Effect of dietary ion supplementation on the survival and growth of *Penaeus vannamei* during the pre-nursery stage in well water

Efecto de la suplementación dietaria con iones sobre el crecimiento y supervivencia de *Penaeus vannamei* en precría con agua de pozo

Efeito da suplementação dietética com íons sobre o crescimento e a sobrevivência de *Penaeus vannamei* em pré-cria com água de poço´

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Abstract

Aquaculture has become an essential activity to ensure global food security, with white shrimp (*Penaeus vannamei*) standing out as one of the most important species due to its high demand and adaptability to diverse environments. This study aimed to evaluate the effect of dietary ion supplementation on the survival, growth and feed efficiency of *P. vannamei* during the pre-nursery stage using well water. The experiment included a control treatment (T0) with seawater (34 ppt) and no added ions, and three treatments using well water and ion-supplemented diets: T1 (0.1 mg Ca^{2+}, 1.2 mg Mg^{2+}, 0.4 mg K^{+}), T2 (0.2 mg Ca^{2+}, 2.2 mg Mg^{2+}, 0.8 mg K^{+}), and T3 (0.4 mg Ca^{2+}, 4.2 mg Mg^{2+}, 1.4 mg K^{+}). Each treatment consisted of three replicates, with 50 post-larvae per tank, over a 28-day period. Statistically significant differences ($p<0.05$) were observed in survival, growth and feed conversion ratio (FCR). The highest growth (1.03 %) was recorded in T0, followed by T2 (0.91 %) and T3 (0.83 %), while T1 showed the lowest growth (0.68 %) and the best FCR (1.027). Treatment T3 showed a favorable balance between growth and survival (94.5 %) with a competitive FCR (1.091). It is concluded that dietary ion supplementation improves the zootechnical performance of *P. vannamei* cultured in well water, and that appropriate adjustment of Ca^{2+}, Mg^{2+} and K^{+} concentrations in feed can optimize both survival and feed efficiency under low-salinity conditions.

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Resumen

La acuicultura se ha convertido en una actividad esencial para garantizar la seguridad alimentaria global, siendo el camarón blanco (*Penaeus vannamei*) una de las especies más importantes por su alta demanda y capacidad de adaptación a diversos entornos. Este estudio tuvo como objetivo evaluar el efecto de la suplementación dietaria con iones sobre la supervivencia, crecimiento y la eficiencia alimenticia de *P. vannamei* durante la etapa de precría utilizando agua de pozo. El experimento incluyó un tratamiento control (T0) con agua de mar (34 ppt) sin adición de iones, y tres tratamientos con agua de pozo y dietas suplementadas: T1 (0,1 mg Ca^{2+}, 1,2 mg Mg^{2+}, 0,4 mg K^{+}), T2 (0,2 mg Ca^{2+}, 2,2 mg Mg^{2+}, 0,8 mg K^{+}) y T3 (0,4 mg Ca^{2+}, 4,2 mg Mg^{2+}, 1,4 mg K^{+}). Cada tratamiento tuvo tres repeticiones con 50 postlarvas por tanque, durante un periodo de 28 días. Se detectaron diferencias significativas ($p<0,05$) en supervivencia, crecimiento y factor de conversión alimenticia (FCA). El mayor crecimiento (1,03 %) se registró en T0, seguido de T2 (0,91 %) y T3 (0,83 %), mientras que T1 presentó el menor crecimiento (0,68 %) y mejor FCA (1,027). Sin embargo, el tratamiento T3 alcanzó un equilibrio favorable entre crecimiento y supervivencia (94,5 %), con una eficiencia alimenticia competitiva (1,091). Se concluye que la suplementación iónica en dietas permite mejorar el rendimiento zootécnico de *P. vannamei* cultivado en agua de pozo, y que el ajuste adecuado de las concentraciones de Ca^{2+}, Mg^{2+} y K^{+} en el alimento puede optimizar tanto la supervivencia como la conversión alimenticia, especialmente en condiciones de baja salinidad.

Palabras clave: supervivencia, conversión alimenticia, suplementación mineral, postlarvas, baja salinidad.

