± 0.68
T5	11.42 ± 2.94	9.82 ± 1.20	15.20 ± 2.10 ^{b}	45.87 ± 6.10	39.10 ± 3.80	80.00 ± 10.60	1.11 ± 0.11	4.00 ± 0.40
T6	10.75 ± 2.71	9.72 ± 1.10	15.10 ± 3.80 ^{ab}	33.48 ± 4.00	26.71 ± 1.00	85.00 ± 11.00	1.51 ± 0.42	4.20 ± 0.14

* 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. Superscript letters indicate significant differences between the experimental groups with "a" as the lowest value.

3.2. Centesimal Chemical Composition of Feeds and Shrimps

Regarding the centesimal body chemical composition based on natural matter, the parameters of dry matter, ether extract, and ash were affected by the treatments ($p = 0.02$, $p = 0.04$, $p = 0.04$, respectively). For dry matter, all treatments differed from each other, with the highest dry matter content observed in T4 (Aquabite®) ($p > 0.05$). For ether extract, the highest values were observed in the control, feather hydrolysate, CPH, and Aquabite® treatments ($p = 0.04$) (Table 3). In treatment T3, the ether extract was higher, being statistically similar to T1, T2, and T4 and significantly higher than T5 and T6. There was no significant difference among treatments T2, T4, T5, and T6. Likewise, a higher ash

value was observed in T3, which was statistically similar to that in T2 and higher than that in T1, T4, T5, and T6.

Table 3. Mean values of centesimal chemical composition parameters of shrimp carcasses based on natural matter during the grow-out phase.

Treatment *	Dry Matter (%)	Crude Protein (%)	Ethereal Extract (%)	Ash (%)	Crude Energy (kcal kg^{-1})
T1	19.14 ± 0.00 ^{f}	14.98 ± 1.60	0.49 ± 0.25 ^{a}	1.84 ± 0.28 ^{a}	950.00 ± 18.21
T2	20.92 ± 0.01 ^{e}	16.68 ± 0.90	0.44 ± 0.14 ^{ab}	2.18 ± 0.12 ^{ab}	1030.00 ± 27.53
T3	22.69 ± 0.00 ^{c}	18.01 ± 1.00	0.61 ± 0.16 ^{a}	2.47 ± 0.07 ^{b}	1127.00 ± 116.98
T4	23.32 ± 0.00 ^{a}	18.34 ± 0.69	0.49 ± 0.19 ^{ab}	2.22 ± 0.01 ^{b}	1165.00 ± 26.23
T5	21.62 ± 0.02 ^{d}	17.09 ± 0.40	0.40 ± 0.02 ^{b}	2.15 ± 0.09 ^{a}	1076.00 ± 38.75
T6	23.08 ± 0.11 ^{b}	18.24 ± 0.10	0.40 ± 0.07 ^{b}	2.22 ± 0.06 ^{b}	1124.00 ± 28.61

* 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. Superscript letters indicate significant differences between the experimental groups with "a" as the lowest value.

3.3. Enzymatic Activity Assessment

Regarding the analysis of digestive enzyme activity, the treatments influenced the activities of amylase and trypsin ($p < 0.05$). With respect to amylase, treatment T2 (CPH) differed from treatment T5 (CPH + maltodextrin), with the lowest values observed under treatment T2 and the highest under treatment T5. With respect to trypsin, treatments T2 (CPH) and T3 (enzymatic hydrolysate of chicken feathers) differed from treatments T4 (Aquabite®), T5 (CPH + maltodextrin), and T6 (CPH with added yeast), but were similar to the control (Table 4).

Table 4. Mean and standard deviation of digestive enzyme activity evaluated.

