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High protein krill meal as a tool to optimize low cost formulas for juvenile *Litopenaeus vannamei* diets farmed under semi-intensive conditions

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ABSTRACT

To investigate the potential of high-protein krill meal (HPK) to improve growth in low-cost diets for Pacific white shrimp (*Litopenaeus vannamei*), a commercial control and a 3% HPK diet were compared. To simulate a semi-intensive culture system, a total of 4,500 shrimp with a body weight (BW) of 3.07 ± 0.01 g were stocked with 25 animals/m² in 20 cages in a 2.16-ha pond. After 60 days of rearing, the 3% HPK diet achieved a significantly higher yield ($22,094.0 \pm 130.35$ g/cage) in comparison to the control diet ($19,301.6 \pm 272.28$ g/cage) ($P < 0.05$). When the feed cost per kg shrimp produced was compared, it was significantly lower in the 3% HPK group (US\$1.01/kg shrimp) when compared to the control group (US\$1.11/kg shrimp). The results indicate that low feeding cost diets can profit from the partial replacement of fish meal by HPK to optimize shrimp growth performance without increasing formula cost.

KEYWORDS

Antarctic krill; growth enhancement; high protein krill meal; low-cost diets; shrimp

Introduction

The continuous growth of aquaculture requires the use of sustainable resources and techniques that guarantee growth and success in the long-term. However, there is a need to decrease the fish meal and fish oil dependence in diets for shrimp because of stagnant supplies and increasing prices. Low fish meal feeds can take advantage of alternative protein sources such as plant and rendered animal by-products (Tacon and Metian 2008), but there are concerns because of missing essential nutrients, lower attractability/palatability, and antinutritional factors that can suppress feeding stimulus and reduce nutrient bioavailability (Nunes et al. 2006).

Antarctic krill (*Euphausia superba*), a crustacean related to shrimp, has emerged as a sustainable resource that can compensate for the negative consequences of reducing fish meal in industrially compounded feeds and restore growth performance of shrimp. It has been demonstrated that 3% krill meal (KRM) in low fish

meal diets containing 20% poultry meal increased feed palatability and growth of blue shrimp, *Litopenaeus stylirostris* (Suresh and Nates 2011). In another feeding trial with the white shrimp, *Litopenaeus vannamei*, where only plant proteins were used, only 1% of KRM was enough to stimulate feed intake and at 2% KRM, shrimp growth and yield were significantly increased and the feed conversion ratio (FCR) reduced (Sabry-Neto et al. 2017). A diet high in soybean protein and low in fish meal (3%) was used in a study comparing the addition of 3% KRM to other marine feed attractants such as 3% squid meal, shrimp head meal, squid liver meal, salmon meal, soy protein concentrate, or 5% liquid sardine hydrolyzate (Nunes et al. 2019). In the end of the 10-week feeding trial, shrimp in the KRM group demonstrated the best growth performance with the highest final body weight (BW) when compared to the other feed attractants evaluated.

These feeding studies showed that KRM has the potential to help nutritionists lower the inclusion of fish meal in feed formulations and improve growth performance of shrimp already at low inclusion levels, thereby providing an economically efficient mean of shrimp production. The cost-benefit analysis of shrimp feed formulations is of importance since the biggest and most important cost in aquaculture production is the feed, being over 50% of the overall production expenses. This makes good quality, steady availability, and optimal performance of feed ingredients the defining parameters to ensure profitability of shrimp farming.

Although KRM is the meal from ground whole krill, which is rich in proteins, omega-3 phospholipids, and astaxanthin, the high protein krill meal (HPK), which is a by-product from the krill oil extraction for the human nutraceutical market, is defined by higher protein and lower lipid contents. This study was performed to better understand its value in *L. vannamei* diets, and an optimized low-cost feed diet containing 3% HPK was compared to a low-cost commercial diet for its effect on growth performance and profitability under farmlike conditions.

