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Mussel Meal as a Promotor of Growth Performance for the Whiteleg Shrimp (*Litopenaeus vannamei*)

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Abstract: Mussel meal (species *Perna perna*) was evaluated as a potential feed additive for whiteleg shrimp (*Litopenaeus vannamei*) diets to improve growth and cold resistance. Five experimental diets (0, 1, 2, 3 and 4% of mussel meal inclusion) were tested in quadruplicate in whiteleg shrimp, using twenty polyethylene tanks of 400 L. Each tank was stocked with 40 shrimp (3.5 ± 0.5 g), filled with seawater and kept under constant aeration and a temperature of 28.4 ± 0.4 °C. After 8 weeks, the growth and feed efficiency of the shrimp were evaluated, and a thermal shock was administered. The shrimp that were fed with the addition of 3 or 4% mussel meal in their diets showed similar results as the control (0% inclusion), while the addition of 1 or 2% mussel meal in the diet resulted in a significantly higher final weight, weight gain and relative growth rate and a lower feed conversion ratio. Further, no differences were observed in thermal shock resistance and survival among the treatments. In conclusion, mussel meal can be used as a feed additive in whiteleg shrimp diets to improve growth, and quadratic regression models indicate that the best levels of inclusion range from 1.73 to 2.00%.

Keywords: feed additive; feed efficiency; pacific white shrimp; *Perna perna*; shrimp culture; shrimp nutrition; thermal shock

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1. Introduction

In recent decades, global aquaculture production has experienced immense growth in terms of the total quantity, the diversity of farmed species and the production systems used [1]. One of the most important production segments of aquaculture is the group comprising marine crustaceans, which contribute to almost 13% of the total global market, with an 86% increase in total production over the past 10 years [2]. Within this group, the species *Litopenaeus vannamei*, commonly known as whiteleg shrimp or Pacific white shrimp, comprises nearly 84% of the total farmed marine shrimp production worldwide.

Whiteleg shrimp became one of the most important aquaculture species due to their fast growth, tolerance to a wide range of salinity and high (stocking) densities, low dietary protein requirements, high survival rates and high market value [3]. This species is native to the tropical marine habitats of the Eastern Pacific coast and is mostly farmed in countries in East and South Asia (83.4%) and Latin America (16.3%) [2]. The continuous increase in the production of this species requires the development of sustainable production strategies. Current trends in aquaculture production rely on finding sustainable alternative and emergent ingredients that may be used to minimize the inclusion of traditional ingredients, such as fishmeal and fish oil, in aquafeed formulations [4,5]. The search for feed additives that can improve daily production management by promoting the shrimp's robustness within this new paradigm of feed formulation is becoming popular [3,5].

Mussel meal may be a unique local alternative ingredient and/or feed additive. The nutritional characteristics of mussel meal are similar the those of fish meal, with a similar amino acid and lipid profile [6]. Additionally, mussels remove nitrogen and phosphate from water by filtering nutrient particles and microscopic organisms, converting non-food into food [7]. Undersized mussels that are not used for human consumption or are grown specifically to reduce the overload of nutrients should be used for the production of mussel meal. By using mussel meal as an alternative ingredient for aquafeeds, nitrogen and phosphate are eco-cycled, and the mussel shells may be used for poultry feed, thereby contributing to lowering the carbon footprint of production [8].

Several studies have shown that mussel meal (produced from different species) can be utilized as a viable alternative ingredient and is a good option for replacing fishmeal or as a feed additive (e.g., [9–14]). The replacement of 25 to 50% of fishmeal with mussel meal does not impair growth performance and/or feed efficiency in fish species like Artic charr (*Salvelinus alpinus*; [15]), common sole (*Solea solea*; [9]), turbot (*Scophthalmus maximus*; [16]) and Ussuri catfish (*Pseudobagrus ussuriensis*; [10]). The inclusion of mussel meal (10–30%) in soybean meal diets even improved the growth and stimulated the feeding of tiger puffer, *Takifugu rubripes* [13]. The dietary inclusion (up to 20%) of the water-soluble fraction of blue mussel, *Mytilus edulis*, increased the body weight and feed efficiency in Japanese flounder, *Paralichthys olivaceus* [17]. Furthermore, mussel meal has also been investigated for its ability to enhance palatability. In common sole, the replacement of fishmeal with mussel meal enhanced diet palatability [18]. However, mussel meal presented only a medium/low attractant potential in Senegalese sole (*Solea senegalensis*), rockfish (*Sebastes schlegeli*) and rainbow trout (*Oncorhynchus mykiss*) [19–21]. In the case of turbot, the addition of small amounts (2 to 8%) of mussel meal to a rapeseed-based diet resulted in improved palatability [11]. Additionally, also in turbot, the inclusion of mussel meal (1%) in high-plant diets increased the mRNA expression of ghrelin in the intestine [22]. In shrimp, Cavalli et al. [12] evaluated the growth and feed utilization of *Farfantepenaeus paulensis* fed with different marine protein sources (fishmeal, squid meal and mussel meal). The results indicated that growth and feed utilization were similar in shrimp fed with fishmeal- and mussel meal-based diets. Also, the dietary inclusion of mussel meal (up to 60%) did not affect the growth, feed efficiency and survival of giant tiger prawn (*Penaeus monodon*) [14].