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	32.59 ± 6.05 ^{ab}	0.17 ± 0.04	14.97 ± 1.37	1405.98 ± 239.55	380.82 ± 67.86	0.04 ± 0.01 ^{ab}
T2	27.29 ± 4.39 ^{a}	0.32 ± 0.04	14.76 ± 0.73	1537.42 ± 248.07	200.95 ± 61.79	0.05 ± 0.01 ^{b}
T3	35.74 ± 11.85 ^{ab}	0.21 ± 0.07	14.02 ± 0.94	1279.81 ± 113.11	422.99 ± 44.21	0.05 ± 0.01 ^{b}
T4	46.47 ± 15.59 ^{ab}	0.31 ± 0.18	15.62 ± 2.33	1098.34 ± 115.42	286.84 ± 53.24	0.02 ± 0.00 ^{a}
T5	49.52 ± 9.26 ^{b}	0.24 ± 0.08	13.81 ± 0.31	1120.58 ± 409.94	407.10 ± 153.42	0.03 ± 0.00 ^{a}
T6	36.55 ± 3.13 ^{ab}	0.27 ± 0.08	13.44 ± 0.78	1161.62 ± 171.74	361.28 ± 162.43	0.03 ± 0.00 ^{a}

* 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. Superscript letters indicate significant differences between the experimental groups with "a" as the lowest value.

3.4. Total Hemocyte Count

No significant differences were found among the treatments with respect to total hemocyte count ($p > 0.05$) (Table 5).

Table 5. Total hemocyte count of *Penaeus vannamei* during the grow-out phase.

Treatment *	Total Hemolytic Cells
T1	$9 \times 10^6 \pm 5 \times 10^5$
T2	$10 \times 10^6 \pm 4 \times 10^5$
T3	$10 \times 10^6 \pm 7 \times 10^5$
T4	$11 \times 10^6 \pm 5 \times 10^5$
T5	$12 \times 10^6 \pm 6 \times 10^5$
T6	$11 \times 10^6 \pm 4 \times 10^5$

* 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 from different sources are viable ingredients to be used in the nutrition of aquatic organisms as a replacement of fish meals. These ingredients can improve the growth rate and feed conversion of fish and crustaceans, as well as enhance non-specific immunity and be used as a source of bioactive peptides [33]. They also enhance the metabolic utilization of the protein fraction, reducing the excretion of nitrogen compounds and favoring water quality in production systems. Additionally, they improve the palatability of diets without affecting the feeding behavior of the animals [34]. It has been reported that the inclusion of hydrolyzed protein from poultry and pork by-products in *P. vannamei* diet alters the enzymatic activity of the hepatopancreas and the composition of the intestinal microbiota. The inclusion of this protein hydrolyzate in the diet changed the enzymatic activity of the shrimps when compared to the control group ($p < 0.05$). Amylase activity increased proportionally to the increase in the percentage of protein replacement in the diet. Furthermore, a metagenomic analysis revealed changes in the shrimp intestinal microbiome: increasing levels of replacement led to greater richness and diversity in the intestinal microbiota in the 75% and 100% inclusion level treatments. This was mainly related to changes in abundances in the Rhodobacteraceae and Flavobacteriaceae families. A decrease in the abundance of the Vibrionaceae family was also observed, showing that the protein hydrolyzate demonstrated beneficial changes when added to the *P. vannamei* diet [10].

In the present study with the grow-out phase of *P. vannamei,* neither the partial replacement (6%) of fish meals with enzymatic hydrolysate of chicken feathers, with or without additives, nor the enzymatic hydrolysate of chicken feathers affected the zootechnical performance of the animals. This confirms that the diets were nutritionally balanced and that these ingredients could be used in the feeding of the species in question. Additionally, the feed conversion rate did not differ among the treatments, which was similar to or better than the results obtained in similar studies with different protein hydrolysates [35,36], indicating good digestibility of nutrients and the ability of the shrimp to utilize them.

Regarding animal welfare, the substitution of fish meals (6%) with enzymatic hydrolysate of chicken feathers or protein hydrolysate of chicken feathers combined with maltodextrin and/or yeast had a positive effect on antenna length. Antenna length is used as an indicator of animal welfare because stressful environmental changes can damage the antennae [37,38]. Therefore, long and intact antennae indicate that the animals were in a suitable environment. Animals kept under treatment T4 showed shorter antenna length than animals kept under the other treatment conditions. This may be related to the nutritional composition of the offered diet, considering that all other conditions were identical among 3 treatments.

