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Inclusion of Hydrolyzed Feather Meal in Diets for Giant River Prawn (*Macrobrachium rosenbergii*) During the Nursery Phase: Effects on Growth, Digestive Enzymes, and Antioxidant Status

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Featured Application

This study demonstrates the potential use of hydrolyzed feather meal as a sustainable alternative to fishmeal in diets for *Macrobrachium rosenbergii* post-larvae, promoting prawn growth and welfare while maintaining health and carcass quality.

Abstract

We evaluated the inclusion of hydrolyzed feather meal (HFM) as a partial replacement for fishmeal in diets for *Macrobrachium rosenbergii* post-larvae (PL) over a 32-day nursery feeding trial. Five experimental diets with increasing HFM levels (control, 1.5%, 3.0%, 4.5%, and 6.0%) were tested. Survival rates ranged from 73.3 ± 5.44% to 83.3 ± 3.84% without significant differences among groups. Dietary HFM inclusion levels above 3.0% significantly improved prawn performance, including final weight (up to 2.18-fold higher than control), length (1.13-fold), antenna length (1.18-fold), biomass gain (2.14-fold), and feed conversion ratio (1.59-fold lower). Prawn-fed diets at 6.0% HFM showed the highest performance among all experimental groups. No significant effects were observed on antioxidant biomarkers or digestive enzymes in prawns hepatopancreas, which suggests no imbalance in the antioxidant system or impairment of digestive function. Likewise, carcass proximate composition remained stable across experimental groups. These findings suggest that HFM at 3.0–6.0% dietary inclusion levels is a potential alternative to fishmeal in nursery-phase diets for *M. rosernbergii* PL, promoting prawn growth and welfare and maintaining health and carcass quality. Notably, to the best of our knowledge, this is the first study demonstrating the potential effective use of HFM in feeding the nursery phase of *M. rosernbergii*.

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1. Introduction

The giant river prawn (*Macrobrachium rosenbergii*) is among the most important freshwater crustaceans in global aquaculture due to its high commercial value and favorable production performance [1]. Its appeal lies in several biological and zootechnical advantages, including high fertility, adaptability to a wide range of environmental conditions, tolerance to fluctuations in water quality, and rapid growth rates [2,3]. In 2020, the global production of *M. rosenbergii* reached approximately 294,000 tons, making a significant contribution to total crustacean aquaculture output [4]. This tropical species has gained increasing attention not only for its market demand but also for its potential role in developing more sustainable aquaculture systems [5].

In recent years, considerable research efforts have been devoted to improving productivity and profitability in *M. rosenbergii* farming. Strategies have included the use of growth enhancers, dietary immunostimulants [6,7], and the dietary inclusion of alternative protein sources to reduce reliance on fishmeal, a limited and costly marine-derived resource [8,9]. Among these alternatives, hydrolyzed animal proteins have shown encouraging results across multiple crustacean species. For example, in Pacific white shrimp (*Penaeus vannamei*), dietary replacement of fishmeal with poultry by-products and swine liver hydrolysates (up to 25%) improved growth performance, feed conversion ratio, and nutrient retention [10], while also modulating the intestinal microbiota by decreasing the relative abundance of Vibrionaceae [11]. Furthermore, the inclusion of protein hydrolysates—such as hydrolyzed feather meal—at 6% maintained growth, digestive enzyme activity, health status, and body composition in *P. vannamei* during both nursery and grow-out phases [12,13].

Hydrolyzed feather meal (HFM) has emerged as a promising candidate among animal protein hydrolysates due to its high protein content and potential to support circular economy principles. For instance, the current global market of feather meal is USD 619.1 million [14], and the European annual production of feather meal is approximately 175,000 tons [15]. In terms of current market price, HFM trades for USD 661.8 metric ton^{-1}, which is substantially cheaper than fishmeal (USD 1788.4 metric ton^{-1}) [16].

Derived from poultry industry by-products, HFM offers a sustainable mean of reducing aquaculture dependence on marine protein sources. Prior studies have demonstrated the feasibility of partially replacing fishmeal with HFM in diets for *P. vannamei* [17,18] and *Penaeus monodon* [19] without compromising growth performance. However, HFM digestibility can be lower than that of fishmeal, potentially due to the presence of disulfide-rich keratin structures [20,21]. Then, HFM may present low methionine content and the potential accumulation of undesirable residues if not properly processed [20,22].

The use of HFM in freshwater crustacean diets remains underexplored. While limited work has been conducted on juvenile white shrimp [13,23] and juvenile longer river prawn [24], no studies to date have evaluated the use of HFM in *M. rosenbergii*, particularly during early developmental stages. Therefore, the present study aims to investigate the effects of increasing dietary inclusion levels (0 to 6%) of hydrolyzed feather meal as a partial replacement for fishmeal on the growth performance, digestive enzyme activity, and antioxidant status of *M. rosenbergii* post-larvae during the nursery phase.

2. Materials and Methods

2.1. Experimental Conditions and Animals

This study was carried out in the Laboratory of Prawn Culture of the Nucleus for Research and Development in Sustainable Aquaculture at the Federal University of Paraná (UFPR; Maripá, Paraná, Brazil).

A total of 1600 mixed-sex *Macrobrachium rosenbergii* post-larvae aged 20 days (initial mean weight: 0.021 ± 0.006 g) were obtained from the hatchery of the Prawn Culture Laboratory. Prawns were randomly distributed across 20 experimental tanks (250 L each), corresponding to five dietary treatments with four replicates per treatment. Prior to the start of the experiment, the post-larvae underwent a 7-day acclimation period in a 1000-L holding tank maintained under the same environmental and water conditions as those used during the experimental phase. During acclimation, animals were fed the control diet.

The experimental facility consisted of a clear water recirculating aquaculture system, comprising 20 individual tanks. integrated into a recirculating aquaculture system, equipped with mechanical filtration using a Bead Filter (A19 Skid—Altamar Aquatic Systems, Santos, Brazil).

The system maintained a recirculating flow of 1200 L h^{-1}. All tanks were housed in a temperature-controlled room with a 12 h light/12 h dark photoperiod.

Feeding was administered five times daily at 9:00, 11:00, 14:00, 17:00, and 21:00. The initial feeding rate was set at 10% of the total biomass and adjusted daily based on observed feed consumption. Prawns were monitored daily to record feed intake and mortality, and rations were adjusted accordingly. Uneaten feed and waste were removed thirty minutes after feeding through siphoning to maintain optimal water quality.

The feeding trial lasted for 32 days, a period consistent with common nursery durations for *M. rosenbergii* in tropical indoor systems, which typically range from two to eight weeks, with 30 days being standard [25].

2.2. Experimental Design and Diet Formulation

A completely randomized experimental design was used, comprising five dietary treatments with four replicates per treatment. The diets were formulated to include different levels of HFM as a partial replacement for fishmeal, specifically at inclusion rates of 0% (control diet), 1.5%, 3.0%, 4.5%, and 6.0%. The HFM was obtained from BRF. S.A. (Toledo, Paraná, Brazil) and its chemical composition are available in Table S1 of Supplementary Materials. To the best of the authors' knowledge, no research has investigated the recommended dietary inclusion level of HFM for *M. rosenbergii*. Thus, the dietary inclusion levels tested in the present trial were selected based on a precautionary approach, that is, we started with a low dosage aiming at minimizing the risk of eventual negative impact on animal health, welfare, and performance, and ensuring a safe evaluation of HFM potential.

