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Research Article

Combined feeding frequency and ration size effects on juvenile *Penaeus vannamei* performance fed diets supplemented with fish hydrolysates

Manuel Espinoza-Ortega[1], Cesar Molina-Poveda[1]
Roberto Civera-Cerecedo[2] & Miguel Jover-Cerdá[3]
[1]Research and Development Department, Guayas, Ecuador
[2]Laboratorio de Nutrición Acuícola, Centro de Investigaciones Biológicas del Noroeste (CIBNOR)
La Paz, B.C.S., México
[3]Research Group of Aquaculture and Biodiversity, Institute of Animal Science and Technology
Universitat Politècnica de València, València, Spain
Corresponding author: Roberto Civera-Cerecedo (rcivera04@cibnor.mx)

ABSTRACT. Optimum feed ratio and frequency ensure maximum growth and efficient feed utilization across feeding management strategies. The present study evaluated the effects of feeding frequency and ration restriction on *Penaeus vannamei* juveniles (0.8 ± 0.06 g) fed two diets over 53 days. Feeding frequency included two (10:00, 16:00 h) and four times a day (10:00, 16:00, 22:00, 04:00 h), using isonitrogenous diets (35% protein) formulated with fish hydrolysates produced via external (FHEE) or internal (FHIE) enzymes. Feed was supplied at 100 and 80% of apparent satiation. At the end of the experiment, survival was not different among treatments ($P > 0.05$). Shrimp fed twice showed a significantly higher weight gain than those fed four times (6.27 ± 0.43 *vs*. 5.76 ± 0.38 g, respectively); no significant difference ($P > 0.05$) in feed conversion ratio (FCR) was found between two and four times a day treatment at 100% ration size. However, feed efficiency was significantly affected by feeding frequency ($P < 0.05$) at 80% of apparent satiation, with four daily feedings showing improved FCR (1.55 ± 0.07) compared to two feedings per day (1.73 ± 0.08). The results demonstrated improved feed efficiency at 80% satiation compared with 100% satiation (FCR = 1.64 ± 0.07 *vs*. 1.93 ± 0.12, respectively), at the expense of growth (5.79 ± 0.31 *vs*. 6.23 ± 0.51, respectively). No differences in weight gain were observed between the distinct diet types (FHEE or FHIE). The results highlight the significant impact of ration size and feeding schedules, demonstrating that restricted rations (80%) improve feed efficiency at the expense of growth and that increasing feeding frequency at night does not provide additional benefits compared to daytime feeding.

Keywords: *Penaeus vannamei*; shrimp aquaculture; feed utilization; feed restriction; feed conversion ratio (FCR); growth performance; sustainable aquaculture

INTRODUCTION

The Pacific white shrimp *Penaeus vannamei* is the most valued species in aquaculture worldwide, representing an estimated production of 6.8 million metric tons (mt) in 2022 (FAO 2024).

In Ecuador, shrimp exports reached 1.06 million mt in 2022, rising to 1.21 million mt in 2023 (CNA 2023). In 2022, Ecuador's total shrimp exports amounted to US$ 10.1 billion, underscoring the country's central role in global farmed shrimp production (FAO 2024).

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Feed is one of the most important factors affecting aquaculture profits and accounts for 40-60% of production costs (Tan et al. 2005, Tacon et al. 2013). Since feed accounts for these high production costs, proper feed management is critical for maximizing profits by optimizing feed efficiency, enhancing growth, and mitigating the environmental impacts of shrimp farming (Weldon et al. 2021).

Feeding frequency strategies are critical in determining production cost-effectiveness (Arnold et al. 2016). However, feed partitioning is a contradictory subject to date, with some authors suggesting that multiple feedings may not be advantageous or have no effect on performance (Velasco et al. 1999, Smith et al. 2002); others indicated that higher feeding frequency may improve growth and enhance feed utilization (Napaumpaiporn et al. 2013, Aalimahmoudi et al. 2016, Nunes et al. 2019). Given the contradictory information about feeding frequency, the effects of other variables have not been addressed to date, such as ration size or formula composition, which impact this strategy.

Additionally, studies reporting interactions between feeding frequency and diet restriction are limited. Arnold et al. (2016) described the benefits of a restricted ration on feed efficiency in *Penaeus monodon* juveniles, while Carvalho & Nunes (2006) reported the possibility of moderately reducing daily feeding rates without affecting performance in juvenile *P. vannamei*. Nutrition is one of the main factors affecting growth, survival, and feed efficiency. Recent years have seen growing interest in the inclusion of hydrolysate in *P. vannamei* formulations and in the role of peptides in animal nutrition. The various hydrolysis processes, including chemical, enzymatic, or microbial, are attractive for generating high-quality peptides of suitable size with physiological and nutritional functions in crustaceans (Hou et al. 2017).

Despite the potential of hydrolysates as chemostimulants to increase ingestion and thereby affect survival and growth (Yuangsoi et al. 2025), limited information is available on their effects on *P. vannamei* performance (Nunes et al. 2006).

Some marine ingredients (squid, tuna, or crustaceans) can generate a high level of low-molecular-weight nitrogen compounds when hydrolyzed under the right conditions, which are highly palatable and possess bioactive and functional properties that improve performance.

Marine-derived protein hydrolysates have been demonstrated to generate low-molecular-weight nitrogenous components with enhanced palatability and bioactive functionality, leading to improved performance in aquaculture feeds (Shahidi & Saeid 2025).

The enzymatic hydrolysis of marine product residues is normally carried out under conditions that yield final high functionality products (Kristinsson & Rasco 2000). In this regard, some authors have reported improvements in *P. vannamei* growth when enzymatic fish hydrolysates were used (Córdova-Murueta & García-Carreño 2002, Forster et al. 2004, 2011, Nguyen et al. 2012).