Centesimal body chemical composition analysis is used to determine the health and physiological parameters of aquatic organisms because their body composition is directly affected when there are nutritional deficiencies in the provided food [39]. In this study, the body dry matter of the shrimps was affected by the inclusion of different hydrolysates, with the highest content observed under the treatment with the commercial product Aquabite®, and the lowest under the control treatment. The ether extract was higher in the control treatment, chicken protein hydrolyzate, enzymatic hydrolyzate of chicken feathers, and Aquabite® treatments ($p < 0.05$), while the ash content was higher in the treatments with protein hydrolyzate of chicken feathers and enzymatic hydrolyzate of chicken feathers ($p < 0.05$). Although there was a significant difference among the treatments with respect to these parameters, the bromatological analysis did not indicate any abnormalities, suggesting that the studied diets provided the necessary nutrients to the shrimps in the grow-out phase; all the tested ingredients showed good nutrient absorption by the shrimps without compromising their zootechnical performance and health.

Digestive enzyme regulation in shrimps highly influences their digestive capacities and, therefore, the ingredients that can be included in the diets [40]. In this study, variations in amylase and trypsin enzyme activities were detected. Amylase is a digestive enzyme

responsible for hydrolyzing α (1,4) glycosidic bonds in polysaccharides, releasing simple sugars [41]. Higher amylase enzyme activity was observed under the treatment with CPH + maltodextrin, but the other treatments, except for CPH, showed similar activities. This indicates that *P. vannamei* has the capacity to modulate the digestive activity according to the ingredients used.

Among the most important digestive proteases is trypsin, which catalyzes the separation of peptide bonds on the carboxyl side of the amino acids lysine and arginine [42]. In the present study, higher trypsin activity was observed in the diets containing fish meals, protein hydrolysate of chicken, and enzymatic hydrolysate of chicken feathers ($p < 0.05$). The low-molecular-weight peptides present in protein hydrolysates may have acted as functional ingredients, similarly to hormones, thus regulating the shrimp enzyme activity [11]. The high trypsin activity promotes the digestion and absorption of dietary protein, thereby promoting shrimp growth [4].

Among the immune parameters, the total hemocyte count (THC) is one of the most affected by stress conditions, whether environmental or due to infections or molting periods, and serves as an indicator of the animal's health status [43]. In the study, the use of hydrolyzate did not cause significant changes in the hemocyte counts when compared to the control treatment for the species and production phase.

5. Conclusions

In conclusion, the partial replacement of fish meals with different protein hydrolysates in the diet of *P. vannamei* in the grow-out phase did not affect zootechnical performance and animal health. This demonstrates that this species has the ability to modulate the activities of amylase and trypsin enzymes according to the ingredients used, ensuring the availability of nutrients for the metabolism of the cells. The positive effect on the shrimp antenna length documented when the fish meal was replaced (6%) with enzymatic hydrolysate of chicken feathers suggests a factor related to animal welfare, as any environmental change that may cause stress can damage antennae [37,38]. Therefore, all hydrolysates and combinations of additives tested, as well as the commercial product, included up to 6% of the total ingredients of the diet of *P. vannamei* in the grow-out phase, maintained satisfactory growth, animal welfare, carcass chemical composition, and digestive enzyme activity, equivalent to those achieved using fish meals. Additionally, this study provides important information for the formulation of nutritionally balanced diets with reduced dependence on fish meal for the Pacific white shrimp.