Diet formulation was carried out using SuperCrac® 6.1 software (Viçosa, Minas Gerais, Brazil), ensuring that all diets were iso-nitrogenous (44.2 ± 0.4% crude protein) and isolipidic (14.8 ± 0.1% crude lipids) to meet the nutritional requirements of *M. rosenbergii* post-larvae [26]. The ingredients and proximate composition of the experimental diets are detailed in Table 1, as well as the diet amino acid (Table 2) and fatty acid profiles (Table 3).

Table 1. Ingredients (%) and proximate composition (% DM; mean ± SD) of the experimental diets for *Macrobrachium rosenbergii* post-larvae containing different levels of hydrolyzed feather meal.

	Experimental Diets				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
HFM	0.00	1.50	3.00	4.50	6.00
Fish meal	12.16	10.62	9.05	7.49	5.92
Soybean meal	35.00	33.75	32.50	31.25	30.00
Bone and meat meal	12.00	12.00	12.00	12.00	12.00
Poultry viscera meal	10.00	10.00	10.00	10.00	10.00
Wheat bran	7.04	8.00	8.94	9.87	10.81
Red blood cell meal	4.00	4.00	4.00	4.00	4.00
Ground cord	5.00	5.00	5.00	5.00	5.00
Soy lecithin	2.00	2.00	2.0	2.00	2.00
Fish oil	8.31	8.36	8.39	8.41	8.44
Binder	0.50	0.50	0.50	0.50	0.50
Limestone	0.81	0.92	1.02	1.11	1.21
Potassium chloride	0.55	0.59	0.63	0.66	0.70
Dicalcium phosphate	0.40	0.62	0.83	1.05	1.26
Vitamin and mineral premix [1]	0.80	0.80	0.80	0.80	0.80
Salt	0.87	0.77	0.67	0.57	0.47
DL—methionine	0.35	0.37	0.38	0.40	0.41
Lysine	0.09	0.06	0.11	0.17	0.31
Antifungal	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02	0.02	0.02
Magnesium sulphate	0.00	0.01	0.02	0.02	0.03
Total	100%	100%	100%	100%	100%
Proximate composition					
Dry matter (DM), %	93.5 ± 0.04	93.9 ± 0.04	94.2 ± 0.01	93.5 ± 0.04	93.4 ± 0.02
Crude protein, % DM	44.4 ± 0.09	44.2 ± 1.06	45.0 ± 0.26	43.7 ± 0.56	43.9 ± 0.01
Crude lipids, % DM	14.8 ± 0.30	14.5 ± 0.08	14.3 ± 0.06	15.2 ± 0.01	15.3 ± 0.05
Ash, % DM	16.4 ± 0.65	15.8 ± 0.03	15.9 ± 0.35	15.5 ± 0.06	15.3 ± 0.04
Crude fiber, % DM	2.90 ± 0.21	3.21 ± 0.15	2.65 ± 0.69	3.49 ± 0.15	3.21 ± 0.14

HFM: Hydrolyzed feather meal. [1] Vitamin and mineral premix (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.

Table 2. Amino acid profile (mg/100 g; mean ± SD) of the experimental diets containing different levels of hydrolyzed feather meal and fed *Macrobrachium rosenbergii* during the nursery phase.

	Experimental Diets				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
Samples, n	2	2	2	2	2
Alanine	2110 ± 7	2246 ± 128	2309 ± 160	2080 ± 26	2296 ± 437
Arginine	2181 ± 25	2316 ± 129	2335 ± 66	2179 ± 31	2339 ± 251
Aspartic Acid	3398 ± 29	3568 ± 139	3756 ± 199	3374 ± 126	3714 ± 783
Cysteine	394 ± 3	449 ± 25	480 ± 22	498 ± 15	577 ± 54
Glutamic Acid	5907 ± 70	6249 ± 271	6556 ± 336	5930 ± 209	6544 ± 1285
Glycine	2734 ± 39	2925 ± 262	2869 ± 181	2649 ± 84	2900 ± 356
Histidine	1095 ± 23	1119 ± 17	1127 ± 16	1051 ± 29	1121 ± 81
Isoleucine	971 ± 26	1183 ± 52	1220 ± 24	1106 ± 44	1188 ± 188
Leucine	2445 ± 28	2653 ± 119	2786 ± 90	2552 ± 84	2791 ± 425
Lysine	2317 ± 23	2494 ± 76	2659 ± 172	2381 ± 101	2723 ± 673
Methionine	610 ± 50	585 ± 45	551 ± 31	568 ± 44	590 ± 52

Table 2. *Cont.*

	Experimental Diets				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
Phenylalanine	1581 ± 4	1679 ± 103	1711 ± 11	1603 ± 31	1724 ± 100
Proline	2049 ± 4	2204 ± 162	2268 ± 144	2123 ± 5	2386 ± 362
Serine	1643 ± 7	1743 ± 98	1888 ± 55	1847 ± 22	2104 ± 240
Threonine	1318 ± 25	1396 ± 67	1463 ± 53	1356 ± 36	1497 ± 198
Tryptophan	334 ± 10	344 ± 3	337 ± 9	317 ± 8	286 ± 18
Tyrosine	612 ± 21	590 ± 12	598 ± 11	561 ± 7	587 ± 40
Valine	1384 ± 42	1699 ± 77	1753 ± 61	1615 ± 49	1735 ± 274

HFM: Hydrolyzed feather meal.

Table 3. Fatty acid profile (% total fatty acids; mean ± SD) of the experimental diets containing different levels of hydrolyzed feather meal and fed *Macrobrachium rosenbergii* during the nursery phase.