This result is attributed to a higher absorption efficiency of the hydrolysate and to the presence of hydrolysis products, such as amino acids and low-molecular-weight compounds, which enhance attractability and, consequently, increase intake (Berge & Storebakken 1996, Aksnes et al. 2006). More recently, several studies have demonstrated that hydrolysates improve feeding efficiency and can contribute to better feed conversion ratios, a critical factor in shrimp nutrition (Hlordzi et al. 2022, Bøgwald et al. 2024).

Enzymatic hydrolysis is widely recognized as the preferred method for producing fish protein hydrolysates (FPH) for aquafeeds, as it enables controlled cleavage of proteins into a targeted mixture of peptides and free amino acids while preserving nutritional quality and avoiding undesirable modifications often associated with chemical hydrolysis (Siddik et al. 2021). By using specific proteases and carefully adjusting hydrolysis conditions, it is possible to tailor the molecular weight profile and functional properties of the hydrolysate, thereby enhancing digestibility and palatability in aquatic species (Chinnakkannu et al. 2023). Compared to chemical hydrolysis, enzymatic methods provide superior control over peptide size distribution and preserve bioactive properties, making them particularly suitable for high-value applications in aquaculture feeds. Moreover, dietary inclusion of enzymatically derived hydrolysates has been reported to improve growth performance, feed utilization efficiency, and immune responses in fish and shrimp, supporting their use as functional ingredients in precision nutrition strategies for aquaculture (Siddik et al. 2021).

Chemoattractants play a crucial role in the formulation of shrimp feeds. These compounds, derived from marine ingredients such as fish hydrolysates, contain bioactive peptides and amino acids that enhance palatability and feed intake, which are essential for maximizing growth and feed efficiency (Carr 1988, Aksnes et al. 2006). Including chemoattractants can improve shrimp performance and optimize the cost-benefit ratio, thereby reducing losses from unconsumed feed and its environmental impact. However, the literature on the specific effects of different types of fish hydrolysates on *P. vannamei* remains limited, especially in combination with feeding management strategies and ration restriction.

Therefore, the present research aimed to evaluate feeding frequency and ration size effects on the *P. vannamei* growth, survival, and feed conversion in diets supplemented with two marine chemoattractants.

MATERIALS AND METHODS

Experimental design

The experimental design was a three-way factorial with two diets, two feeding frequencies, and two feeding ratios, yielding eight treatments with three replicates each. Two diets were formulated with different hydrolysates: fish hydrolysate produced via external enzymes (FHEE, pH = 4.36 ± 0.68) with 67% of peptides <1000 Da, and fish hydrolysate produced via natural internal enzymes (FHIE, pH = 3.87 ± 0.12) with 88% of peptides with a molecular size <1000 Da (Fig. 1). A factorial array was applied to feeding frequency and ration, which included two feeding frequencies-two times and four times per day- and two feeding rations -100 and 80% satiation-.

Experimental system

Shrimp (n = 5,000) were collected from a grow-out pond (GranMar, Baja California Sur, México) and transported to Centro de Investigaciones Biológicas del Noroeste (CIBNOR, La Paz, Baja California Sur, México), where they were stocked in an indoor facility with 18 holding tanks: three 1,300-L rectangular tanks (140×240×50 cm) and 15 700-L circular tanks (ø = 94 cm). Before stocking, shrimp were acclimated to laboratory conditions (temperature 27 ± 0.5°C, salinity 38 ± 1, and dissolved oxygen >5 mg L^{-1}) and held for one week in these tanks supplied with filtered seawater (50% water replacement daily). Shrimp were fed a commercial diet containing 35% protein twice daily until they reached approximately 0.8 g. Immediately before stocking, 100 shrimp were randomly selected and individually weighed to the nearest 0.01 g (Ohaus Scout® Pro Balance, USA) to estimate the mean and standard deviation (SD).

The experimental system consisted of continuous water circulation through 24 fiberglass aquaria (50×34×38 cm) with a 50 L water volume; each tank was equipped with a 250-W submersible heater, an air stone, and seawater filtered supply via a sand filter, a cartridge filter (10 μm), and a UV light. Water was replenished daily at a rate of 75%. A 12:12 h light:dark cycle (photophase 07:30 to 19:30 h) was maintained throughout the experiment, using 28-W tubes to achieve ~490 lux.

Shrimp were weighed individually, selected according to required weight based on experimental design, and randomly stocked (10 shrimp per aquarium; 47 shrimp m^{-2}) in each 50-L aquarium. The global variation coefficient (VC) was under 10%, and the mean ± SD across all aquaria was 0.8 ± 0.01 g.

Diets

Two pelletized diets were prepared at a laboratory scale and formulated to fulfill or exceed the National Research Council (NRC 2011) requirements for shrimp and consistent with standard industry diets used in Ecuador. The diets contained fish hydrolysates at 2% dietary inclusion level (Table 1). The hydrolysate (FHEE) was manufactured from a fish mix that included: *Katsuwonus pelamis*, *Scomber japonicus*, *Opisthonema* spp., *Etrumeus teres*, *Cetengraulis mysticetus*, *Auxis* spp., and *Engraulis ringens*. The hydrolysate (FHIE) was obtained from *Salmo salar*. The composition of hydrolysates is detailed in Table 2.

Before preparing the experimental diets, all macro-ingredients were ground in a laboratory mill (Pavan®, Italy) and passed through a 0.25 mm mesh sieve. The micro-ingredients were mixed in a plastic container and then added to the macro-ingredients. The dry ingredients for each diet were mixed thoroughly in a food processor (KitchenAid®, USA) before soy lecithin was added. After the soy lecithin was dispersed, water was added (approximately 30% of the total "as is" ingredient weight), and the mixture was finally mixed. The resulting mixture was pressure-processed in a meat grinder (Torrey® México) through a die with 2 mm-diameter holes, as described by Civera & Guillaume (1989). Pellets were dried to a moisture content of 8-10% in a forced-air oven at 60°C for approximately 8 h and stored at 4°C until used.