Author Contributions: Conceptualization and design of the research, provision of funding, and supervision of the study, E.L.C.B., L.H.C. and W.R.B.; formal analysis, C.N., C.H.d.N.F., R.O.K., R.L.F. and L.C.d.S.; investigation, C.N., C.H.d.N.F., R.O.K., R.L.F., M.C.R. and L.C.d.S.; data curation, C.N., C.H.d.N.F. and R.O.K.; writing—original draft preparation, M.T.M. and C.N.; writing—review and editing, M.T.M., E.L.C.B., L.H.C. and W.R.B.; visualization, E.L.C.B., L.H.C. and W.R.B.; supervision, E.L.C.B., L.H.C., M.T.M. and W.R.B.; project administration, E.L.C.B. and M.T.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study with invertebrates does not require ethical approval according to Brazilian federal law N° 11.794/08.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors would like to thank BRF S.A. for supplying the tested protein hydrolysates. E.L.C. Ballester (PQ process: 311456/2020-0) thanks the National Council for Scientific and Technological Development (CNPq) for the researcher productivity grant.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Food and Agriculture Organization. *The State of World Fisheries and Aquaculture. Towards Blue Transformation*; FAO: Rome, Italy, 2022. [CrossRef]
2. Richardson, A.; Dantas-Lima, J.; Lefranc, M.; Walraven, M. Effect of a black soldier fly ingredient on the growth performance and disease resistance of juvenile Pacific White Shrimp (*Litopenaeus vannamei*). *Animals* **2021**, *11*, 1450. [CrossRef]
3. Araripe, M.N.B.A.; Araripe, H.G.A.; Lopes, J.B.; Castro, P.L.; Braga, T.E.A.; Ferreira, A.H.C.; Abreu, M.L.T. Redução da proteína bruta com suplementação de aminoácidos em rações para alevinos de tambatinga. *R. Bras. Zootec.* **2011**, *40*, 1845–1850. [CrossRef]
4. Shao, J.; Zhao, W.; Liu, X.; Wang, L. Growth performance, digestive enzymes, and TOR signaling pathway of *Litopenaeus vannamei* are not significantly affected by dietary protein hydrolysates in practical conditions. *Front. Physiol.* **2018**, *9*, 998. [CrossRef] [PubMed]
5. Médale, F.; Kaushik, S. Les sources protéiques dans les aliments pour les poissons d'élevage. *Cah. Agric.* **2009**, *189*, 103–111. [CrossRef]
6. National Research Council–NRC. *Nutrient Requirements of Fish and Shrimp*; Animal Nutrition Series, National Research Council of the National Academies; The National Academies Press: Washington, DC, USA, 2011; 376p.
7. González-Félix, M.L.; Perez-Velazquez, M.; Ezquerra-Brauer, J.M.; Bringas-Alvarado, L.; Sánchez-Sánchez, A.; Torres-Arreola, W. Evaluation of jumbo squid (*Dosidicus gigas*) byproduct hydrolysates obtained by acid-enzymatic hydrolysis and by autohydrolysis in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *J. Food Sci. Technol.* **2014**, *34*, 552–558. [CrossRef]
8. Niu, J.; Zhang, Y.; Liu, Y.; Tian, L.; Lin, H.; Chen, X.; Yang, H.; Liang, G. Effects of graded replacement of fish meal by fish protein hydrolysate on growth performance of early post-larval Pacific white shrimp (*Litopenaeus vannamei*, Boone). *J. Appl. Anim. Res.* **2014**, *42*, 6–15. [CrossRef]
9. Quinto, B.P.T.; Albuquerque, J.V.; Bezerra, R.S.; Peixoto, S.; Soares, R. Replacement of fishmeal by two types of fish protein hydrolysate in feed for post larval shrimp *Litopenaeus vannamei*. *Aquac. Nutr.* **2017**, *24*, 768–776. [CrossRef]