	Experimental Diets				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
Samples	2	2	2	2	2
C6:0	0.53 ± 0.03	0.89 ± 0.01	0.86 ± 0.04	0.83 ± 0.02	0.76 ± 0.16
C7:0	0.08 ± 0.02	0.14 ± 0.03	0.16 ± 0.01	0.12 ± 0.02	0.11 ± 0.01
C8:0	0.08 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
C9:0	0.13 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.21 ± 0.01	0.16 ± 0.01
C10:0	0.06 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
C12:0	0.11 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.01
C13:0	0.11 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.15 ± 0.04	0.14 ± 0.01
C17:0	0.52 ± 0.01	0.57 ± 0.01	0.56 ± 0.01	0.56 ± 0.01	0.51 ± 0.01
C16:0	29.9 ± 0.10	31.6 ± 0.01	31.5 ± 0.21	30.9 ± 0.17	29.5 ± 0.06
C15:0	0.71 ± 0.01	0.73 ± 0.04	0.71 ± 0.01	0.71 ± 0.02	0.67 ± 0.01
C14:0	3.42 ± 0.04	3.46 ± 0.09	3.37 ± 0.02	3.30 ± 0.01	3.22 ± 0.01
C18:0	9.99 ± 0.08	10.6 ± 0.39	10.6 ± 0.05	10.5 ± 0.02	9.61 ± 0.01
C22:0	0.22 ± 0.01	0.25 ± 0.08	0.29 ± 0.10	0.25 ± 0.08	0.23 ± 0.02
C20:0	0.60 ± 0.01	0.73 ± 0.01	0.71 ± 0.01	0.73 ± 0.01	0.67 ± 0.01
C14:1 n-7	0.35 ± 0.02	0.40 ± 0.03	0.41 ± 0.01	0.34 ± 0.07	0.35 ± 0.07
C17:1 n7	0.31 ± 0.01	0.30 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.33 ± 0.01
C16:1 n-7	5.26 ± 0.01	4.85 ± 0.12	4.89 ± 0.01	4.97 ± 0.01	5.22 ± 0.04
C16:1 n-9	0.61 ± 0.01	0.55 ± 0.02	0.56 ± 0.01	0.57 ± 0.01	0.60 ± 0.01
C15:1 n-9	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C18:1 n-9	34.2 ± 0.15	31.7 ± 0.18	32.2 ± 0.30	33.6 ± 0.13	34.7 ± 0.03
C18:1 n-7	3.38 ± 0.01	3.18 ± 0.01	3.18 ± 0.01	3.26 ± 0.01	3.28 ± 0.01
C18:1 n-5	0.47 ± 0.01	0.53 ± 0.01	0.49 ± 0.02	0.49 ± 0.01	0.45 ± 0.01
C19:1 n-9	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
C22:1 n-9	0.38 ± 0.01	0.35 ± 0.01	0.38 ± 0.07	0.33 ± 0.05	0.42 ± 0.01
C20:1 n-9	2.16 ± 0.02	2.21 ± 0.11	2.07 ± 0.03	2.21 ± 0.01	2.15 ± 0.01
C20:1 n-12	0.58 ± 0.02	0.77 ± 0.04	0.72 ± 0.01	0.73 ± 0.01	0.67 ± 0.01
C18:3 n-3	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.02	0.16 ± 0.01	0.26 ± 0.01
C20:3 n-3	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.05 ± 0.01	0.07 ± 0.01
C18:2 c9t11	0.18 ± 0.01	0.24 ± 0.01	0.22 ± 0.02	0.19 ± 0.03	0.18 ± 0.02
C18:3 n-6	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.09 ± 0.01
C18:2 n-6	3.50 ± 0.04	2.89 ± 0.03	2.76 ± 0.06	2.37 ± 0.03	3.73 ± 0.01
C20:2 n-6	0.23 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.22 ± 0.01
C20:3 n-6	0.08 ± 0.01	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.01
C20:4 n-6	0.13 ± 0.06	0.19 ± 0.04	0.21 ± 0.13	0.18 ± 0.05	0.10 ± 0.01
C20:5 n-3	0.30 ± 0.00	0.36 ± 0.03	0.32 ± 0.03	0.31 ± 0.01	0.29 ± 0.01
C22:5 n-3	0.11 ± 0.03	0.12 ± 0.02	0.10 ± 0.03	0.09 ± 0.02	0.13 ± 0.02

Table 3. *Cont.*

	Experimental Diets				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
C21:5 n-3	0.21 ± 0.02	0.32 ± 0.07	0.27 ± 0.01	0.27 ± 0.01	0.23 ± 0.01
C22:6 n-3	0.24 ± 0.02	0.20 ± 0.03	0.34 ± 0.03	0.32 ± 0.03	0.15 ± 0.02
C22:2 n-6	0.14 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.04 ± 0.01
C22:5 n-6	0.13 ± 0.02	0.15 ± 0.01	0.20 ± 0.02	0.11 ± 0.04	0.14 ± 0.03
C22:4 n-6	0.06 ± 0.01	0.09 ± 0.02	0.03 ± 0.05	0.00 ± 0.00	0.03 ± 0.01
SFA	46.5 ± 0.10	49.7 ± 0.32	49.5 ± 0.06	48.6 ± 0.01	45.9 ± 0.01
MUFA	47.9 ± 0.12	45.0 ± 0.12	45.4 ± 0.23	47.0 ± 0.18	48.0 ± 0.01
PUFA	5.67 ± 0.02	5.32 ± 0.20	5.16 ± 0.29	4.44 ± 0.17	5.77 ± 0.01
PUFA n-6	4.54 ± 0.04	4.04 ± 0.09	3.86 ± 0.21	3.24 ± 0.17	4.63 ± 0.02
PUFA n-3	1.13 ± 0.02	1.28 ± 0.11	1.30 ± 0.08	1.21 ± 0.01	1.14 ± 0.03

HFM: Hydrolyzed feather meal; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

For diet preparation, all ingredients were individually milled using a knife mill fitted with a 0.5-mm sieve to achieve uniform particle size. The ground ingredients were then mixed thoroughly according to the specific formulations. Pelleting was performed through cold extrusion to prevent nutrient degradation. The resulting pellets were stored at 4 °C under refrigeration to preserve quality until use.

2.3. Recordings and Samplings

Water quality parameters were maintained within optimal ranges for *M. rosenbergii* culture throughout the experiment. Dissolved oxygen was measured daily, while ammonia and nitrite concentrations were monitored weekly. Hardness and alkalinity were assessed every two weeks. Mean values (± SD) were as follows: temperature (29.35 ± 0.91 °C), dissolved oxygen (6.10 ± 1.05 mg L^{-1}), pH (8.34 ± 0.09), total hardness (108 ± 19 mg $CaCO_3$ L^{-1}), alkalinity (107 ± 11 mg $CaCO_3$ L^{-1}), total ammonia nitrogen (m, and nitrite (0.03 ± 0.01 mg L^{-1}).

Survival rate, weight gain, biomass production, and feed conversion ratio (FCR) were determined at the conclusion of the feeding trial, following the procedures described by de Souza Valente et al. [27]. To quantify actual feed intake, the amount of feed offered to the prawns was recorded at each feeding time, and the uneaten feed was collected and weighed at the end of each feeding time to calculate the difference. Body length was measured as the straight-line distance from the tip of the rostrum to the end of the telson. Additionally, total antenna length was recorded.

At the end of the feeding trial, prawns were sampled for hepatopancreas enzyme activities and prawn proximate composition, as well as fatty acid and amino acid profiles, as detailed hereafter.

2.4. Antioxidant and Enzyme Activity Assessment in Prawn Hepatopancreas

At the end of the feeding trial, a total of 100 prawns (20 per dietary treatment; 5 prawns per tank, samples analyzed individually) were randomly selected for hepatopancreas sampling to assess antioxidant and digestive enzyme activity, as well as lipid peroxidation levels. Prawns were euthanized by thermal shock (immersion in ice water for 5 min), and the body surface was disinfected using 70% (v/v) ethanol. The hepatopancreas was carefully dissected and immediately stored at −20 °C until further biochemical analysis.

Tissue samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4) using an electric homogenizer (IKA® T10 basic; IKA®, Staufen, Germany). All procedures were performed on ice to maintain enzyme stability. The homogenates were centrifuged at 12,000× g for

10 min at 4 °C in a refrigerated centrifuge (Sigma 3-16 KL; Sigma, St. Louis, MO, USA), and the resulting supernatant was collected for enzymatic assays.