Experimental conditions

During the 53 days of the trial, oxygen and temperature were measured every day, pH and salinity were measured and recorded once a week, and ammonia and nitrites were measured once every 15 days. A 100% feeding ration treatment was administered slightly above expected satiety, and 80% was calculated based on a 100% feeding ration. Uneaten feed and feces were removed in all treatments by siphoning each tank daily at 08:00 h. The amount of uneaten feed was scored by counting pellets and multiplying by the average pellet

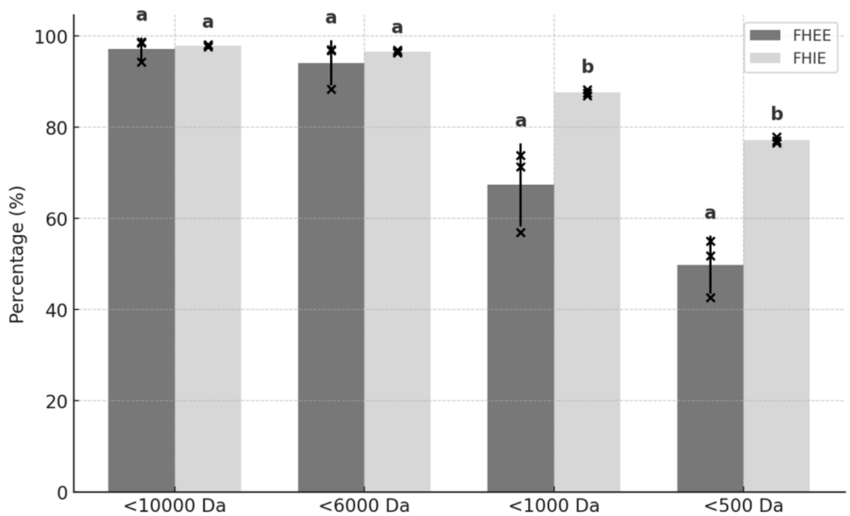

Figure 1. Peptide size profiles of the two fish hydrolysates-external enzyme (FHEE) and natural internal enzyme (FHIE)-used in the experimental diets. Different lowercase letters indicate significant differences among peptide size fractions (Dalton classes) between hydrolysates ($P < 0.05$). Error bars represent mean ± standard deviation (SD; n = 3). The 'x' symbols represent the individual values from each of the three replicate determinations.

Table 1. Ingredients and proximate composition of experimental diets (g 100 g^{-1} as fed basis). [1]FHEE: hydrolysate produced by controlled enzymatic hydrolysis of the fresh fish by-products via external enzymes. [2]FHIE: enzymatically produced, hydrolyzed fish protein with fish produced via natural fish enzymes. [3]The complete formulation is not disclosed, as only representative ingredient categories and proximate composition are shown to describe the experimental diets.

	FHEE[1]	FHIE[2]
Ingredients[3]		
Vegetable-sourced meal	71.04	71.04
Marine animal products	10.00	10.00
Poultry by-products	7.45	7.45
Fish hydrolysate	2.00	2.00
Fish oil	1.74	1.74
$CaCO_3$, $Ca(H_2PO_4)_2$, NaCl	3.99	3.99
Lecithin Mix	1.29	1.29
Water	1.21	1.21
Binder	0.52	0.52
Antifungal and mycotoxin sequestrant	0.20	0.20
Vitamin-Mineral Premix	0.56	0.56
Proximate composition		
Dry matter (g 100 g^{-1} as fed)	89.83	89.30
Protein	35.43	35.20
Ether extract	5.70	5.90
Ash	10.30	10.70
Nitrogen-free extract	17.80	17.90

weight; this was used to adjust the following day's ration accordingly.

Within each treatment, the following feeding schedules were applied: two feeding times/day (10:00, 16:00 h) and four feeding times/day (10:00, 16:00, 22:00, 04:00 h), selected based on previous experimental evidence from our laboratory (Espinoza-Ortega et al. 2024). The entire daily ration was weighed and divided manually into similar volumes. All feed rations were distributed in uniform portions, and the amount allotted to each aquarium was recorded. The first two rations were administered manually, while the following rations in each treatment group were fed using Fish Mate® F14 Automatic feeders (Pet Mate®, Surrey, England).

At the beginning of the day, the automatic feeders were visually checked, and any feed remaining was recorded. The number of shrimp was recorded daily in each aquarium, which was aerated with a single air diffuser. Dissolved oxygen and temperature were monitored daily. At the beginning of the experiment, feed was supplied at approximately 8% of the biomass, ensuring a marginal excess in 100% treatments. At the end of the trial, feed supply was 5.9 and 4.8% of biomass for 100 and 80% rations, respectively.

Table 2. Proximate composition of fish hydrolysates produced via external enzymes (FHEE), and fish hydrolysates produced via internal enzymes (FHIE) (g 100 g^{-1} as fed basis).

Diet	Moisture (%)	pH	Protein (%)
FHEE	59.93 ± 4.83	4.36 ± 0.68	19.80 ± 3.41
FHIE	56.45 ± 0.07	3.87 ± 0.12	31.10 ± 0.71

Ammonia, nitrate, and nitrites in water were analyzed by a chemical colorimetric kit (Mars Fishcare North America, Chalfont, PA, USA).