Material and methods

Shrimp and experimental conditions

The experiment was carried out in a shrimp experimental farm located in Bacorehuis Bay, Ahome, Sinaloa State in Mexico. This facility provided farmlike conditions with similar challenges as for farmed shrimp such as environmental parameter changes, natural productivity, and exposure to opportunistic pathogens. A batch of 4.5 million postlarvae from the FITMAR commercial production laboratory in Sinaloa, Mexico, were confined to raceways of the Costa Pacífico farm with a stocking density of 16 PL/L. There the organisms were maintained for a period of 28 days until 200–250 mg of BW, and then 216,500 shrimp were transferred to a pond with a stocking density of 10 shrimp/m² until they reached

3 g. For seeding the cages, a total of 4,500 shrimp with a BW of 3.07 ± 0.01 g were selected and stocked at 25 animals/m² in 20 cages of 9 m³ providing 10 cages per treatment that were randomly assigned to the cages. The cages were installed in a 2.16-ha pond using four docks with five cages attached to each, which ensured that water quality parameters were equal for all cages due to access to the same fluctuations and water exchanges, which were around 10–15% per day. Shrimp were fed 2–3 times daily on 70 × 70 cm feeding trays for 60 days with declining feeding rates from 5% of the total shrimp biomass for 3 g shrimp to 2.3% for 16 g shrimp taking into account body weight, biomass, and feed consumption. The feeding trays were used to inspect shrimp feed intake, and the feeding rates were increased by 30, 20, and 10% over three days, if there was no feed leftover observed. If there was leftover feed, then the feeding rates were reduced by 10% the following day, 20% on the second day, and 30% on the third day.

For the evaluation of physical-chemical water conditions, temperature and dissolved oxygen (DO) were measured with an ISY PRO-20 Oximeter daily at 8:00 a.m. and 5:00 p.m. An aqua-line refractometer was used to measure salinity, and Aquafauna brand digital pH meter to measure pH twice a week. The total ammonia nitrogen (TAN), nitrite, and nitrate concentrations were measured once a week using an ISY 9500 Photometer.

Growth and feed conversion assessments

For the evaluation of the productive parameters, the individual weights of the animals were taken at the time of stocking and harvest. Weekly average shrimp BWs in each cage were determined from a sample of 25 to 50 animals taken at random using an Ohaus scale with an accuracy of 0.1 g. Mortality was monitored daily, and shrimp final survival (SR, %) was calculated by the equation: $SR = (POPf/POPi) \times 100$, where POPi = number of shrimp at stocking, and POPf = number of shrimp at harvest.

Accumulated food consumption was calculated from the quantities of food supplied in each cage during the experiment. The FCR was calculated as the product of the cumulative consumption of food among the gained biomass during 60 days of observation for each of the cages. Growth performances were estimated through the assessment of weight gain, average daily weight gain, FCR, and mortality rate using the following formulas:

$$\text{Weight gain} = BW_t - BW_0,$$

$$\text{average daily gain (g/d)} = (BW_t - BW_0) / \text{rearing days and}$$

$$FCR = \text{total dry feed delivered} / (BW_t - BW_0),$$

where BW_t is the body weight per shrimp per tank obtained at time t

Experimental design and diet preparation

Two experimental diets with 10 replicates each were compared in this feeding trial, i.e., a control diet based on a commercial semi-intensive 35% crude protein (CP) feed and one krill diet, where 3% HPK was included into the control diet replacing parts of fish meal resulting in fish meal inclusions of 8.54% (Table 2). The control, commercial feed, is made with the following ingredients: soybean meal, wheat meal, fish meal, canola meal, corn gluten meal, sorghum meal, fish oil, salt, binder, soybean oil, calcium carbonate, mineral premix, vitamin premix, synthetic lecithin, and vitamin C achieving 35% crude protein, 8% crude fat, 8% ash, and 9% moisture. HPK was provided by Aker BioMarine Antarctic ASA (Lysaker, Norway). It is a commercial product named QRILL™ High Protein, with a typical composition presented in Table 1. Feeds were manufactured through pelleting at an industrial shrimp feed mill. Briefly, ground ingredients and feed additives (minerals, vitamins, and amino acids) were weighed, mixed, and transferred to a feeder conditioning for steam cooking. The conditioned mash was then transferred to a pellet mill, where molding and cutting of pellets took place. Subsequently, the moist pellets were subjected to postconditioning and then dried and cooled in a cooler before the 1.8 mm pellets were collected in bags. Besides the low fish meal inclusion, feed formulation was based on a high dietary inclusion of plant ingredients and 3% fish oil (Tables 2–4). Soybean protein was used at 38%; dietary inclusion of wheat flour, canola meal, and corn gluten meal reached 25.76, 10, and 5% respectively (Table 2). Dietary CP, lipid, fiber, and ash levels reached 36.7, 8, 3.6, and 8.2 (% of the diet), respectively (Table 3). The diets were isocaloric (control: 18.08 and 3% HPK: 18.14 KJ g⁻¹) and contained similar levels of available phosphorus (control: 0.63 and 3% HPK: 0.6%) (Table 3).