Total protein concentration in the homogenates was quantified using the Bradford method [28], employing bovine serum albumin as a standard and measuring absorbance at 595 nm. Protein concentration values were used to normalize the enzymatic activity measurements.

Glutathione peroxidase (GPx) activity was evaluated based on the oxidation of NADPH during the reduction of cumene hydroperoxide, with absorbance read at 340 nm [29,30]. Glutathione reductase (GR) activity was determined by quantifying NADPH oxidation during the conversion of glutathione disulfide to reduced glutathione, also measured at 340 nm [28,31].

Reduced glutathione (GSH) levels were analyzed using the non-protein thiol detection method with Ellman reagent [31,32]. Glutathione S-transferase (GST) activity was measured through the conjugation of GSH with 1-chloro-2,4-dinitrobenzene, monitored spectrophotometrically [33,34]. Lipid peroxidation was determined by the quantification of malondialdehyde (MDA) via its reaction with thiobarbituric acid reactive substances (TBARS) [35,36].

The activities of digestive enzymes, such as maltase (U/mg protein), sucrase (U/mg protein), chymotrypsin (µmol/min/mg protein), and trypsin (nmol/min/mg protein), were also quantified to evaluate the digestive capacity of *M. rosenbergii* post-larvae.

The activities of maltase and sucrase were measured following the protocol by Retcheski et al. [37]. After enzyme incubation, glucose concentration was determined using a commercial colorimetric kit (Gold Analisa®, Belo Horizonte, MG, Brazil), following the manufacturer's instructions. Results were expressed in µmol/min/mg protein.

Chymotrypsin and trypsin activities were measured following the BCW method [37,38], adapted for use with a 96-well microplate. Chymotrypsin activity was assessed using benzoyl tyrosine ethyl ester (BTEE) as the substrate, with kinetic absorbance readings taken at 256 nm and 30 °C. The reaction product, ethanol, was quantified using a molar extinction coefficient of 964 M/cm. Trypsin activity was evaluated using α-ρ-toluenesulphonyl-L-arginine methyl ester hydrochloride (TAME) as the substrate. Samples were incubated for 2 min, and kinetic readings were taken at 247 nm and 30 °C to detect the formation of Na-p-Tosyl-L-Arginine, with a molar extinction coefficient of 540 M/cm.

2.5. Analysis of Prawns and Diets: Proximate Composition and Fatty Acid and Amino Acid Profiles

At the end of the feeding trial, another 50 prawns (n = 10 prawns per experimental group) were randomly collected and euthanized, as previously described. Then, prawns were minced, freeze-dried, and stored in vials (2 pools of 5 prawns each per experimental diet) before subsequent analysis for proximate composition, fatty acid profile, and amino acid profile. Samples (n = 10; 2 samples per diet) of the experimental diets were also grounded and submitted to the same analyses.

The samples of prawn and experimental diets were analyzed using standard procedures [39] to assess dry matter (method 934.01), crude protein (method 2001.11), ash content (method 967.05), and crude fiber (987.10) (only for the experimental diets). Ether extract was quantified following acid hydrolysis, in accordance with Commission [40] guidelines. Chitin content in the prawns was determined following Stelmock et al. [41].

The lipid fraction was extracted from freeze-dried prawn and diet samples using an accelerated solvent extraction method, following the protocol outlined in Application Note 334 (ASE®, Dionex, Sunnyvale, CA, USA). The extraction was performed in two cycles using petroleum ether as the solvent, at 125 °C and 10.3 MPa. Each cycle included a 6 min heating phase followed by a 2 min extraction phase.

Following extraction, 10 mL of sodium sulfate solution (0.47% in water) was added to the lipid-containing samples, which were then stored at 4 °C for 30 min. The resulting supernatant, comprising petroleum ether and extracted lipids, was transferred into pre-weighed vials. Lipid solvents were evaporated under a nitrogen stream (Genevac EZ-2, SP Industries, Warminster, PA, USA), and the remaining lipid residue was weighed.

Subsequently, 2 mL of 2% sulfuric acid in methanol was added for transesterification, as described by Christie [42]. Samples were incubated overnight at 50 °C. To extract the resulting fatty acid methyl esters (FAMEs), hexane was added at a ratio of 1 mL per 20 mg lipids, followed by the addition of 5 mL of 2% potassium bicarbonate. After centrifugation, the supernatant was collected for analysis. The complementary protocol is described in Supplementary Materials (Protocol 1).

2.6. Statistical Analysis

The data were assessed for homogeneity using Levene's test and for normality using the Shapiro–Wilk test. After confirming that the assumptions were met, a one-way ANOVA was performed considering the experimental diet as the main effect, followed by Tukey's post-hoc test using RStudio software version 2025.05.1 + 513. Results are presented as mean ± SEM, and a p-value < 0.05 was considered statistically significant. Results related to diet and prawn proximate composition, fatty acid profile, and amino acid profile are presented using descriptive statistics (mean ± SD).

3. Results

3.1. Zootechnical Performance and Welfare

Survival rates ranged from 73.3 ± 5.44% to 83.3 ± 3.84% without significant differences among treatments ($p > 0.05$). The zootechnical performance of prawn post-larvae was significantly improved by the inclusion of HFM in the diets. In addition, prawns fed the diet containing HFM 6.0% showed the highest final weight (0.35 g; $p < 0.001$), final length (3.15 cm; $p < 0.001$), and biomass gain (20.2 g; $p < 0.001$) compared to the prawns of the other groups. Antenna length and feed conversion ratio were the lowest in prawns fed the control diet and in those fed the diet with 1.5% HFM, whereas they significantly increased in prawns fed diets with 6.0% HFM inclusion (Table 4).

Table 4. Zootechnical performance (mean ± SEM) of *Macrobrachium rosenbergii*-fed diets containing different HFM levels during the nursery phase.

Treatment	Final Weight (g)	Final Length (cm)	Antenna Length (cm)	Biomass Gain (g)	FCR [2]
HFM [1] 0%	0.16 ± 0.04 ^{c}	2.78 ± 0.3 ^{b}	2.2 ± 0.79 ^{c}	8.98 ± 0.19 ^{c}	2.68 ± 0.06 ^{c}
HFM 1.5%	0.24 ± 0.09 ^{b}	3.04 ± 0.4 ^{a}	2.2 ± 0.78 ^{c}	11.2 ± 0.47 ^{bc}	2.48 ± 0.08 ^{c}
HFM 3.0%	0.20 ± 0.05 ^{b}	2.61 ± 0.3 ^{b}	2.4 ± 0.97 ^{bc}	11.8 ± 0.18 ^{b}	2.37 ± 0.07 ^{bc}
HFM 4.5%	0.20 ± 0.06 ^{b}	2.63 ± 0.3 ^{b}	2.9 ± 0.83 ^{a}	12.4 ± 0.32 ^{b}	2.17 ± 0.06 ^{b}
HFM 6.0%	0.35 ± 0.13 ^{a}	3.15 ± 0.4 ^{a}	2.6 ± 1.25 ^{ab}	20.2 ± 0.75 ^{a}	1.68 ± 0.04 ^{a}
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

[1] HFM: hydrolyzed feather meal. [2] FCR: feed conversion ratio. Different superscript letters in the same column indicate significant differences. One-way ANOVA followed by post-hoc Tukey's test, $p < 0.05$.