Calculations

Growth performance and *P. vannamei* survival for all the groups were calculated with the following equations:

$$\text{Survival (\%)} = \text{final number of shrimp / initial number of shrimps} \times 100$$

$$\text{Weight gain (g)} = (Wf - Wi)$$

$$\text{Feed conversion ratio (FCR)} = Tf / (Wf - Wi)$$

$$\text{Final biomass (g)} = \text{final number of shrimp} \times Wf$$

where Wf represents the final body weight (g), Wi is the initial body weight (g), and Tf represents total feed consumption (g)

Statistical analyses

Statistical analyses were performed using Statgraphics® Centurion™ XVII© (Copyright 1982-2014 Statpoint Technologies, Inc). The data were verified for normality using the Shapiro-Wilk test before performing analysis of variance (ANOVA). Growth, survival, and feed efficiency were analyzed by means of one-way analysis of variance ANOVA; final weight, final biomass, survival, FCR, growth rate, weight gain, and total consumed feed were analyzed by three-way ANOVA. The model included diet, frequency, and ration size as main effects and their interactions. Subsequently, a t-test was applied to find significant differences within each treatment.

RESULTS

Water quality

Averages of water quality parameters are shown in Table 3. Water temperature in all treatments remained constant (27 ± 0.06ºC), as did salinity (37 ± 0.28) and dissolved oxygen (5.81 ± 0.08 mg L^{-1}). Ammonia, nitrate, and nitrite did not exceed the limits set by Boyd (2016).

Shrimp performance

The effect of the type of attractant, ration size, and feeding frequency is presented in Tables 4 and 5. A three-way ANOVA analysis revealed significant main effects ($P < 0.05$) for frequency and ration on weight gain and FCR. However, no significant difference in survival was observed. No significant interactions ($P > 0.05$) were detected between diet × frequency × ration in any of the zootechnical parameters.

After 53 days, survival was high, averaging 95 ± 7% (ranging from 87 ± 12 to 100 ± 0%), and no significant differences ($P > 0.05$) among treatments were detected (Table 6). Mean shrimp final weight of each treatment ranged from 6.38 ± 0.13 to 7.17 ± 0.23 g (Table 6), and mean weight gain ranged from 5.58 ± 0.13 to 6.41 ± 0.23 g per week (Table 6).

Regarding FCR, the trend indicated that 80% restriction treatments tended to show better feed efficiency (1.53 ± 0.08 to 1.77 ± 0.10), while 100% treatments showed higher values (1.83 ± 0.04 to 2.07 ± 0.13).

As expected, a higher FCR was observed in treatments with 100% diet, with the highest feed intake observed in the FHIE treatment at two doses and 100% ration size (130 ± 1.47 g). In comparison, the lowest consumption was observed with both FHEE and FHIE treatments at four doses and 80% satiety (84 ± 1.55 and 86 ± 1.49 g, respectively).

Feeding frequency and ration size

Average weight gain was statistically greater in the treatments with a 100% ration ($P < 0.05$) compared to an 80% ration size (Table 7). FCR was also the highest when no restriction was applied ($P < 0.05$) (Table 8). When the organisms were fed twice per day, average weight gain was significantly greater ($P < 0.05$) than that observed when the diet was distributed four times per day (6.27 ± 0.43 *vs*. 5.76 ± 0.38) ($P < 0.05$). Additionally, a significant average weight increment ($P < 0.05$) was observed when the ration increased from 80% (5.79 ± 0.31) to 100% (6.23 ± 0.51), with a consistent effect across both diets (Table 6).

When feeding frequency was analyzed for each feeding ration, weight gain (g) was highest with two feedings (6.40 ± 0.28 *vs*. 5.85 ± 0.43), but no significant difference was observed at 80%.

A significant effect ($P < 0.05$) of frequency and ration size on FCR was observed (Table 8) after 53 days of testing. Ration size was reduced to 80%, resulting in a statistically lower average FCR than 100% (1.64 ± 0.07 *vs*. 1.93 ± 0.12, respectively). FCR decreased from

Table 3. Mean (± standard deviation) minimum and maximum values of water quality parameters throughout juvenile shrimp *Penaeus vannamei* (0.8 g initial weight) rearing period stocked at 47 shrimp m^{-2} and grown for 53 days in a clear water system. [1]According to Boyd (2000).

Water parameters	Accepted Range[1]	Mean	Minimum	Maximum
Temperature (ºC)	18-33	27.05 ± 0.06	26.91	27.14
pH	6-9	7.97 ± 0.03	7.90	8.08
Dissolved oxygen (mg L^{-1})	2.5-10	5.81 ± 0.08	5.65	5.98
Salinity (g L^{-1})	1-50	37.28 ± 0.28	36.83	37.62
Ammonia (NH_3/NH_4^+, mg L^{-1})	0.1-1.0	0.44 ± 0.10	0.30	0.6
Nitrate (NO_3^-, mg L^{-1})	0.2-10	0.61 ± 0.49	0	1
Nitrite (NO_2^-, mg L^{-1})	<1.0	0.1 ± 0.00	0.10	0.10

Table 4. Three-way analysis of *Penaeus vannamei* (0.8 g initial weight) variance of diet × frequency × ration effects on weight gain of stocked at 47 shrimp m^{-2} and grown for 53 days in a clear water system.

	Sum sq.	df	Mean square	F-value	*P*-value
A: diet	0.0611	1	0.0611	0.5300	0.4753
B: frequency	1.0621	1	1.0621	9.2800	0.0077
C: ration	0.8489	1	0.8489	7.4200	0.0150
Diet × frequency	0.0003	1	0.0003	0.0000	0.9594
Diet × ration	0.0089	1	0.0089	0.0800	0.7843
Frequency × ration	0.0920	1	0.0920	0.8000	0.3831
Diet × frequency × ration	0.0636	1	0.0636	0.5600	0.4668
Residuals	1.8310	16	0.1140		

Table 5. Three-way analysis of variance of *Penaeuss vannamei* (0.8 g initial weight) diet × frequency × ration effects on the feed conversion ratio stocked at 47 shrimp m^{-2} and grown for 53 days in a clear water system.