Statistical analysis and ethics statement

All data obtained were analyzed by a nonparametric one-way ANOVA Kruskal Wallis test taking the “treatment” as an explanatory variable and initial BW, final BW, weight gain, survival, initial biomass, final yield, feed

Table 1. A typical composition of QRILL™ high-protein (HPK), the krill meal used in this study.

Nutritional composition (%)	HPK
Moisture	4
Crude protein	72
Crude fat	12
Ash	12
<i>% of total lipids</i>	
Phospholipids	22
EPA	9
DHA	6

Note. Docosahexaenoic acid = DHA; Eicosapentaenoic acid = EPA.

Table 2. Experimental diet formulation with 3% high-protein krill meal (HPK). In addition, a commercial grower feed with proprietary ingredient composition has been used in the study as comparison.

Ingredients (%)	3% HPK
Soybean meal, 46% crude protein	38.00
Wheat flour	25.69
Fish meal, 64% crude protein ^a	8.54
Canola meal	10.00
Corn gluten meal	5.00
Sorghum	4.00
Fish oil	3.00
High-protein krill meal (HPK) ^b	3.00
Salt, NaCl	1.00
Pellet binder	0.50
Soybean oil	0.49
Calcium carbonate	0.40
Mineral premix	0.10
Vitamin premix	0.10
Synthetic lysine	0.06
Vitamin C	0.06
Lipid additive	0.05

^aMexican local fish meal; ^bQRILLTM High Protein, Lysaker, Norway.

consumption, and FCR as dependent variables, respectively. The statistical analyses were performed with R software. All reported P values are two-sided, and $P < 0.05$ was considered significant.

The study was performed in compliance with the Mexican ethical guidelines (https://www.conacyt.gob.mx/images/conacyt/sinecty/CODIGO_ETICA.pdf).

Results

Two low-cost diets were compared for their effects on growth performance of juvenile *L. vannamei*, i.e., a control diet (commercial feed developed for semi-intensive farming systems) and a diet containing 3% HPK (based on the control diet but partially replacing fish meal) (Tables 2–4). For the feeding trial, a stocking density of 25 animals/m² with 3.07 ± 0.01 g BW on average was chosen to represent a reasonable semi-intensive culture system. The water conditions in the uncontrolled pond simulated the conditions of a commercial crop with temperatures ranging between 29.2°C to 31.5°C and DO concentration of 4.0 to 8.0 mg/L. Transparency was in the range of 35–60 cm measured with a Secchi disc, and pH was between 8.54 and 8.62. Salinity fluctuated between 35 and 50 ppt, reaching an average of 39.8 ppm. TAN values peaked in the third week of the trial (0.0403 mg/L), which is far below the

Table 3. Proximate and amino acid composition (% , as-fed basis), as well as raw material cost (USD/MT) of feed used in the study.

Proximate (%) and amino acid compositions (g/100 g sample)	Control	3% HPK
Energy (KJ/g)	18.08	18.14
Crude protein	36.1	36.7
Crude fat	7.9	8
Ash	8.0	8.2
Moisture	9.6	9.0
Fiber	3.6	3.5
Phosphorus	0.63	0.60
Calcium (mg/kg)	9.5	9.8
Triacylglycerol ^a	66	69
Free fatty acids ^a	14	9.0
Cholesterol esters ^a	1.4	1.3
Phosphatidylethanolamine ^a	2.1	1.4
Phosphatidylcholine ^a	4.3	3.4
Lyso-phosphatidylcholine ^a	0.9	0.8
Total polar lipids ^a	7.3	5.6
Total neutral lipids ^a	81.4	84.9
Aspartic acid	2.9	2.9
Glutamic acid	6.0	6.0
Hydroxyproline	0.32	0.33
Serine	2.2	2.2
Glycine	1.9	2.0
Histidine	0.67	0.68
Arginine	2.2	2.2
Threonine	1.3	1.4
Alanine	1.7	1.7
Proline	2.4	2.4
Tyrosine	1.1	1.1
Valine	1.8	1.8
Methionine	0.49	0.51
Isoleucine	1.5	1.5
Leucine	2.8	2.8
Phenylalanine	1.6	1.6
Lysine	1.6	1.7
Tryptophan	0.39	0.39

^ag/100 extracted fat.

concentration considered lethal to organisms (0.400 mg/L). The concentration of nitrite (NO₂) in the water varied between 0.001 and 0.123 mg/L; the nitrate (NO₃) fluctuated between 16.15 and 36.14 mg/L.