Due to its high economic value, the areas in which whiteleg shrimp are farmed have been expanded to sub-tropical areas. Therefore, low temperatures have become some of the major constraining factors in culturing whiteleg shrimp, affecting the health of the shrimp by suppressing their immune system and disturbing physiological processes [23–25]. In Southern China, shrimp farming has been affected by winter mortality for several decades, especially in 2008 [26]. Also, in Brazil, especially in Southern Brazil, where the weather is quite unstable, cold stress plays an important role as a natural trigger for disease outbreaks [27]. Mussels have high levels of some free amino acids [28] and polyunsaturated fatty acids (PUFAs) [29] and are a rich source of vitamins and minerals [8]. In fish, several of these nutrients have been implicated in cold resistance [30,31].

This study aims to identify whether mussel meal (species *Perna perna*) can be used as a feed additive in whiteleg shrimp (*L. vannamei*) diets to improve their growth and resistance to thermal shock.

2. Materials and Methods

This experiment was carried out at the Marine Shrimp Laboratory (Laboratório de Camarões Marinhos (LCM/UFSC)) at Barra da Lagoa, Florianópolis, Brazil.

2.1. Shrimp

The shrimp were acquired from Aquatec®, a commercial laboratory located in Cangueretama, RN, Brazil. The shrimp were maintained in a 50 m^3 nursery tank and cultured in a biofloc system until they reached the initial weight required for the experiment (3.5 ± 0.5 g).

2.2. Mussel Meal Preparation

Mussels were bought in the south of Florianópolis (Brazil) at Paraíso das Ostras (27°49′00.5″ S 48°33′49.6″ W) and transported to the Marine Shrimp Laboratory. The mussel meal was prepared via the protocol developed by Dr Antón Salgado from CSIC-IMM, Vigo, Spain (personal communication, 2022). First, the mussels were cooked for 10 min, and the shells were removed. The cooked mussels were placed in a ventilated oven at 70 °C for 48 h. After, the dried mussel meat (comprising 95.8% dry matter, 56.9% crude protein, 12.7% crude fat and 11.6% ash) was minced with a commercial mincer until a powder was obtained and sieved to <600 µm.

2.3. Diets

Five diets were formulated via the animal feed formulation software Optimal Fórmula 2000® (version 19.102.009, Optimal, Campinas, SP, Brazil) to fulfil the nutritional requirements of whiteleg shrimp [32]. Based on the formulation for a commercial-like diet for this species, experimental diets with different levels of inclusion of mussel meal (*P. perna*) were formulated—0 (control), 1, 2, 3 or 4% mussel meal—at the expense of small amounts of other protein sources (Table 1). All five diets were formulated to be isoproteic, isolipidic and isoenergetic. The experimental diets were produced at the Laboratório de Nutrição de Espécies Aquícolas (LABNUTRI, Florianópolis, Brazil), where the selected ingredients were collected, weighed and sieved using a 600 µm sieve. First, the dry ingredients were mixed in a concrete mixer. After mixing the dry ingredients, soybean oil, soy lecithin and fish oil were added and mixed using a concrete mixer for 10 min. The resulting mixture was pelletized with an extruder at 70 °C and a 2.5 mm sieve. Then the pellets were placed in an oven (50 °C) and dried for approximately 24 h until the moisture content was <10%.

The diets were analyzed to obtain their proximate compositions (dry matter, ash, crude protein, crude fat and gross energy), following the procedures standardized by the Association of Official Analytical Chemists [33] and described in Teodósio et al. [34]. Following acid hydrolysis, the dietary amino acid composition (Table 2) of each diet was analyzed via ultra-performance liquid chromatography, according to the procedures described by Aragão et al. [35].