	Sum sq.	df	Mean square	F-value	*P*-value
A: diet	0.0180	1	0.0180	2.0100	0.1752
B: frequency	0.1271	1	0.1271	14.2500	0.0017
C: ration	0.4896	1	0.4896	54.8900	0.0000
Diet × frequency	0.0284	1	0.0284	3.1800	0.0933
Diet × ration	0.0000	1	0.0000	0.0000	0.9869
Frequency × ration	0.0066	1	0.0066	0.7400	0.4033
Diet × frequency × ration	0.0186	1	0.0186	2.0800	0.1685
Residuals	0.1427	16	0.0089		

1.86 ± 0.16 when average feeding frequency was considered, when the organisms were fed twice a day, and 1.71 ± 0.18 when fed four times a day (Table 8); FCR decreased from 1.73 ± 0.08 when two doses were fed and at 80% to 1.55 ± 0.07 when fed four doses (Table 8). However, this pattern was not the same at 100% of the ration, where FCR (1.99 ± 0.13) was not significantly different ($P > 0.05$) with two times a day from that obtained with four times a day (1.87 ± 0.11).

Considering the average across two frequencies, reducing the ration size from 100 to 80% led to a lower combined FCR (1.93 ± 0.12 *vs.* 1.64 ± 0.07). The same pattern was also observed where FCR at 100% (1.99 ± 0.13) was higher at two times per day than at 80% satiation (1.73 ± 0.08) at four times per day when the ration decreased from 100 (1.87 ± 0.11) to 80% satiation (1.55 ± 0.07), which led also to a significant difference in FCR ($P < 0.05$).

Attractant type effects

No statistical differences ($P > 0.05$) were observed in growth, feed conversion, and survival between shrimp fed with the different marine hydrolysates. Nevertheless, the consumed FHIE was significantly higher than the FHEE diet (107.01 ± 1.76 *vs.* 99.99 ± 1.76, respectively), mainly in the two-meal frequency (Fig. 2).

Table 6. Mean ± standard deviation of *Penaeus vannamei* growth, survival, and feed efficiency after 53 days of rearing fed two different diets at two feeding frequencies and two ration levels. Different letters in the same row are significantly different ($P < 0.05$); FHEE: hydrolyzed fish with external enzymes, FHIE: hydrolyzed fish with natural internal enzymes, FCR: feed conversion rate.

Diet	FHEE				FHIE			
Frequency	2		4		2		4	
Ration size	100%	80%	100%	80%	100%	80%	100%	80%
Initial weight (g shrimp^{-1})	0.80 ± 0.00	0.80 ± 0.00	0.80 ± 0.01	0.80 ± 0.001	0.80 ± 0.01	0.80 ± 0.00	0.80 ± 0.00	0.80 ± 0.00
Final weight (g shrimp^{-1})	7.17 ± 0.23	6.61 ± 0.18	6.52 ± 0.33	6.41 ± 0.37	7.21 ± 0.30	6.77 ± 0.35	6.77 ± 0.56	6.38 ± 0.13
Final biomass (g)	66.87 ± 1.41^{ab}	61.68 ± 2.85^{ab}	56.43 ± 6. 25^{a}	64.08 ± 3.69^{ab}	69.67 ± 5.56^{b}	67.70 ± 3.50^{ab}	63.14 ± 4.94^{ab}	61.70 ± 3.99^{ab}
Weight gain (g)	6.38 ± 0.32	5.81 ± 0.17	5.72 ± 0.33	5.61 ± 0.36	6.41 ± 0.29	5.97 ± 0.35	5.97 ± 0.55	5.57 ± 0.13
Consumed feed (g)	116.77 ± 4.11^{ab}	95.39 ± 1.89^{cd}	101.98 ± 12.96^{bcd}	83.83 ± 1.55^{d}	130.38 ± 1.47^{a}	105.27 ± 1.38^{abc}	106.05 ± 9.96^{abc}	86.33 ± 1.49^{d}
FCR	1.89 ± 0.05^{cd}	1.70 ± 0.03^{abc}	1.91 ± 0.16^{cd}	1.53 ± 0.09^{a}	2.07 ± 0.13^{d}	1.77 ± 0.10^{abc}	1.83 ± 0.04^{bcd}	1.57 ± 0.06^{ab}
Survival (%)	93 ± 6	93 ± 6	87 ± 12	100 ± 0	97 ± 6	100 ± 0	93 ± 6	97 ± 6

Table 7. Mean (± standard deviation SD) of juvenile *Penaeus vannamei* weight gain (g shrimp^{-1}) combined by frequencies (2 and 4 feedings) and ration sizes (80 and 100%) after 53 days of rearing fed two different diets at two frequencies and two satiation levels. Values are presented as means ± SD of three replicates for each feed-frequency-ration size combination. The frequency averages with different superscripts (uppercase per row and lowercase per column) are significantly different ($P < 0.05$).

		Ration		
	Frequency	100%	80%	Frequency mean
Combined	2 feedings	6.40 ± 0.28^{Aa}	5.89 ± 0.26^{B}	6.27 ± 0.43^{a}
	4 feedings	5.85 ± 0.43^{Ab}	5.59 ± 0.25^{B}	5.76 ± 0.38^{b}
	Ration mean	6.23 ± 0.51^{A}	5.79 ± 0.31^{B}	6.01 ± 0.36

Table 8. Mean (± standard deviation SD) feed conversion rate combined by frequencies (2 and 4 feedings) and ration sizes (80 and 100%) after 53 days of rearing juvenile *Penaeus vannamei* fed two different diets at two feeding frequencies and two satiation levels. Values for each diet-frequency-ration combination are means ± SD of three replicates. The frequency averages with different superscripts (uppercase per row and lowercase per column) are significantly different ($P < 0.05$).