At the 60-day harvest, the results indicated a high shrimp survival with a tendency of higher survival in the 3% HPK (91.7%) compared to the control group (88.3%) (Table 5, P > 0.05).

Shrimp final BW and yield of the 3% HPK group were significantly different and better than the control group (P < 0.05, Table 5). Mean final BW of the 3% HPK-supplemented diet group was 14.08 ± 0.24 g and of the control group 13.19 ± 0.50 g (P < 0.05). The feed intake was higher for the 3% HPK group (2,623.67 ± 58.87 g) when compared to the control group (2,526.85 ± 75.08). The lowest FCR was achieved with shrimp fed the 3% HPK diet (1.20 ± 0.09); the control diet (1.34 ± 0.21) showed a higher FCR albeit not significantly different (P < 0.05).

Table 4. Fatty acid profile (% of total dietary fatty acids, dry matter basis) of feeds used in this study.

Fatty acids (%)	Control	3% HPK
14:0	1.0	1.0
16:0	13.6	13.4
18:0	3.7	3.7
20:0	0.4	0.3
22:0	0.2	0.2
16:1 n-7	1.4	1.4
18:1 (n-9) + (n-7) + (n-5)	19.1	19.0
20:1 (n-9) + (n-7)	0.5	0.5
22:1 (n-11) + (n-9) + (n-7)	0.3	0.3
24:1 n-9	0.1	0.1
16:2 n-4	0.1	0.1
16:3 n-4	0.1	0.1
18:2 n-6	31.1	31.0
18:3 n-6	0.1	0.1
20:2 n-6	0.1	0.1
20:3 n-6	0.1	0.1
20:4 n-6	0.2	0.2
22:4 n-6	0.1	0.1
18:3 n-3	3.8	3.8
18:4 n-3	0.2	0.2
20:3 n3	0.1	0.1
20:4 n-3	0.1	0.1
20:5 n-3 (EPA)	1.4	1.3
21:5 n-3	0.1	0.1
22:5 n-3	0.3	0.2
22:6 n-3 (DHA)	1.3	1.2

Table 5. Growth performance and feed utilization of *L. vannamei* after 60 days of rearing. P values are calculated with nonparametric one-way ANOVA Kruskal Wallis test.

Parameter	Days	Control	3% HPK	P value
Initial biomass (g/cage)	0	690.6 ± 4.44 ^a	694.2 ± 13.70 ^a	.732
Final yield (g)	60	19,301.97	22,081.13	
Final yield (g/cage)	60	1,930.20 ± 271.95 ^a	2,208.11 ± 130.87 ^a	.013
Initial body weight (g)	0	3.07 ± 0.02 ^b	3.09 ± 0.06 ^b	.85
Final body weight (g)	60	13.19 ± 0.50 ^b	14.08 ± 0.24 ^b	.000
Feed consumption (g/cage)	60	2,526.85 ± 75.08 ^a	2,623.67 ± 58.87 ^a	.005
Feed conversion ratio	60	1.34 ± 0.21 ^a	1.20 ± 0.09 ^a	.078
Survival (%)	60	88.3 ± 8.05 ^a	91.7 ± 4.46 ^a	.404

^aMean ± standard deviation.^bMean ± standard error.

Shrimp grew significantly faster when fed the 3% HPK diet (11.02 ± 0.28 g) compared to the control (10.12 ± 0.49) diet ($P < 0.05$). Hence, a significantly higher gained yield was obtained with the 3% HPK diet ($2,208.11 \pm 130.87$ g/cage) in comparison to the control diet ($1,930.20 \pm 271.95$ g/cage) ($P < 0.05$).

The feed formulation costs were similar in the two diets, but when the feed costs per kg shrimp produced were compared, a lower cost was obtained in the 3% HPK group with US\$1.08 versus US\$1.19/kg shrimp of the control group (Table 6). The differences in cost of feed/kg shrimp produced and yield/ha

Table 6. Economic analysis of the three diets and their performance after 60 days of rearing. Price of feed and shrimp were taken at the time of harvest in Mexico in 2019.