3.2. Antioxidant Status

The antioxidant profile of prawn hepatopancreas was not affected by the dietary treatment ($p > 0.05$). Specifically, the average levels observed were thiobarbituric acid reactive substances (TBARS) at 0.94 ± 0.13 µM/mg protein; reduced glutathione (GSH) at 1.70 ± 0.37 µM/mg protein; glutathione S-transferase (GST) at 0.18 ± 0.04 nMol thioether/min/mg protein; glutathione

peroxidase (GPx) at 0.88 ± 0.16 µM NADPH/min/mg protein; and glutathione reductase (GR) at 0.73 ± 0.18 µM NADPH/min/mg protein (Table 5).

Table 5. Biomarkers of antioxidant status (mean ± SEM) in the hepatopancreases of *Macrobrachium rosenbergii*-fed diets with different levels of HFM during the nursery phase.

Treatment	TBARS [2]	GSH [3]	GST [4]	GPx [5]	GR [6]
HFM [1] 0%	1.10 ± 0.14	1.59 ± 0.45	0.20 ± 0.04	1.25 ± 0.29	1.08 ± 0.26
HFM 1.5%	0.98 ± 0.12	1.67 ± 0.45	0.11 ± 0.02	0.65 ± 0.09	0.41 ± 0.10
HFM 3.0%	0.93 ± 0.21	1.60 ± 0.34	0.13 ± 0.02	0.86 ± 0.16	0.70 ± 0.18
HFM 4.5%	0.85 ± 0.09	1.81 ± 0.27	0.24 ± 0.07	1.06 ± 0.20	1.05 ± 0.29
HFM 6.0%	0.86 ± 0.08	1.82 ± 0.36	0.20 ± 0.06	0.58 ± 0.07	0.39 ± 0.07
p-value	0.78	0.99	0.56	0.29	0.21

[1] HFM: hydrolyzed feather meal; [2] TBARS: thiobarbituric acid reactive substances (µM mg protein); [3] GSH: reduced glutathione (µM mg protein); [4] GST: glutathione S-transferase (nMol tioeter/min/mg protein); [5] glutathione peroxidase (µM NADPH/min/ mg protein); [6] GR: glutathione reductase (µM NADPH/min/mg protein).

3.3. Digestive Enzyme Activity

As for antioxidant biomarkers, the digestive enzyme activity in prawn hepatopancreas was not affected by the dietary treatments (Table 6). On average, recorded values were: maltase, 11341 ± 2902 U/mg protein; sucrase, 4266 ± 1233 U/mg protein; chymotrypsin, 4.94 ± 0.73 µmol/min/mg protein; and trypsin, 1.54 ± 0.25 nmol/min/mg protein.

Table 6. Digestive enzyme activity (mean ± SEM) in the hepatopancreases of *Macrobrachium rosenbergii*-fed diets containing different levels of HFM during the nursery phase.

Treatment	Maltase [2]	Sucrase [2]	Chymotrypsin [3]	Trypsin [4]
HFM [1] 0%	16,706 ± 4617	5339 ± 1289	4.47 ±0.76	1.40 ± 0.22
HFM 1.5%	9926 ± 2087	3265 ± 774	3.73 ± 0.69	1.70 ± 0.27
HFM 3.0%	11,048 ± 2838	4808 ± 1478	5.85 ± 0.81	1.40 ± 0.29
HFM 4.5%	12,629 ± 3705	4736 ± 1690	6.07 ± 0.72	1.40 ± 0.16
HFM 6.0%	6397 ± 1265	3180 ± 936	4.59 ± 0.69	1.80 ± 0.31
p-value	0.51	0.87	0.48	0.92

[1] HFM: hydrolyzed feather meal; [2] Expressed as U/mg protein; [3] expressed as µmol/min/mg protein; [4] expressed as nmol/min/mg protein.

3.4. Prawn Composition

No notable changes in carcass composition were observed (Table 7; Tables S2 and S3 of Supplementary Materials).

Table 7. Proximate composition (mean ± SD) of prawns (*Macrobrachium rosenbergii*) fed diets with different levels of hydrolyzed feather meal during the nursery phase.

	Treatment				
	HFM 0%	HFM 1.5%	HFM 3.0%	HFM 4.5%	HFM 6.0%
Samples, n	2	2	2	2	2
Dry matter, %	91.0 ± 0.08	91.7 ± 0.44	91.7 ± 0.28	91.7 ± 0.32	91.8 ± 0.16
Crude protein, %	72.7 ± 0.69	68.7 ± 1.28	69.6 ± 0.75	74.4 ± 2.69	71.1 ± 4.18
Crude lipids, %	6.50 ± 0.05	6.73 ± 0.02	7.35 ± 0.91	5.16 ± 0.01	5.99 ± 0.14
Ash, %	18.1 ± 0.26	14.5 ± 0.49	18.1 ± 0.02	16.8 ± 0.23	17.5 ± 0.16
Chitin, %	6.61 ± 0.49	7.07 ± 0.45	8.44 ± 0.84	6.53 ± 0.45	7.58 ± 0.48

HFM: hydrolyzed feather meal.

4. Discussion

To the best of our knowledge, this is the first study evaluating the effects of the inclusion of HFM in diets for *M. rosenbergii*. Unlike poultry by-product meal (PBM), which has been extensively studied in aquafeed, HFM remains poorly characterized, particularly in the nursery stage of freshwater crustaceans. The present study fills a critical gap by evaluating HFM in *M. rosenbergii* post-larvae and contributes novel evidence that supports the dietary use of HFM as a viable protein source in aquafeed.

Previous work demonstrated the feasibility of PBM as a substitute for fishmeal in prawn diets. In detail, up to 75% replacement of fishmeal with PBM did not adversely impact growth performance, survival rate, proximate body composition, or intestinal digestive enzyme activities of *M. rosenbergii* juveniles [43]. These findings and the results observed in our trials align with previous research in various crustacean species, including *Penaeus vannamei* [44,45], *Cherax quadricarinatus* [46], and *Macrobrachium nipponense* [47], where PBM has been shown to effectively replace a substantial proportion of fishmeal without compromising prawn or shrimp growth and digestive functions. In contrast, the stable amino acid profile of prawns in the present study across all experimental groups suggests that the partial replacement of fishmeal with HFM preserved the amino acid profile of prawns fed with HFM.

Regarding antioxidant status, consistent with our results, studies have reported no adverse effects on antioxidant defense mechanisms when incorporating poultry by-products in the diets of various aquatic species. For instance, Yang et al. [48] found that the inclusion of poultry by-product meal in the diets of gibel carp (*Carassius auratus* gibelio) did not negatively impact antioxidant status. Chaklader et al. [49] observed no significant changes in antioxidant capacity and immune response in juvenile barramundi (*Lates calcarifer*) when fishmeal was entirely replaced with poultry by-product meal.