		Ration		
	Frequency	100%	80%	Frequency mean
Combined	2 feeds	1.99 ± 0.13^{A}	1.73 ± 0.08^{aB}	1.86 ± 0.16
	4 feeds	1.87 ± 0.11^{A}	1.55 ± 0.07^{bB}	1.71 ± 0.18
	Ration mean	1.93 ± 0.12^{A}	1.64 ± 0.07^{B}	1.78 ± 0.19

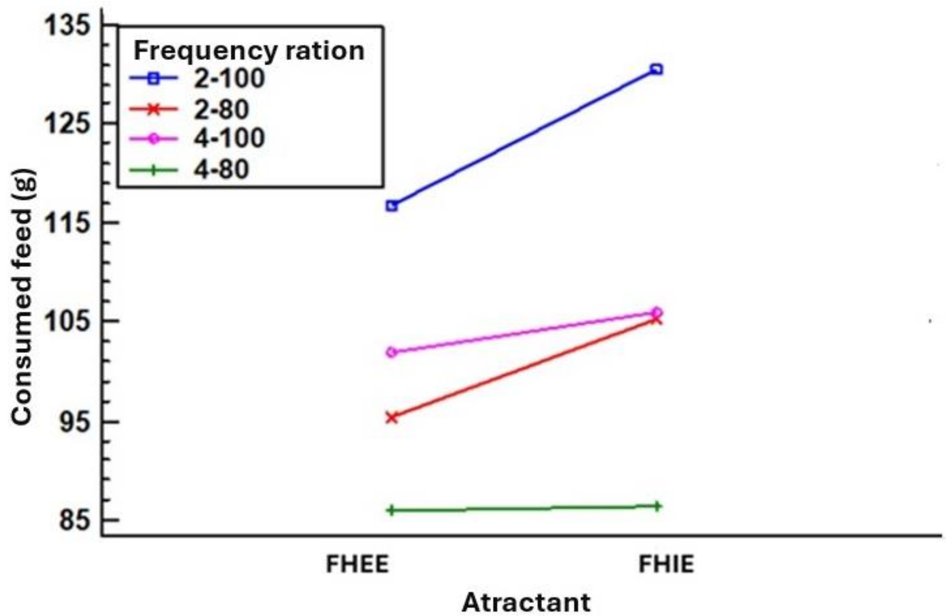

Figure 2. Attractant effect on consumed feed (g) by shrimp fed two feeding frequencies and rations. FHEE: fish hydrolysates produced via external enzymes, FHIE: fish hydrolysates produced via internal enzymes.

Additionally, regarding consumed feed, significant differences were observed ($P < 0.05$) between FHIE at two doses treatment and 100% of ration size, compared to all other treatments at 80% satiety and even compared to FHEE at four doses treatment and 100% ration size. The only exception occurred when FHIE at two doses and 100% satiety was compared with FHIE at two doses and 80% satiety, in which no significant differences were observed ($P > 0.05$), even though this treatment involved a 20% restriction.

DISCUSSION

The present study found significant effects of feeding frequency and ration restriction size on juvenile *P. vannamei* growth and FCR. Feeding shrimp four times per day, rather than twice a day, significantly improved FCR but did not enhance growth; rather, a significantly lower growth rate was observed under these conditions, including night feeding, because feed consumption was reduced. These findings reveal a notable impact of feeding and ration size schedules on production efficiency during shrimp rearing, highlighting the need to understand this correlation when planning feed management schemes, as noted by Arnold et al. (2016).

Feeding frequency

Under the present study conditions, our results clearly show that increasing feeding frequency during night hours does not improve growth performance.

The higher weight gain observed with two daily feedings compared to four is likely explained by the feeding schedule rather than the number of meals. In our study, the two meals were offered at times coinciding with periods of higher feeding activity in *P. vannamei*, thereby optimizing feed intake and utilization. In contrast, when feedings were distributed into four events, part of the ration was supplied during less favorable periods, which may have reduced feeding efficiency, highlighting the importance of synchronizing feeding schedules with the shrimp's natural activity rhythms, rather than simply increasing feeding frequency.

The highest weight gain was observed when feed was administered during daylight hours (10:00 and 16:00 h). In contrast, no additional benefit was observed when part of the ration was distributed during night hours (22:00 and 04:00 h).

Findings indicate that *P. vannamei*, as observed in early studies by Reymond & Lagardère (1990) and Nunes et al. (1996), exhibit feeding behaviors that differ from those of other penaeid shrimps. While most grooved penaeids are typically nocturnal and burrow during the night (Hindley, 1975), *P. vannamei* displays a more flexible activity pattern. Recent studies indicate that while *P. vannamei* exhibits a relatively uniform distribution and exploration during nighttime; their feeding behavior does not necessarily intensify at night. Instead, marked peaks of feeding activity occur during daylight hours immediately after feed is provided, especially under regimes with multiple daily rations, when shrimp rapidly congregate in feeding areas (Hindley 1975, Darodes de Tailly et al. 2025).

P. vannamei showed to be more active in feeding during daylight hours as reported by Pontes et al. (2006) and Nunes et al. (2019). In terms of FCR, more feedings per day resulted in more efficient feed utilization by shrimp, as reported by Aalimahmoudi et al. (2016), as evidenced by lower feed conversion values.