2.4. Experimental Set Up

Twenty 400 L polyethylene tanks were used, and each tank was stocked with 40 shrimp (3.5 ± 0.5 g). A 12 h light/12 h dark regime was used, and each tank was filled with seawater and kept under constant aeration and temperature. Every day, 100% of the water was exchanged to keep the water quality parameters within ideal values. During the water exchange, dead shrimp, shrimp carcasses and feces were removed. Each experimental diet (inclusions of 0, 1, 2, 3 and 4% mussel meal) was randomly assigned to four replicate (n = 4) tanks and evaluated for 8 weeks.

The shrimp were fed four times a day (at 8h00, 11h00, 14h00 and 17h00) with their respective diets following the feeding table by Van Wyk and Scarpa [36]. The experimental diets were placed in a feeding tray (0.03 m^2) underwater in the tank. After 1.5 h, the feeding tray was checked for leftovers.

Oxygen and temperature were measured twice a day (8h00 and 16h30), using a YSI Pro 20 meter that was calibrated once a month. The temperature was kept at 28.4 ± 0.4 °C and the dissolved oxygen was maintained at 6.33 ± 0.23 mg L^{-1}. Weekly, water samples were taken to measure the total ammonia nitrogen (0.98 ± 0.41 mg L^{-1}), nitrite (0.04 ± 0.09 mg L^{-1}), alkalinity (123 ± 3 mg L^{-1}), pH (8.09 ± 0.07) and salinity (30.6 ± 0.27 ‰). The total ammonia nitrogen (TAN) was measured via the indophenol with trione method [37]. Nitrite was measured via the Griess reaction method [38]. Alkalinity was measured via titration using the APHA method 2320-B [39]. pH was measured via a Tecnal pH meter (Tecnal, Piracicaba, SP, Brazil), and the salinity was measured using a YSI EcoSense® probe, model EC300A (YSI, Yellow Springs, OH, USA).

Table 1. Formulations and proximate compositions of the experimental diets containing 0 (control), 1, 2, 3 and 4% mussel meal (*P. perna*).

	Diets				
Ingredients ($g\ kg^{-1}$)	**0%**	**1%**	**2%**	**3%**	**4%**
Mussel meal	0.0	10.0	20.0	30.0	40.0
Soybean meal	324.3	320.3	316.3	312.3	310.3
Fish meal	126.0	125.0	122.0	119.0	115.0
Poultry meal	150.0	145.0	142.0	139.0	135.0
Wheat flour	150.0	150.0	150.0	150.0	150.0
Fish oil	20.0	20.0	20.0	20.0	20.0
Soybean oil	10.0	10.0	10.0	10.0	10.0
Carboxymethyl cellulose	5.0	5.0	5.0	5.0	5.0
Soy lecithin	25.0	25.0	25.0	25.0	25.0
Vitamin C [a]	0.7	0.7	0.7	0.7	0.7
Vitamin premix [b]	5.0	5.0	5.0	5.0	5.0
Mineral premix [c]	17.0	17.0	17.0	17.0	17.0
Monocalcium phosphate	25.0	25.0	25.0	25.0	25.0
Magnesium sulphate	15.0	15.0	15.0	15.0	15.0
Kaolin	100.0	100.0	100.0	100.0	100.0
Sodium chloride	12.0	12.0	12.0	12.0	12.0
Potassium chloride	10.0	10.0	10.0	10.0	10.0
DL-Methionine	5.0	5.0	5.0	5.0	5.0
Proximate composition * (% as fed)					
Moisture	8.9	6.8	9.7	10.8	8.2
Crude protein	36.2	37.0	35.9	35.2	36.3
Crude fat	7.2	7.2	7.8	7.5	7.9
Ash	22.2	22.4	21.8	21.5	22.2
Gross energy ($MJ\ kg^{-1}$)	16.1	15.7	15.2	15.1	15.5

[a] L-ascorbic acid-2-monophosphate 35%: DSM Produtos Nutricionais Brasil (São Paulo, Brazil). [b] Vitamin premix: In Vivo mix (Paulínia, Brazil)—vitamin A, 3,000,000 IU; vitamin D3, 1,000,000 IU; vitamin E, 70,000 IU; vitamin K3, 14 g; vitamin B1, 30 g; vitamin B2, 20 g; vitamin B6, 33 g; vitamin B12, 50,000 µg; pantothenic acid, 40 g; biotin, 750 mg; nicotinic acid, 70 g; folic acid, 3000 mg; excipient for 1000 g. [c] Mineral premix: InVivo mix (Paulínia, Brazil)—potassium, 6100 mg; copper, 23,330 mg; zinc, 10,000 mg; manganese, 20,000 mg; selenium, 125 mg; iodine, 1000 mg; cobalt, 50 mg; excipient for 1000 g. * All values are reported as means of duplicate analysis.