Parameters	Control	3% HPK
Cost of feed per kg produced (US\$)	1.19	1.08
Shrimp biomass produced (kg)	19.3	22.1
Shrimp biomass per ha (kg)	2,144.66	2,453.80
Feed cost (US\$/ha)	2,386.47	2,477.91
Cost of feed & larvae (US\$)	3,511.47	3,602.91
Fixed costs assumption (US\$)	950	950
Shrimp sale price (US\$)	8,237.09	8,552.70
Net profit (US\$)	5,132.51	5,335.88
Increased profit (US\$)	–	203.37
Increased profit (%)	–	4.0

resulted in increased profits by dietary supplementation of 3% HPK when compared to the control diet of US\$203.37/ha. This corresponded to an increased profit of 4% when including 3% HPK in the diet (Table 6).

Discussion

Overall shrimp survival in this study was high, which can be explained by good adaptation of shrimp to pond conditions and the absence of stress and disease, as well as environmental variations that were within the optimal ranges for shrimp growth (Martinez-Cordova et al. 1998; Samocha 2019). The study showed that dietary inclusion of 3% HPK into shrimp feed significantly improved shrimp final BW by 0.9 g, survival by 3.4%, yield by 278 g/cage, and FCR by -0.14 compared to the control diet after 60 days of rearing. Moreover, these growth performance benefits of HPK inclusion were obtained at similar feed cost. Feed manufacturers can take advantage of these benefits to increase performance of low-cost diets, thereby positioning them as a better alternative to competing low-cost feed without incurring additional costs but instead providing differentiation and a market advantage.

Moreover, HPK can be used as a tool to enable the reduction of fish meal in shrimp diets and offsets fish meal quality variations. Reductions of 5 to 10% of fish meal opens space in the feed formulation to include lower cost, sustainable protein sources such as from plant (soybean meal, wheat, sunflower, rapeseed, etc.) and animal (pork, poultry, blood, etc.) sources. This will decrease the pressure on wild fish stocks and increase cost effectiveness and sustainability of commercial shrimp feed. Since alternative protein sources might have different organoleptic profiles that change attractability and palatability of feed than fish meal, krill meal can act as a feed attractant, overcoming low feed intake as described previously (Sá et al. 2013; Sabry-Neto et al. 2017; Smith et al. 2005; Suresh and Nates 2011; Williams et al. 2005). In particular for low-cost diets

with varying feed quality, the dietary inclusion of krill meal might provide a safety net that ensures high feed intake and thereby optimal growth performance.

Direct benefits to the farmers of a 3% HPK diet include better growth performance at the same cost, leading to a US\$203.37/ha profit. For a 100-ha farmer, this will translate into an incremental benefit of US\$20,337 per crop, which improves profitability and long-term sustainability of the business.

Since more than 50% of the production cost is associated with feed, it is of interest to keep the FCR, which measures how much feed is used to produce 1 kg of harvested shrimp, as low as possible. In this study, inclusion of 3% HPK reduced the FCR when compared to the control diet. The same diet demonstrated the highest growth, suggesting that higher feed consumption helped the shrimp to take maximal advantage of the feed and its nutrients for conversion into BW. Besides, better FCR means less waste pollution because of leaching nutrients and improved water and soil quality, which eventually will reduce the proliferation of opportunistic bacteria and the risk of disease outbreaks. Deterioration of water quality would require additional costs because of the need of increasing water exchange to maintain water quality and additional soil treatment to reduce organic matter at the end of the cycle.

The strength of the study lies in the simulated farmlike conditions; however, there were optimal environmental conditions and no disease outbreak throughout the study period. For a future study, it would be of interest to test the dietary inclusion of HPK during more challenging conditions to determine its full potential for shrimp growth performance.

Conclusion

This study aimed to demystify the popular belief that krill meal is an ingredient that can only be used in high-performance diets. Instead, krill meal gives benefits at low inclusion levels such as higher feed consumption, leading to growth acceleration at equal feed formulation costs. Krill meal as a formulation tool to decrease the reliance on fish meal opens opportunities to the use of alternative ingredients that improve the cost efficiency and sustainability of feeds. In short, HPK can be used at 3% in low-cost feeds and at higher levels up to 10% in high-performance/functional diets for shrimp.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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