Growth improvement with two feed doses instead of four could be explained by enzyme activity profiles throughout the day, suggesting an oscillatory pattern with feeding peaks at times when maximum feeding activity occurs. In crustaceans, certain biological phenomena have been observed to occur rhythmically around the same time (Casillas-Hernández et al. 2006). Circadian rhythms affect enzymatic activity, which depends on several exogenous factors, such as age, size, protein sources, and molting and endogenous causes (such as ontogenics, metabolic rates, and circadian rhythms) (Lemos et al. 2000, Molina et al. 2000). The results of the present study suggest that circadian rhythms might underlie variations in performance at different frequencies.

Casillas-Hernández et al. (2006) studied the enzymatic profile of juvenile *P. vannamei* under continuous feeding and found fluctuations in proteolytic activity. In an experiment with juvenile *P. vannamei*, a biphasic circadian rhythm was observed, with diurnal and nocturnal enzymatic peak activities at 10:00 and 20:00 h, respectively. Moreover, as observed, feeding 2 h before these peaks (08:00 and 18:00 h) resulted in significantly higher growth rate, final size, survival, and biomass. These findings suggest that there are periods throughout the day during which feeding effort should be concentrated, and that the specific hours depend on *P. vannamei's* physiological status. The results also confirm synchrony between feeding activity and feed use.

The biphasic circadian rhythm might explain why no effect on growth was observed when feeding frequency was increased at night. In the present study, shrimp fed two times per day had higher weight gain than those fed four times per day at 100% of the ration size (Table 7), which is also in agreement with Pontes et al. (2008), who reported that increased temporal space between feeding stimulates searching for and ingesting feed.

Moreover, regarding the feeding schedule, Pontes & Arruda (2005) studied *P. vannamei* feeding behavior as a function of artificial light and dark photoperiods and observed that feeding time was higher during the light phase, while swimming occurred mostly during the dark phase. Along the same lines, Sick et al. (1973) reported that the ingestion rate was proportional to light intensity and inversely related to the time the feed was immersed in water in juvenile *Penaeus setiferus*. Furthermore, Robertson et al (1993) reported that day feedings produce more growth in juvenile *P. vannamei* held in pond enclosures than those at night feedings. Seemingly, *P. vannamei* are inherently more active during the day than at night.

More recently, Reis et al. (2021) reported that, across outdoor and indoor systems, *P. vannamei* does not appear to have a specific feeding schedule preference as long as environmental conditions and overall feeding rates are appropriate. However, night feeding may be less efficient than day feeding, mainly due to limitations on dissolved oxygen. During the night, biological oxygen demand increases due to feeding activity, while oxygen levels naturally drop. It increases the risk of oxygen depletion, which may require adjustments such as reduced feed rations or more frequent use of mechanical aerators, thereby increasing electrical costs. In terms of growth, shrimp fed exclusively at night or on a 24-h schedule showed slightly slower growth and lower average final weights than day feeders or those under the AQ1 (acoustic, on-demand) feeding system (Reis et al. 2021).

In brief, an oscillating pattern in feeding habits suggests that an increase in feeding frequency should be based on natural behavior and physiological status, with higher activity during daytime hours. Our findings show that growth can be sustained by reducing feeding frequency when excess feed is provided to meet nutritional requirements. However, it is important to note that this approach comes with a trade-off: feeding excess -even marginal- compromises feed efficiency (Arnold et al. 2016).

In terms of FCR, feeding shrimp four times daily resulted in a more efficient feed utilization (1.71 ± 0.18) compared to feeding twice daily (1.86 ± 0.16). These findings align with those of Aalimahmoudi et al. (2016), who also observed improved FCR with increased feeding frequency. However, this improved efficiency did not translate into higher growth under our experimental conditions, suggesting that the benefits of increased feeding frequency are context-dependent and should be evaluated alongside environmental and operational factors.

Ration size

The present study demonstrates a detrimental effect of a restricted ration (20% below satiation) in juvenile *P. vannamei*, even when feeding frequency increased from 2 to 4 times per day, resulting in reduced feed consumption. Feeding at apparent satiety -as demonstrated in the present study- led to improved growth, since shrimp can consume feed, allowing them to meet their nutritional requirements. Additionally, on-demand feeding reduces the stress associated with limited feeding. In this strategy, shrimp are less likely to compete for feed or exhibit aggressive behavior. Reduced stress levels can contribute to better overall health and increased disease resistance.

On the other hand, reducing the ration size to 80% improves feed efficiency, which was consistent across both diets. Restrictive feeding maximizes feed utilization, though it comes at the expense of overall weight gain. Increased feed conversion ratios observed in the present study, such as increased growth rate, indicate that the feeding input plateau was likely approached (Weldon et al. 2021).

When the maximum weight gain is reached, an inflection point occurs at 101% of the feeding rate; beyond this point, growth and feed utilization decrease rapidly, resulting in a higher FCR and diminished growth gains. Therefore, aiming for maximum gain is counteracted by the elevated FCR (Weldon et al. 2021). Our results are consistent with those of Arnold et al. (2016), who reported similar trends in FCR and growth when ration size was restricted. In this study, Arnold et al. (2016) reported a decrease in FCR when the ration was reduced from 100% satiation (1.52 ± 0.05) to 80% satiation (1.26 ± 0.04). In agreement with our results, the present study also found a reduction in FCR from 1.93 ± 0.12 at 100% satiation to 1.64 ± 0.07 at 80% ration size.

The effect of reducing FCR as ration size decreases contrasts with that reported by Venero et al. (2007), where feeding at 100 and 75% led to significantly different yields for the two satiation levels: 6482 kg ha^{-1} at 100% *vs*. 5054 kg ha^{-1} at 75%, respectively, but similar to FCRs for both treatments. On the other hand, the results obtained in the present study are very similar to those reported by Nunes et al. (2018), observing that feeding shrimp 4.5% of biomass with 0% restriction, against 3.4% of biomass with 25% restriction, resulted in improved growth performance with higher but still acceptable FCRs. Nunes et al. (2006) found no differences between apparent satiation and 25% restriction, suggesting the possibility of moderately reducing daily feeding without detrimental effects on growth. In contrast, in the present study, an adverse effect on growth performance was observed at 20% feed restriction.