Table 2. Amino acid compositions of the experimental diets containing 0 (control), 1, 2, 3 and 4% mussel meal (*P. perna*).

Amino Acids	Diets				
(% as Fed)	**0%**	**1%**	**2%**	**3%**	**4%**
Arginine	2.3	2.4	2.3	2.2	2.3
Histidine	0.8	0.8	0.8	0.8	0.8
Lysine	2.1	2.2	2.1	2.0	2.1
Threonine	1.4	1.4	1.4	1.3	1.4
Isoleucine	1.7	1.8	1.7	1.6	1.7
Leucine	2.5	2.6	2.5	2.4	2.5
Valine	1.7	1.7	1.7	1.6	1.7
Methionine	1.3	1.3	1.3	1.2	1.3
Phenylalanine	1.8	1.8	1.8	1.7	1.8
Cystine	0.9	1.0	0.9	0.9	1.0
Tyrosine	1.7	1.9	1.8	1.6	1.7
Aspartic acid	3.2	3.3	3.2	3.0	3.2
Glutamic acid	5.4	5.5	5.4	5.1	5.4
Alanine	1.7	1.8	1.7	1.6	1.7
Glycine	2.2	2.2	2.2	2.1	2.1
Proline	1.9	1.9	1.9	1.8	1.8
Serine	1.6	1.7	1.6	1.6	1.6
Taurine	0.2	0.2	0.3	0.3	0.3

All values are reported as means of duplicate analyses.

2.5. Biometry

Once a week, the total biomass for each tank was determined, and the number of shrimp was counted. The weekly biometry measurements were conducted 1.5 h after the first or second round of feeding. The lid, inlet and aeration pipe of each tank were removed, and the water level was reduced to ±15 cm to make it easier and faster to catch the shrimp and thus reduce stress during the biometric analysis. The shrimp were weighed using a scale (Marte AD2000, Marte Científica, Santa Rita do Sapucai, MG, Brazil) with an accuracy of 0.01 g. After weighing, the shrimp were placed back in the tank where they were counted. With the total weight of the shrimp, the new feeding amount was calculated until the next biometry measurements were obtained.

2.6. Shrimp Growth Performance

On the final day of the experiment (the 56th day), the number of shrimp and the tank biomass were determined. The growth performance, feed conversion ratio (FCR) and survival of the shrimp were calculated using the following formulas [40–42]:

$$\text{Weight gain (\% initial weight)} = 100 \times \text{wet weight gain} \times \text{initial weight}^{-1}, \\ \text{where the wet weight gain is the final weight} - \text{initial weight.} \tag{1}$$

$$\text{Weekly weight gain (g week}^{-1}\text{)} = \text{wet weight gain} \times \text{number of weeks}^{-1}. \tag{2}$$

$$\text{Productivity (kg m}^{-3}\text{)} = \text{final biomass} \times \text{tank volume}^{-1}. \tag{3}$$

$$\text{Relative growth rate: RGR (\% day}^{-1}\text{)} = (e^{g} - 1) \times 100, \\ \text{where } g = (\ln Wt - \ln Wi) \times t^{-1}, \text{ Wt and Wi are the final and initial wet weights,} \\ \text{respectively and t is the duration of the trial in days.} \tag{4}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake} \times \text{wet weight gain}^{-1}. \tag{5}$$

$$\text{Feed intake (g shrimp}^{-1}\text{): total feed} \times \text{number of shrimp}^{-1}. \tag{6}$$

$$\text{Survival (\%)} = 100 \times \text{final number of shrimp} \times \text{initial number of shrimp}^{-1}. \tag{7}$$

2.7. Thermal Shock

On day 57 (one day after finishing the experiment), 10 shrimp from each tank were subjected to an abrupt, potentially lethal thermal stress (cold shock). The thermal shock test was applied to show the possible effects of the addition of mussel meal on the immune system of the shrimp in the face of sudden temperature changes that are common in tropical and subtropical regions [43]. The experimental design considered the parameters that occur in practice in Brazilian farms and the challenges that the shrimp may experience. The 10 shrimp (18.0 ± 1.8 g) were simultaneously transferred from tanks with seawater at a temperature of 28.4 ± 0.4 °C to a 60 L aquarium filled with ±25 L of seawater at a temperature of 10.9 ± 0.1 °C under constant aeration, where they were maintained for 1 h. After this period, the shrimp were transferred to tanks containing ±30 L of seawater at a temperature of 28.5 ± 1.0 °C, and mortality was monitored for 48 h. The seawater used in the thermal shock trial was from the same reservoir as the experiment, at a salinity of 30.5 ‰.