The absence of significant differences in biomarkers of antioxidant status (MDA, GSH, GST, GPx, and GR) in the hepatopancreas of *M. rosenbergii* post-larvae across the dietary treatments with increasing levels of HFM corroborates these findings. This suggests that the inclusion of HFM, up to a 6.0% inclusion level, is safe in terms of oxidative stress management. The hepatopancreas plays a crucial role in the antioxidant defense mechanisms in decapods by producing and secreting antioxidant enzymes [50,51]. Maintaining a balanced antioxidant status is essential for preventing oxidative damage to cellular components, ensuring overall health and optimal performance [52].

Notably, prawns fed diets containing 3.0% to 6.0% HFM showed improved performance and welfare. The improved final weight, antenna length, biomass gain, and feed conversion ratio with increasing HFM levels suggest increasing efficient utilization of nutrients in the diets, reflecting better overall health and welfare. Similarly, Badillo-Zapata et al. [24] demonstrated that replacing fishmeal with HFM in diets for juvenile *Macrobrachium tenellum* improved muscle fatty acid profiles without compromising growth, further supporting the use of HFM as a viable alternative protein source in prawn aquaculture. Likewise, Negrini et al. [12] reported that the inclusion of chicken protein hydrolysates in diets for *P. vannamei* maintained the fatty acid profile of shrimps during nursery and grow-out phases. Comparable positive results have also been reported in fish. Campos et al. [53] observed that the HFM dietary inclusion up to 12.5% maintains growth performance, nutrient utilization, and feed efficiency in European seabass (*Dicentrarchus labrax*). Unlike unprocessed feathers, HFM presents high crude protein content and digestibility [22]. Thus, HFM protein can more easily be absorbed and used for tissue maintenance.

Although fishmeal is often considered the gold standard due to its balanced amino acid profile, HFM offers a competitive alternative. The amino acid profile of the HFM-based diets used in the present study showed high levels of crucial amino acids, such as

arginine, leucine, lysine, and valine. These amino acids are essential for prawns' physiological functions, growth, and immune responses [20]. Although methionine levels in HFM-based diets were slightly lower than in standard fishmeal [26], this limitation was compensated through the addition of DL-methionine in the formulations, ensuring nutritional adequacy. Moreover, the final carcass proximate composition and stable amino acid profile observed in prawn carcasses (see Supplementary Materials) further suggest that the amino acid composition of HFM-based diets effectively supported tissue building and protein metabolism during the nursery phase and that dietary HFM did not impact prawn flesh nutritional quality.

Additionally, the antenna length serves as a physical indicator of welfare in decapods. Assessing this feature offers a non-invasive approach to evaluating the welfare of farmed prawns. Long and intact antennae reflect good health and appropriate husbandry conditions [54]. Prawns fed diets with the highest levels of HFM inclusion exhibited longer antennae, reflecting an improved welfare.

The maintenance of digestive enzyme activities and antioxidant status in the hepatopancreas of *M. rosenbergii* post-larvae when incorporating HFM as a partial replacement for fishmeal is particularly significant in the context of the growing demand for sustainable and cost-effective alternative protein sources in aquaculture feeds. Poultry by-products, such as feather meal, represent a promising alternative due to their high protein content, balanced amino acid profile, and widespread availability [55,56]. The absence of differences in the digestive enzymes, including maltase, sucrase, chymotrypsin, and trypsin, among dietary treatments suggests that HFM did not impair digestive function and, thus, nutrient assimilation. Nevertheless, further research is needed to corroborate this hypothesis through digestibility trials.

While the present study demonstrates that dietary inclusion of HFM up to 6.0% enhances growth performance and maintains physiological and nutritional balance in *M. rosenbergii* PL, the feasibility of higher inclusion levels needs to be confirmed. Limitations may include the relatively low digestibility of residual keratin, the methionine deficiency, and the risk of bioaccumulation of undesirable compounds if processing quality is suboptimal. Higher HFM inclusion levels may also affect diet palatability, which could lead to reduced feed intake, particularly in early life stages. Therefore, while our results are promising, further studies are suggested to investigate the effect of higher HFM inclusion levels, the long-term effect of HFM, including in more advanced stages of the culture cycle (e.g., juveniles and adult prawns), and its economic value in commercial aquaculture farms.

5. Conclusions

This study demonstrates the potential of HFM to partially replace fishmeal in nursery diets for *M. rosenbergii* PL, contributing to sustainable freshwater farming. Dietary inclusion level of 6.0% significantly improved prawn performance and welfare without affecting antioxidant status, digestive enzyme activity, or nutritional composition. Notably, to the best of our knowledge, this is the first study evaluating the effects of dietary HFM on the growth, health, and welfare of *M. rosenbergii* PL.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/app15158627/s1. Table S1: Bromatological analysis of hydrolyzed feather meal used in diets for giant river prawn (*Macrobrachium rosenbergii*) post-larvae. Table S2: Amino acid profile (mg/100 g; mean ± SD) of prawns (*Macrobrachium rosenbergii*) fed diets with different levels of hydrolyzed feather meal during the nursery phase. Table S3: Fatty acid profile (% total fatty acids; mean ± SD) of prawns (*Macrobrachium rosenbergii*) fed diets with different levels of hydrolyzed feather meal during the nursery phase.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because the current Brazilian animal protective legislation (Law No. 11,794, Brazil) does not require ethical approval by an Animal Welfare Committee when the study encompasses the use of decapod crustaceans. The feeding trial was conducted in a research facility of a Brazilian federal university. This study followed the recommended commercial and academic parameters for prawn farming, including adequate feeding and water quality. Prawns were euthanized following torpor with thermal shock.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