The findings of the present study suggest that a restricted feeding scheme reduced growth rate by limiting the amount of feed available to shrimp. With this scheme, shrimp growth may be compromised. A restrictive feeding scheme might create a competitive feeding environment, leading to increased stress, aggression, and dominance behavior among shrimp. Stress can negatively affect their overall health and well-being. Moreover, restrictive feeding might lead to variations in individual growth rates within a population. Some shrimp may consume more feed than others due to dominant behavior (Luan et al. 2020, Bardera et al. 2021, Wilke et al. 2025), leading to size disparities and uneven growth distribution within the group.

Restrictive feeding schemes may also fail to provide all the essential nutrients required for optimal shrimp growth and health, thereby affecting various physiological functions. Efficient feeding practices ensure the correct amount of feed is provided. However, overfeeding should be avoided; thus, the release of excess nutrients into the water is important. By providing the precise feed quantity, farmers can minimize nutrient and organic matter release, thereby reducing the environmental impact of shrimp farming (Boyd et al. 2017, Weldon et al. 2021).

Type of diet

No significant differences were observed using either fish processed with internal (FHIE) or external enzymes (FHEE) in terms of growth and survival. Still, hydrolysates may enhance aquafeed palatability even though a variety of studies suggest that peptides with lower molecular weights facilitate easier absorption (Carvalho et al. 2004), since the hydrolysis process releases smaller peptides that can stimulate feeding response and improve feed intake.

Feed consumption was consistently higher in shrimp fed the FHIE diet, particularly at 100% ration size and two feedings per day (130.38 ± 1.47 g for FHIE *vs.* 116.77 ± 4.11 g for FHEE), which suggests that the FHIE hydrolysate may enhance feed palatability, even though this did not translate into significant differences in growth or FCR under the present study conditions.

Hydrolysates with a suitable peptide distribution in the 500-1,000 Da range appear to be effective in increasing feed palatability. Studies have shown that smaller peptides improve absorption efficiency and stimulate feeding responses. For example, Carvalho et al. (2004) found that low-molecular-weight peptides enhance nutrient utilization in fish larvae. Similarly, Kristinsson & Rasco (2000) reported that hydrolysates with targeted peptide sizes improve palatability and functional properties. Aksnes et al. (2006) also demonstrated the role of size-fractionated fish hydrolysates in enhancing feed intake and efficiency in aquaculture diets. Finally, Hou et al. (2017) further highlighted that peptides within this molecular range are bioactive and functional, which may contribute to their effectiveness as feed attractants.

Even though in the present study, no effect on growth or survival is observed when FHIE is compared with FHEE. Feed consumption seems to be different for diets with two hydrolysates with an average of 99.5 g for FHEE and 107 g for FHIE, which is manifested in homologous diets, 117 *vs.* 130 (2/100%), 95 *vs.* 105 (2/80%), and 102 *vs.* 106 (4/100%), except for 4/80%, which does not have an effect into growth or feed conversion. Further research is required to evaluate different peptide distributions in hydrolysates, as well as a narrower range of ration levels (100 and 90%) in combination with a wider feeding frequency series (2, 4, 8, 16 times a day), followed by confirmation of the findings in commercial scenarios.

Optimal feeding strategies are essential to minimize feed wastage while ensuring adequate nutrient intake. Studies have shown that improper feed management can increase production costs and environmental impact by causing uneaten feed to accumulate. Summarizing, the three aspects studied: type of feed, feeding frequency, and ration size have significant implications on the biological performance of *P. vannamei* and the final feed cost. In our study, twice-daily feeding resulted in higher growth, while four daily feedings improved feed efficiency (FCR). From an economic perspective, these differences can translate into variations in management decisions and operational costs, such as feed use and labor in farms. The existing literature suggests that proper management of these variables can reduce costs associated with feed waste and energy use during nocturnal feeding, especially when consumption is lower (Reis et al. 2021). Thus, our recommendations highlight the importance of evaluating the relationship between operational cost and biological performance to design more profitable and sustainable feeding strategies.

While hydrolysates can add to formulation costs, their ability to enhance feed intake and nutrient absorption may improve overall profitability by reducing the total feed required per unit of shrimp biomass produced (Hlordzi et al. 2022).

CONCLUSION

The present study demonstrated that the feed efficiency benefits of restricted rations are achieved, but at the cost of reduced growth, even when feeding frequency was increased. The main outcomes highlight the significance of the daylight feeding schedule and indicate that *P. vannamei* shows no beneficial effect of restricting or increasing feeding frequency at night hours, and that the positive effects of daylight feeding occur at hours close to juvenile *P. vannamei*'s reported maximum enzymatic activities. The findings of the present research suggest that, under laboratory conditions, feeding at a higher level provides the most advantageous option for optimizing production performance. No significant differences in growth or FCR were observed between diets supplemented with fish hydrolysates produced with internal or external enzymes. However, FHIE showed slightly higher feed intake, suggesting enhanced palatability.

Credit author contribution

M. Espinoza-Ortega: conceptualization, methodology, formal analysis, writing and original draft, project administration, supervision, data curation, review and editing; C. Molina-Poveda: formal analysis, review and editing; R. Civera-Cerecedo: conceptualization, methodology, formal analysis; project administration, supervision, review and editing; M. Jover-Cerdá: conceptualization, methodology, formal analysis, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

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

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