2.8. Statistical Analysis

The results are presented as means ± standard deviations (SDs). After analyzing the assumptions of normality (Shapiro–Wilk test) and homoscedasticity (Levene test), a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test, was used to identify significant differences among the treatments for growth performance parameters, feed efficiency and survival. For this statistical analysis, the tanks were considered the experimental units (n = 4). Regression analyses between the dietary mussel meal content and performance indicators were performed using a quadratic model. The mortality data after the thermal shock treatment were analyzed via a Kaplan–Meier test. All tests used a significance level of $p < 0.05$, and all results expressed as a percentage were previously arcsine transformed [44]. All statistical tests were performed using the software program SPSS 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Growth Performance

After 56 days of the experiment, the shrimp that were fed with the 1 and the 2% mussel meal diets had significantly ($p < 0.05$) higher final weights, weight gain, average weekly weight gain and relative growth rates than the shrimp fed with the 0, 3 and 4% diets (Table 3). The productivity was the highest for the shrimp fed the 2% diet, while the lowest values were found for the shrimp fed the 0 and 4% diets. The shrimp fed with the 2% diet presented the highest feed intake and the lowest FCR, which were significantly different ($p < 0.05$) from the 0, 3 and 4% treatments, whilst the shrimp fed the 1% diet presented intermediate values (Figure 1).

Table 3. Growth performance of *L. vannamei* fed diets containing 0, 1, 2, 3 and 4% mussel meal for 56 days.

	Diets				
Growth Indicators	**0%**	**1%**	**2%**	**3%**	**4%**
Initial weight (g)	3.50 ± 0.01	3.51 ± 0.01	3.51 ± 0.03	3.50 ± 0.00	3.50 ± 0.01
Final weight (g)	18.50 ± 0.30 ^{b}	19.63 ± 0.18 ^{a}	20.34 ± 0.54 ^{a}	18.11 ± 0.31 ^{b}	18.45 ± 0.38 ^{b}
Weight gain (% initial weight)	429.1 ± 9.0 ^{b}	459.8 ± 5.0 ^{a}	479.8 ± 18.5 ^{a}	418.0 ± 9.2 ^{b}	426.7 ± 10.7 ^{b}
Weekly weight gain (g week^{-1})	1.88 ± 0.04 ^{b}	2.02 ± 0.02 ^{a}	2.10 ± 0.07 ^{a}	1.83 ± 0.04 ^{b}	1.87 ± 0.05 ^{b}
Productivity (kg m^{-3})	1.74 ± 0.10 ^{b}	1.85 ± 0.08 ^{ab}	1.93 ± 0.05 ^{a}	1.80 ± 0.01 ^{ab}	1.76 ± 0.04 ^{b}
RGR (% day^{-1})	3.02 ± 0.03 ^{b}	3.12 ± 0.02 ^{a}	3.19 ± 0.06 ^{a}	2.98 ± 0.03 ^{b}	3.01 ± 0.04 ^{b}
Survival (%)	93.8 ± 4.1	94.4 ± 4.1	95.0 ± 3.5	98.8 ± 1.3	95.6 ± 1.1

RGR: Relative growth rate. Results are means ± SDs (n = 4). Absence of letters indicate no significant differences among the treatments. Different letters indicate significant differences ($p < 0.05$) among the treatments.

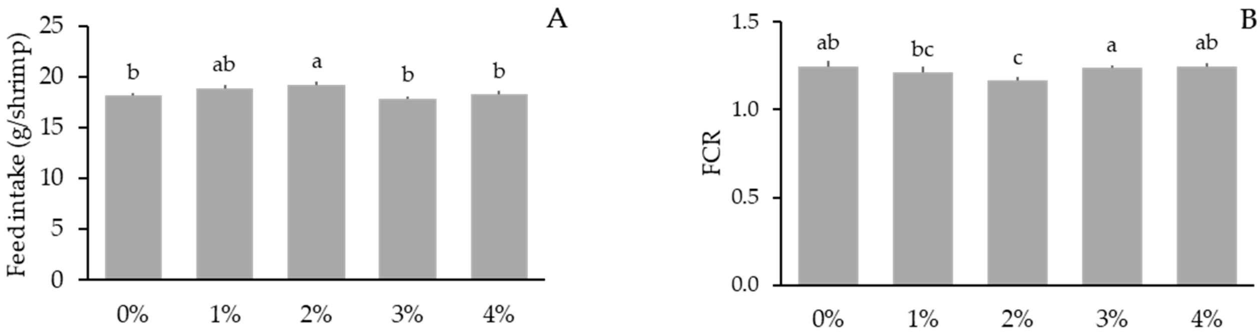

Figure 1. Feed intake (**A**) and feed conversion ratio (**B**) of *L. vannamei* fed diets containing 0, 1, 2 3 and 4% mussel meal for 56 days. Results are means ± SDs (n = 4). Different letters indicate significant differences ($p < 0.05$) among the treatments.