10. Soares, M.; Rezende, P.C.; Corrêa, N.M.; Rocha, J.S.; Martins, M.A.; Andrade, T.C.; Fracalossi, D.M.; Vieira, F.N. Protein hydrolysates from poultry by-product and swine liver as an alternative dietary protein source for the Pacific white shrimp. *Aquac. Rep.* **2020**, *17*, e100344. [CrossRef]
11. Soares, M.; Gonçalves, P.; Schleder, D.D.; Delgadillo-Diaz, M.; Gullian-Klanian, M.; Vieira, F.N. Protein hydrolysate of poultry by-product and swine liver in the diet of pacific white shrimp. *Bol. Inst. Pesca* **2021**, *47*, e657. [CrossRef]
12. Hou, Y.; Wu, Z.; Dai, Z.; Wang, G.; Wu, G. Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 24. [CrossRef]
13. Chalamaiah, M.; Kumar, B.D.; Hemalatha, R.; Jyothirmayi, T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem.* **2012**, *135*, 3020–3038. [CrossRef]
14. Santos, R.A.; Piovesan, M.R.; Oliveira, S.R.; Hattori, J.F.A.; Souza, O.J.; Boscolo, W.J.; Signor, A.; Bittencourt, F. Atratividade e palatabilidade da proteína hidrolisada de penas para juvenis de tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*). *Res. Soc. Dev.* **2022**, *11*, e19111637352. [CrossRef]
15. Bui, H.T.D.; Khosravi, S.; Fournier, V.; Herault, M.; Lee, K.J. Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (*Pagrus major*) fed diets supplemented with protein hydrolysates. *Aquaculture* **2014**, *418–419*, 11–16. [CrossRef]
16. Chalamaiah, M.; Jyothirmayi, T.; Diwan, P.V.; Kumar, B.D. Antioxidant activity and functional properties of enzymatic protein hydrolysates from common carp (*Cyprinus carpio*) roe (egg). *J. Food Sci. Technol.* **2015**, *52*, 5817–5825. [CrossRef]
17. Hlordzi, V.; Wang, J.; Kuebutornye, F.K.A.; Yang, X.; Tan, B.; Li, T.; Cui, Z.; Lv, S.; Lao, T.; Chi, S. Hydrolysed fish protein powder is better at the growth performance, hepatopancreas and intestinal development of Pacific white shrimp (*Litopenaeus vannamei*). *Aquac. Rep.* **2022**, *23*, 101025. [CrossRef]
18. Mahdy, M.A.; Jamal, M.T.; Al-Harb, M.; Al-Mur, B.A.; Haque, M.F. Use of yeasts in aquaculture nutrition and immunostimulation: A review. *J. Appl. Biol.* **2022**, *10*, 59–65. [CrossRef]
19. Fawzya, Y.N.; Nursatya, S.M.; Susilowati, R.; Chasanah, E. Characteristics of fish protein hydrolysate from Yellowstripe Scad (*Selaroides leptolepis*) produced by a local microbial protease. *E3S Web Conf.* **2020**, *147*, e03017. [CrossRef]
20. American Public Health Association–APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
21. Boyd, C.E. *Water Quality in Warmwater Fish Ponds (n°. 639.3 B6923w Ej. 1 009523)*; Auburn University: Auburn, AL, USA, 1979.
22. Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mountain, J.; Scarpa, J. *Farming Marine Shrimp in Recirculating Freshwater Systems*; Harbor Branch Oceanographic Institution: Ft. Pierce, FL, USA, 1999.
23. Cheng, W.; Liu, C.H.; Kuo, C.M. Effects of dissolved oxygen on hemolymph parameters of freshwater giant prawn, *Macrobrachium rosenbergii* (de Man). *Aquaculture* **2003**, *220*, 843–856. [CrossRef]
24. Bett, C.; Vinatea, L. Combined effect of body weight, temperature and salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. *Braz. J. Oceanogr.* **2009**, *57*, 305–314. [CrossRef]
25. Kuhn, D.D.; Smith, S.A.; Boardman, G.D.; Angier, M.W.; Marsh, L.; Flick Jr, G.J. Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: Impacts on survival, growth, antennae length, and pathology. *Aquaculture* **2010**, *309*, 109–114. [CrossRef]
26. Association of Official Analytical Chemist—AOAC. *Official Methods of Analysis of AOAC International*, 16th ed.; AOAC Inc.: Arlington, VA, USA, 1995.