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

References

1. FAO. *The State of World Fisheries and Aquaculture 2024: Blue Transformation in Action*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2024. [CrossRef]
2. New, M.B. *Macrobrachium rosenbergii*. In *Cultured Aquatic Species Fact Sheets*; Food and Agricultural Organization of the United Nations: Rome, Italy, 2009.
3. Rasid, R.; Brown, J.H.; Pratoomyot, J.; Monroig, O.; Shinn, A.P. Growth performance, nutrient utilisation and body composition of *Macrobrachium rosenbergii* fed graded levels of phytic acid. *Aquaculture* **2017**, *479*, 850–856. [CrossRef]
4. FAO. The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation. In *The State of World Fisheries and Aquaculture (SOFIA)*; Food and Agricultural Organization of the United Nations: Rome, Italy, 2022; p. 266.
5. New, M.B.; D'Abramo, L.R.; Valenti, W.C.; Singholka, S. *Sustainability of Freshwater Prawn Culture*; Wiley: Hoboken, NJ, USA, 2000.
6. Jahanbakhshi, A.; Pourmozaffar, S.; Adeshina, I.; Vayghan, A.H.; Reverter, M. Effect of garlic (*Allium sativum*) extract on growth, enzymological and biochemical responses and immune-related gene expressions in giant freshwater prawn (*Macrobrachium rosenbergii*). *J. Anim. Physiol. Anim. Nutr.* **2022**, *106*, 947–956. [CrossRef]
7. Naksing, T.; Rattanavichai, W.; Teeka, J.; Kaewpa, D.; Borthong, J.; Areesirisuk, A. Biological activities and potential of organic banana (*Musa acuminata*) peel extract in enhancing the immunity of giant freshwater prawn (*Macrobrachium rosenbergii*). *Aquac. Res.* **2022**, *53*, 2645–2656. [CrossRef]
8. Lopes Ferreira, R.; de Souza Valente, C.; Rosa Silva, L.C.; Costa de Sousa, N.; Mauerwerk, M.T.; Cupertino Ballester, E.L. Apparent Digestibility Coefficients of Nutrients and Energy from Animal-Origin Proteins for *Macrobrachium rosenbergii* Juveniles. *Fishes* **2024**, *9*, 341. [CrossRef]
9. Fabrini, S.A.; Stroher, R.; Scheufele, F.B.; Mauerwerk, M.T.; Ballester, E.L.C. Optimization of the enzymatic hydrolysis process of shrimp viscera (*Macrobrachium rosenbergii*) with two commercial enzymes, aiming to produce an alternative protein source for aquaculture feed formulation. *Aquac. Int.* **2023**, *31*, 807–825. [CrossRef]
10. Soares, M.; Rezende, P.C.; Correa, N.M.; Rocha, J.S.; Martins, M.A.; Andrade, T.C.; Fracalossi, D.M.; do NascimentoVieira, F. Protein hydrolysates from poultry by-product and swine liver as an alternative dietary protein source for the Pacific white shrimp. *Aquac. Rep.* **2020**, *17*, 100344. [CrossRef]
11. Soares, M.; Gonçalves, P.; Schleder, D.D.; Delgadillo-Díaz, M.; Gullian-Klanian, M.; do Nascimento Vieira, F. Protein hydrolysate of poultry by-product and swine liver in the diet of pacific white shrimp. *Bol. Inst. Pesca* **2021**, *47*, e657. [CrossRef]
12. Negrini, C.; Nascimento Ferreira, C.H.d.; Kracizy, R.O.; Ferreira, R.L.; Costa, L.; Mauerwerk, M.T.; Retcheski, M.C.; Cazarolli, L.H.; Boscolo, W.R.; Cupertino Ballester, E.L. Partial Replacement of Fish Meal with Protein Hydrolysates in the Diet of Penaeus vannamei (Boone, 1934) during the Nursery Phase. *Fishes* **2024**, *9*, 75. [CrossRef]

13. 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. [CrossRef]
14. Insights, F.M. Feather Meal Market Size and Share Forecast Outlook 2025 to 2035. Available online: https://www.futuremarketinsights.com/reports/feather-meal-market (accessed on 7 July 2025).
15. Serra, V.; Pastorelli, G.; Tedesco, D.E.A.; Turin, L.; Guerrini, A. Alternative protein sources in aquafeed: Current scenario and future perspectives. *Vet. Anim. Sci.* **2024**, *25*, 100381. [CrossRef]
16. Mordor Intelligence. Feather Meal Market Size & Share Analysis—Growth Trends & Forecasts (2025–2030). Available online: https://www.mordorintelligence.com/industry-reports/feather-meal-market (accessed on 7 July 2025).
17. Mendoza; Dios, D.; Vazquez; Cruz; Ricque; Aguilera; Montemayor. Fishmeal replacement with feather-enzymatic hydrolyzates co-extruded with soya-bean meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*). *Aquac. Nutr.* **2001**, *7*, 143–151. [CrossRef]
18. Villarreal-Cavazos, D.A.; Ricque-Marie, D.; Peñaa-Rodríguez, A.; Nieto-López, M.; Tapia-Salazar, M.; Lemme, A.; Gamboa-Delgado, J.; Cruz-Suárez, L.E. Apparent digestibility of dry matter, crude protein, and amino acids of six rendered by-products in juvenile *Litopenaeus vannamei*. *Cienc. Mar.* **2014**, *40*, 163–172. [CrossRef]
19. Luo, L.; Wang, J.; Pan, Q.; Xue, M.; Wang, Y.; Wu, X.; Li, P. Apparent digestibility coefficient of poultry by-product meal (PBM) in diets of P enaeus monodon (*Fabricius*) and *Litopenaeus vannamei* (*Boone*), and replacement of fishmeal with PBM in diets of *P. monodon*. *Aquac. Res.* **2012**, *43*, 1223–1231. [CrossRef]
20. Hertrampf, J.W.; Piedad-Pascual, F. *Handbook on Ingredients for Aquaculture Feeds*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2012.
21. National Renderers Association. *Pocket Information Manual: A Buyer's Guide to Rendered Products*; National Renderers Association: Alexandria, VA, USA, 2003; p. 44.
22. Salehizadeh, M.; Tajabadi Ebrahimi, M.; Mousavi, S.N.; Sepahi, A.A.; Orooji, R. Transforming Feather Meal Into a High-Performance Feed for Broilers. *Vet. Med. Sci.* **2025**, *11*, e70199. [CrossRef] [PubMed]
23. de Carvalho, R.A.; Ota, R.H.; Kadry, V.O.; Tacon, A.G.; Lemos, D. Apparent digestibility of protein, energy and amino acids of six protein sources included at three levels in diets for juvenile white shrimp *Litopenaeus vannamei* reared in high performance conditions. *Aquaculture* **2016**, *465*, 223–234. [CrossRef]
24. Badillo-Zapata, D.; Vega-Villasante, F.; Nolasco-Soria, H.; López-Acuña, L.; Vargas-Ceballos, M.A.; Barreto-Curiel, F. Replacement of fishmeal by hydrolyzed feather meal in diets of juvenile *Macrobrachium tenellum* (river prawns) and its effect on muscle fatty acids. *Lat. Am. J. Aquat. Res.* **2023**, *51*, 703–716. [CrossRef]
25. Coyle, S.D.; Alston, D.E.; Sampaio, C.M.S. Nursery Systems and Management. In *Freshwater Prawns*; Wiley: New York, NY, USA, 2009; pp. 108–126. [CrossRef]
26. NRC. *Nutrient Requirements of Fish and Shrimp*; NRC: Washington, DC, USA, 2011.
27. de Souza Valente, C.; Coates, C.J.; Cagol, L.; Bombardelli, R.A.; Becker, A.G.; Schmidt, D.; Heinzmann, B.M.; Vaz-dos-Santos, A.M.; Baldisserotto, B.; Ballester, E.L.C. Antioxidant status and performance of *Macrobrachium rosenbergii* juveniles fed diets containing non-nutritive *Aloysia triphylla* essential oil. *Aquac. Int.* **2024**, *32*, 7201–7214. [CrossRef]
28. Mannervik, B. Measurement of Glutathione Reductase Activity. *Curr. Protoc. Toxicol.* **1999**, 7.2.1–7.2.4. [CrossRef]
29. Wendel, A. [44] Glutathione peroxidase. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1981; Volume 77, pp. 325–333.
30. Bastolla, C.L.V.; Guerreiro, F.C.; Saldaña-Serrano, M.; Gomes, C.H.A.M.; Lima, D.; Rutkoski, C.F.; Mattos, J.J.; Dias, V.H.V.; Righetti, B.P.H.; Ferreira, C.P.; et al. Emerging and legacy contaminants on the Brazilian southern coast (Santa Catarina): A multi-biomarker approach in oysters Crassostrea gasar (Adanson, 1757). *Sci. Total Environ.* **2024**, *925*, 171679. [CrossRef]
31. Alam, M.N.; Bristi, N.J.; Rafiquzzaman, M. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm. J.* **2013**, *21*, 143–152. [CrossRef] [PubMed]
32. Sedlak, J.; Lindsay, R.H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **1968**, *25*, 192–205. [CrossRef]
33. Habig, W.H.; Pabst, M.J.; Fleischner, G.; Gatmaitan, Z.; Arias, I.M.; Jakoby, W.B. The Identity of Glutathione-S-Transferase B with Ligandin, a Major Binding Protein of Liver. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3879–3882. [CrossRef]
34. Capparelli, M.V.; Pérez-Ceballos, R.; Moulatlet, G.M.; Rodríguez-Santiago, M.A.; Dzul-Caamal, R.; Mora, A.; Suárez-Mozo, N.Y.; Abessa, D.M.; Zaldívar-Jiménez, A. Application of ecotoxicological tools to evaluate the quality status of mangroves under restoration in the Yucatán Peninsula, Mexico. *Mar. Pollut. Bull.* **2024**, *203*, 116386. [CrossRef] [PubMed]
35. Mensah, P.K.; Palmer, C.G.; Muller, W.J. Lipid peroxidation in the freshwater shrimp *Caridina nilotica* as a biomarker of Roundup® herbicide pollution of freshwater systems in South Africa. *Water Sci. Technol.* **2012**, *65*, 1660–1666. [CrossRef] [PubMed]
36. de Almeida, E.A.; Bainy, A.C.D.; de Melo Loureiro, A.P.; Martinez, G.R.; Miyamoto, S.; Onuki, J.; Barbosa, L.F.; Garcia, C.C.M.; Prado, F.M.; Ronsein, G.E. Oxidative stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian

marine environment: Antioxidants, lipid peroxidation and DNA damage. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2007**, *146*, 588–600. [CrossRef] [PubMed]
37. Retcheski, M.C.; Maximowski, L.V.; Escorsin, K.J.S.; de Almeida Rosa Kurosaki, J.K.; 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]
38. Hummel, B.C.W. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. *Can. J. Biochem. Physiol.* **1959**, *37*, 1393–1399. [CrossRef]
39. AOAC. *Official Methods of Analysis of AOAC International*; AOAC International: Gaitherburg, MD, USA, 2005.
40. European Commission. Commission Directive 98/64/EC of 3 September 1998: Establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs, and amending Directive 71/393/EEC. *J. Eur. Union.* **1998**, *19*, 1998.
41. Stelmock, R.L.; Husby, F.M.; Brundage, A.L. Application of Van Soest Acid Detergent Fiber Method for Analysis of Shellfish Chitin1. *J. Dairy Sci.* **1985**, *68*, 1502–1506. [CrossRef]
42. Christie, W.W. *Gas Chromatography and Lipids: A Practical Guide*; Oily Press: Glasgow, UK, 1989.
43. Boateng, A.G.; Zhao, J.; Zhao, J.; Xu, H.; Li, H.; Zhao, M.; Xu, Q.; Liu, C. Effects of Replacement of Fish Meal with Poultry By-Product Meal (PBM) on Growth Performance, Digestive Enzyme, and Immunity of Giant River Prawn (*Macrobrachium rosenbergii*). *Aquac. Res.* **2023**, *2023*, 7500596. [CrossRef]
44. Cruz-Suárez, L.E.; Nieto-López, M.; Guajardo-Barbosa, C.; Tapia-Salazar, M.; Scholz, U.; Ricque-Marie, D. Replacement of fish meal with poultry by-product meal in practical diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. *Aquaculture* **2007**, *272*, 466–476. [CrossRef]
45. Samocha, T.M.; Davis, D.A.; Saoud, I.P.; DeBault, K. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* **2004**, *231*, 197–203. [CrossRef]
46. Saoud, I.; Rodgers, L.; Davis, D.; Rouse, D. Replacement of fish meal with poultry by-product meal in practical diets for redclaw crayfish (*Cherax quadricarinatus*). *Aquac. Nutr.* **2008**, *14*, 139–142. [CrossRef]
47. Yang, Y.; Xie, S.; Lei, W.; Zhu, X.; Yang, Y. Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and immune response of *Macrobrachium nipponense*. *Fish Shellfish. Immunol.* **2004**, *17*, 105–114. [CrossRef]
48. Yang, Y.; Xie, S.; Cui, Y.; Lei, W.; Zhu, X.; Yang, Y.; Yu, Y. Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp, *Carassius auratus gibelio*. *Aquac. Nutr.* **2004**, *10*, 289–294. [CrossRef]
49. Chaklader, M.R.; Siddik, M.A.; Fotedar, R. Total replacement of fishmeal with poultry by-product meal affected the growth, muscle quality, histological structure, antioxidant capacity and immune response of juvenile barramundi, *Lates calcarifer*. *PLoS ONE* **2020**, *15*, e0242079. [CrossRef]
50. Chou, C.-H.; Wang, S.-Y.; Lin, Y.-T.; Chen, Y.-C. Antioxidant activities of chicken liver hydrolysates by pepsin treatment. *Int. J. Food Sci. Technol.* **2014**, *49*, 1654–1662. [CrossRef]
51. Vogt, G. Cytopathology and immune response in the hepatopancreas of decapod crustaceans. *Dis. Aquat. Org.* **2020**, *138*, 41–88. [CrossRef]
52. Lushchak, V.I. Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.* **2011**, *101*, 13–30. [CrossRef] [PubMed]
53. Campos, I.; Matos, E.; Marques, A.; Valente, L.M.P. Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **2017**, *476*, 152–159. [CrossRef]
54. Ballester, E.L.C.; Costa, W.G.d.S.; do Nascimento Ferreira, C.H.; Retcheski, M.C.; Cazarolli, L.H.; Schwengber, G.H.; Mauerwerk, M.T.; Pinheiro, C.G.; Heinzmann, B.M.; Baldisserotto, B.; et al. Dietary *Ocimum gratissimum* Essential Oil Improves the Antioxidant Status and Maintains the Performance of *Macrobrachium rosenbergii* Juveniles. *Appl. Sci.* **2025**, *15*, 2745. [CrossRef]
55. Galkanda-Arachchige, H.S.; Wilson, A.E.; Davis, D.A. Success of fishmeal replacement through poultry by-product meal in aquaculture feed formulations: A meta-analysis. *Rev. Aquac.* **2020**, *12*, 1624–1636. [CrossRef]
56. Romero-Garay, M.G.; Montalvo-González, E.; Hernández-González, C.; Soto-Domínguez, A.; Becerra-Verdín, E.M.; García-Magaña, M.D.L. Bioactivity of peptides obtained from poultry by-products: A review. *Food Chem. X* **2022**, *13*, 100181. [CrossRef] [PubMed]