The quadratic regression analysis (Table S1) was significant ($p < 0.05$) for all performance indicators except for survival and feed intake ($p > 0.05$). Figure 2 shows as an example the quadratic regression of the final weight (A) and FCR (B) of the whiteleg shrimp fed diets containing different levels of mussel meal inclusion. According to the models, the best inclusion level of mussel meal is estimated to be from 1.73 to 2.00% (Table S1).

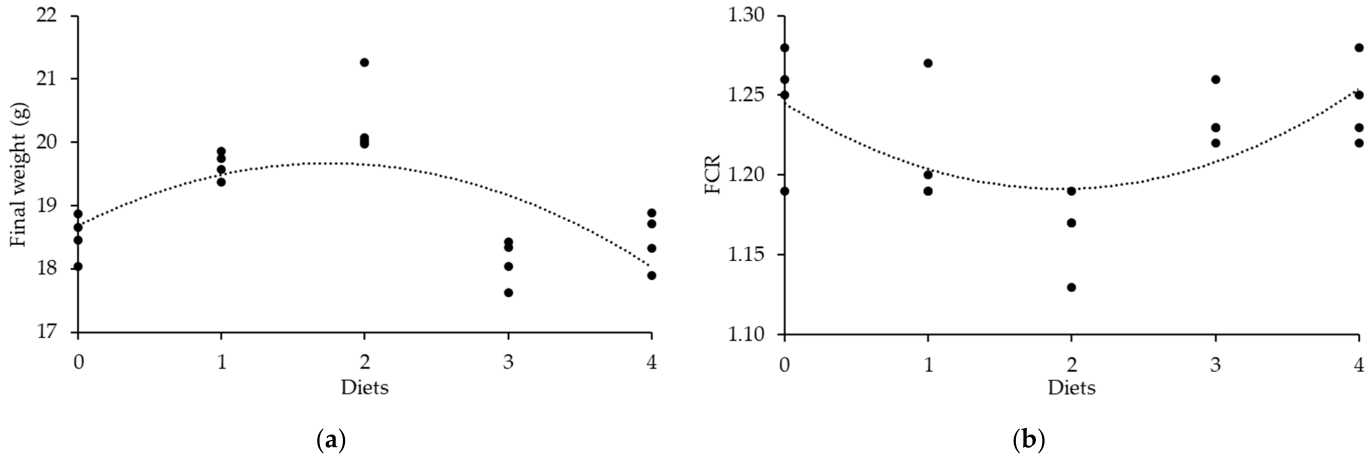

Figure 2. Quadratic regression of final weight (**a**) and feed conversion ratio (**b**) of *L. vannamei* as a function of the dietary mussel meal inclusion (0, 1, 2, 3 and 4%).

3.2. Thermal Shock

The survival of the shrimp 48 h after the thermal shock presented no significant differences among the treatments (Figure 3).

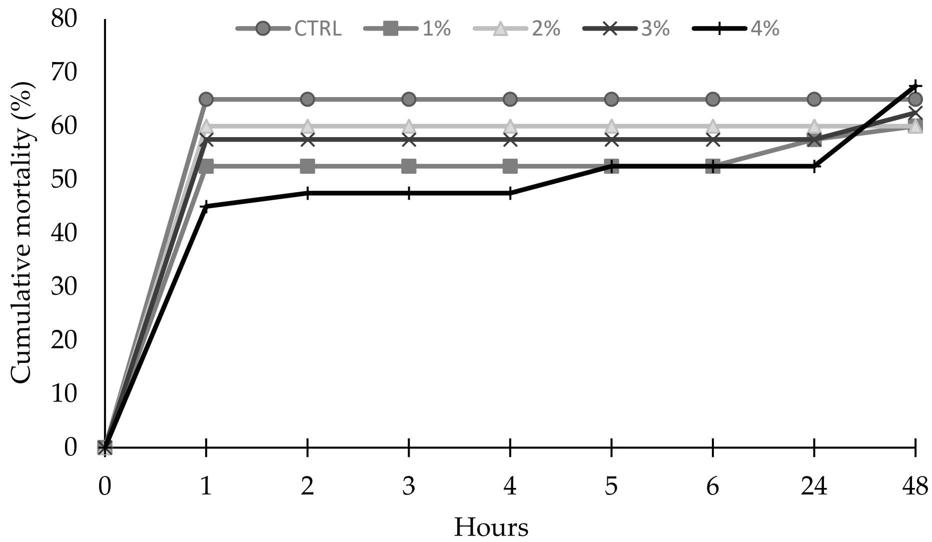

Figure 3. Cumulative mortality (%) of *L. vannamei* during 48 h after the cold shock treatment for treatment groups CTRL (0), 1, 2, 3 and 4%. No significant differences were found among the treatments.