27. Retcheski, M.C.; Maximowski, L.V.; Escorsin, K.J.S.; Kurosaki, J.K.A.R.; Romão, S.; Bitencourt, T.B.; Parra, J.E.G.; Cazarolli, L.H. Yarrowia lipolytica biomass—A potential additive to boost metabolic and physiological responses of Nile tilapia. *Fish Physiol. Biochem.* **2023**, *49*, 655–670. [CrossRef]
28. Hummel, B.C. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. *Can. J. Biochem. Physiol.* **1959**, *37*, 1393–1399. [CrossRef]
29. Niiyama, T.; Toyohara, H. Widespread distribution of cellulase and hemicellulase activities among aquatic invertebrates. *Fish. Sci.* **2011**, *77*, 649–655. [CrossRef]
30. Ashley, P.J. Fish welfare: Current issues in aquaculture. *Appl. Anim. Behav. Sci.* **2007**, *104*, 199–235. [CrossRef]
31. Vieira, F.D.N.; Jatobá, A.; Mouriño, J.L.P.; Buglione Neto, C.C.; Silva, J.S.D.; Seiffert, W.Q.; Vinatea, L.A. Use of probiotic-supplemented diet on a Pacific white shrimp farm. *R. Bras. Zootec.* **2016**, *45*, 203–207. [CrossRef]
32. Zar, J.H. *Biostatical Analysis*, 5th ed.; Pearson Prentice Hall: Upper Saddle River, NJ, USA, 2010.
33. Martínez-Alvarez, O.; Chamorro, S.; Brenes, A. Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. *Food Res. Int.* **2015**, *73*, 204–212. [CrossRef]
34. Alves, D.R.S.; Oliveira, S.R.; Luczinski, T.G.; Paulo, I.G.P.; Boscolo, W.R.; Bittencourt, F.; Signor, A. Palatability of protein hydrolysates from industrial byproducts for Nile tilapia juveniles. *Animals* **2019**, *9*, 311. [CrossRef] [PubMed]
35. Hernández, C.; Olvera-Novoa, M.A.; Aguilar-Vejar, K.; González-Rodríguez, B.; Parra, I.A. Partial replacement of fish meal by porcine meat meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture* **2008**, *277*, 244–250. [CrossRef]
36. Bauer, W.; Prentice-Hernandez, W.; Tesser, M.B.; Wasielesky, W., Jr.; Poersch, L.H.S. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp *Litopenaeus vannamei*. *Aquaculture* **2012**, *15*, 112–116. [CrossRef]
37. New, M.; Valenti, W.; Tidwell, J.; D'Abramo, L.; Kutty, M. *Freshwater Prawns: Biology and Farming*, 1st ed.; Wiley-Blackwell: Chichester, UK, 2010; 544p.
38. Eap, D.; Correa, S.; Ngo-Vu, H.; Derby, C.D. Chemosensory basis of feeding behavior in pacific white shrimp, *Litopenaeus vannamei*. *Biol. Bull.* **2020**, *239*, 115–131. [CrossRef]
39. Ebadi, H.; Zakeri, M.; Mousavi, S.M.; Yavari, V.; Souri, M. The interaction effects of dietary lipid, vitamin E and vitamin C on growth performance, feed utilization, muscle proximate composition and antioxidant enzyme activity of white leg shrimp (*Litopenaeus vannamei*). *Aquac. Res.* **2020**, *52*, 2048–2060. [CrossRef]
40. Carrillo-Farnés, O.; Forrellat-Barrios, A.; Guerrero-Galván, S.; Vega-Villasante, F. A review of digestive enzyme activity in Penaeid shrimps. *Crustaceana* **2007**, *80*, 257–275. [CrossRef]
41. Roy, S.; Kumar, V.; Mitra, A.; Manna, R.K.; Suresh, V.R.; Homechaudhur, S. Amylase and protease activity in shrimps and prawn of Sundarbans, West Bengal, India. *Indian J. Geo Mar. Sci.* **2018**, *47*, 53–59.
42. Aguiñaga-Cruz, J.A.; Sainz-Hernández, J.C.; García-Rodríguez, L.D.; García-Ulloa, M.; García-Gutiérrez, C.; Montoya-Mejía, M. Trypsin polymorphism and modulation in *Penaeus vannamei* (Boone, 1931): A review. *Lat. Am. J. Aquat. Res.* **2019**, *47*, 723–732. [CrossRef]
43. Costa, A.M.; Martins, P.C.C. Análise da contagem total de hemócitos e capacidade coagulante da hemolinfa do camarão *Litopenaeus vannamei* (BOONE, 1931) em cultivos com ocorrência de necrose muscular. *Bol. Inst. Pesca* **2009**, *35*, 545–551.