4. Discussion

The present study investigated whether mussel meal can be used as a feed additive in whiteleg shrimp diets. Small amounts, from 1 to 4%, of mussel meal were added to commercial-like diets to see if it improved the growth of the shrimp. Secondly, this research assessed whether the inclusion of mussel meal had a positive effect on the cold resistance of the shrimp. In this study, significant differences were found in the growth performance between the five different treatments. Treatments 1 and 2% obtained significantly better results than the other treatments, with higher final weights, weight gain and RGRs. The weekly weight gain in the present study was above 2 g per week, similar to the values found by Tacon et al. [45] for whiteleg shrimp fed diets containing 35% crude protein and the inclusion of 2.5% squid meal. Nunes et al. [46], who included 1% krill meal in the shrimp diets, reported a weekly growth of 1.01 ± 0.07 g in a clear water system using the same density as the present study, 100 shrimp m^{-3}. An addition of 3 or 4% mussel meal to the diets showed similar results as the control, with a weekly gain of more or less than 1.85 g. In addition, the mortality that occurred was low and similar in all treatments and intrinsic to the possible stress caused by the management of the intensive system.

Feed costs can represent from 50 to 70% of the total cost of production in aquaculture [47–49]. Currently, the aquafeed industry uses plant ingredients such as soybean meal as the main protein source for shrimp diets since they are less expensive and more readily available compared to fishmeal. However, plant-based diets may be less attractive and palatable to farmed organisms [50,51]. Crustaceans have antennular chemoreception mechanisms to help identify attractive feeds, and amino acids and nucleotides seem to be the main triggers for these mechanisms [52,53]. In this study, despite the feed intake being significantly higher in shrimp from the 2% treatment, the tested experimental diets had very similar amino acid profiles.

The use of marine ingredients, such as krill meal, squid meal and, as in the present study, mussel meal, contain substances that act as chemoattractants and feeding stimulants for shrimp [46,52,54] and may result in increased feed consumption and decreased waste. In this study, the addition of 1 and 2% mussel meal increased feed intake, indicating that up to a certain level, this ingredient may be used as a feeding stimulant. Interestingly, besides the higher feed intake, these shrimp also grew better, resulting in a lower FCR compared to shrimp fed on the other diets. A lower feed conversion ratio was also found in shrimp fed with 3% krill meal in diets with low fishmeal content [46]. In giant tiger prawn, similar zootechnical performance was found between treatments when the prawns were fed a diet containing 20, 40 or 60% of mussel meal (*Mytella charruana*), with no adverse effects [14]. There is a limited body of recent literature exploring the inclusion of mussel meal as a potential ingredient in shrimp diets, highlighting a significant information gap that can be addressed. In this study, we identified that the inclusion of small amounts of mussel meal in the diet of whiteleg shrimp demonstrates a positive potential on zootechnical performance.

Recently, the use of marine bivalves as a potential ingredient for the aquafeed industry was suggested [55]. These resources, in addition to having high levels of protein, vitamins C and B2 and minerals such as iron and magnesium [8], are low-trophic-level filtering species and can be cultivated as tools for mitigating excesses of nutrients in marine environments [7]. For lobster farming, the industry has been testing the inclusion of mussel meal in manufactured diets to reduce reliance on mussels as fresh food [56,57]. In fish, several research studies have already revealed that mussel meal has a high potential for serving as a supplement or a fishmeal replacement in diets, resulting in similar or better growth performances. For example, in juvenile Ussuri catfish, mussel meal protein can successfully substitute 50% of fishmeal protein (~18% maximum inclusion level of mussel meal) without significant effects on growth, nutrient utilization and hepatic IGF-I gene expression [10]. In a study conducted on turbot, it was demonstrated that fishmeal can be substituted with mussel meal up to 25% (11% mussel meal inclusion) without compromising growth or negatively impacting the fish [16]. In Artic charr, similar growth performance, nutrient digestibility and intestinal barrier function were found in groups fed diets with 40% of the fishmeal replaced with mussel meal (22% mussel meal inclusion) compared to the control (0% replacement of fishmeal [15]). In juvenile common sole, a higher specific growth rate, higher feed intake and lower feed conversion ratio were found for groups fed fishmeal replacement diets (up to 75%, a 25% maximum inclusion of mussel meal [9]). Shrimp (*F. paulensis*) fed diets based on fishmeal or mussel meal (40% inclusion for both ingredients) did not present significant differences in growth performance, feed efficiency or survival [12]. Most of these studies investigated the partial or full replacement of fishmeal with mussel meal. Nevertheless, mussel meal was also tested as a feed additive, small amounts of which (2 to 8% of mussel meal inclusion) increased palatability in rapeseed-protein-based diets in turbot [11] or increased the feed intake and specific growth rate in common sole [9]. In the present work with shrimp, increases in feed intake were also found for the 1 and 2% levels of dietary mussel meal inclusion. The quadratic regression models corroborate that the best inclusion levels are from 1.73 to 2.00%, demonstrating that mussel meal can work well as a strategic nutritional additive for marine shrimp diets.

Mussel meal is rich in different compounds like minerals and unsaturated fatty acids that could improve the robustness of shrimp, as shown in previous studies. For instance, the beneficial role of a diet enriched with highly unsaturated fatty acids with respect to tolerance to handling stress and the immune response of whiteleg shrimp juveniles was reported [58]. Also, an improvement in osmoregulation capacity was monitored in whiteleg shrimp juveniles when their diets were supplemented with unsaturated fatty acids [59]. Post-larvae whiteleg shrimp fed with small amounts of brown seaweeds (1% of *Sargassum filipendula* combined with 2% of *Undaria pinnatifidia*) showed lower rates of cumulative mortality when exposed to a cold shock (13.5 ± 0.1 °C) than the control shrimp, which were fed diets without seaweed inclusion [40]. Similar results were found in post-larvae whiteleg shrimp fed with 0.5 and 2% inclusions of *S. filipendula*, showing lower rates of cumulative mortality after cold shock (12.5 ± 0.4 °C) compared with a control treatment [60]. Another study using the same seaweed at a concentration of 0.5%, demonstrated a greater resistance to cold shock in whiteleg shrimp at an even lower temperature (11.5 ± 0.1 °C; [61]). Coelho et al. [62] used the same temperature to subject whiteleg shrimp juveniles to a thermal shock challenge and observed greater survival in shrimp fed with the dietary inclusion of 2% *Ulva ohnoi*. Whiteleg shrimp fed diets enriched with 0.5 and 1% of *Aurantiochytrium* sp. flour, which is rich in docosahexaenoic acid (DHA), showed higher rates of survival for cold shock (12.5–13.0 °C) when compared to a control treatment [63]. In the current experiment, no differences were measured in survival among the treatments after the cold shock (10.9 ± 0.1 °C). This may be related to the final weight of the animals, which was higher than the final weight of the animals in the aforementioned studies. The size of the shrimp plays a significant role in their resistance to cold shock, with juvenile shrimp exhibiting greater resilience compared to post-larvae shrimp [64]. Furthermore, resistance to cold shock may be directly related to the concentration of unsaturated fatty acids in the diet, which are capable of impacting cell membrane fluidity [65,66]. In the present study, a slightly higher lipid content was observed in the diet with the inclusion of 4% mussel meal, and the lowest mortality rate during the first hours of the thermal shock challenge was observed for this treatment, although the accumulated mortality data did not show statistical differences at the end of the 48 h. Mussels are considered marine sources of fatty acids and have considerable amounts of DHA and eicosapentaenoic acid (EPA), which are long-chain unsaturated fatty acids [67,68] that improve immunity and positively impact the growth and zootechnical performance of whiteleg shrimp [69,70], although the nutritional requirement is not well defined [32]. Thus, the impact of mussel meal on the tolerance of shrimp to thermal shock and on their immune responses seem to be worth further investigation.

5. Conclusions

The current study demonstrated that shrimp fed with 1 and 2% mussel meal diets had significantly higher final weights, weight gain, relative growth rates and lower feed conversion ratios than the shrimp fed with the control, 3 and 4% mussel meal diets. The shrimp fed with the 2% mussel meal diet showed the best growth results. After 8 weeks of the experiment, the shrimp from this treatment were significantly heavier than the control shrimp by 10%. Further, no differences were observed in cold resistance and survival among the treatments. In conclusion, mussel meal can be used as a potential additive in whiteleg shrimp diets to promote growth, and inclusion levels between 1.73 and 2.00% are indicated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11091670/s1, Table S1: Results from the regression analyses between the dietary mussel meal content and the performance indicators of *L. vannamei*.

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