Resumo

A aquicultura tornou-se uma atividade essencial para garantir a segurança alimentar global, destacando-se o camarão-branco (*Penaeus vannamei*) como uma das espécies mais importantes devido à sua alta demanda e adaptabilidade a diversos ambientes. Este estudo teve como objetivo avaliar o efeito da suplementação dietética com íons sobre na sobrevivência, crescimento e na eficiência alimentar de *P. vannamei* durante a fase de pré-cria utilizando água de poço. O experimento incluiu um tratamento controle (T0) com água do mar (34 ppt) sem adição de íons, e três tratamentos com água de poço e dietas suplementadas com íons: T1 (0,1 mg Ca^{2+}, 1,2 mg Mg^{2+}, 0,4 mg K^{+}), T2 (0,2 mg Ca^{2+}, 2,2 mg Mg^{2+}, 0,8 mg K^{+}) e T3 (0,4 mg Ca^{2+}, 4,2 mg Mg^{2+}, 1,4 mg K^{+}). Cada tratamento teve três repetições com 50 pós-larvas por tanque, durante um período de 28 dias. Diferenças significativas ($p<0.05$) foram observadas na sobrevivência, crescimento e na taxa de conversão alimentar (TCA). O maior crescimento (1,03 %) foi registrado no T0, seguido de T2 (0,91 %) e T3 (0,83 %), enquanto o T1 apresentou o menor crescimento (0,68 %), e a melhor TCA (1,027). O tratamento T3 demonstrou um equilíbrio favorável entre crescimento e sobrevivência (94,5 %), com uma TCA competitiva (1,091). Conclui-se que a suplementação dietética com íons melhora o desempenho zootécnico de *P. vannamei* cultivado em água de poço, e que o ajuste adequado das concentrações de Ca^{2+}, Mg^{2+} e K^{+} na ração pode otimizar tanto a sobrevivência quanto a eficiência alimentar em condições de baixa salinidade.

Palavras-chave: sobrevivência, conversão alimentar, suplementação mineral, pós-larvas, baixa salinidade.

Introduction

Shrimp aquaculture has emerged as one of the fastest-growing sectors in global aquaculture, with *Penaeus vannamei* (Pacific white shrimp) dominating the market due to its rapid growth, high survival rates, and high salinity tolerance (Morales-Cristobal *et al*., 2022; Campa-Córdova *et al*., 2024). This euryhaline species can thrive in environments ranging from marine waters to near-freshwater conditions (0.5–45 ppt), making it suitable for inland farming systems where access to seawater is limited (Saraswathy *et al*., 2020). The expansion of inland aquaculture using well water offers a promising alternative to traditional coastal systems, helping to reduce the incidence of diseases such as white spot syndrome virus (WSSV), Taura syndrome virus (TSV), and infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Roy *et al*., 2010; Ordoñez-Iglesias *et al*., 2024).

Despite its adaptability, culturing *P. vannamei* in low-salinity or well water presents several physiological challenges, particularly concerning osmoregulation. Shrimp expend considerable energy to maintain osmotic homeostasis when exposed to ionic compositions that differ from their natural habitat. This often results in impaired growth, reduced feed intake, and increased mortality (Huong *et al*., 2010; Saraswathy *et al*., 2020). Ionic imbalances, especially deficiencies in calcium (Ca^{2+}), magnesium (Mg^{2+}), and potassium (K^{+}), have been identified as critical limiting factors in low-salinity shrimp culture systems (Gil-Núñez *et al*., 2020; Valenzuela-Madrigal *et al*., 2017).

The composition of inland waters, particularly well water, often lacks the appropriate ionic ratios essential for optimal shrimp performance (Gil-Núñez *et al*., 2020; Song *et al*., 2025). Studies show that suboptimal concentrations of macrominerals not only affect osmoregulation but also compromise immune responses, nutrient absorption, and muscle function (Valenzuela-Madrigal *et al*., 2017; Saraswathy *et al*., 2020). To mitigate these effects, dietary supplementation with essential ions has been proposed as a viable strategy to support physiological homeostasis in low-salinity environments (Li and Liu, 2017).

Recent research has shown promising results from supplementing shrimp diets with Ca^{2+}, Mg^{2+}, and K^{+} to enhance performance in ion-deficient systems. For instance, Ordoñez-Iglesias *et al*. (2024) reported significant improvements in survival, gut integrity, lipid reserves, and molting frequency in *P. vannamei* postlarvae reared in well water supplemented with these ions. The most favorable outcomes were observed in the treatment containing 0.4 $mg.L^{-1}$ of Ca^{2+}, 4.2 $mg.L^{-1}$ of Mg^{2+}, and 1.4 $mg.L^{-1}$ of K^{+}, which also showed reduced stress markers such as necrosis and expanded chromatophores.

Understanding the osmoregulatory physiology of *P. vannamei* is essential for developing effective ion supplementation strategies. This species exhibits strong hyperosmotic regulatory capacity, maintaining serum osmolality within a narrow range even when ambient salinity drops drastically (Saraswathy *et al*., 2020). The Na^{+}/K^{+}-ATPase enzyme plays an essential role in this process by actively transporting ions across gill membranes, sustaining the ionic gradients required for homeostasis (Lucu and Towle, 2003). In parallel, dietary formulation also influences shrimp performance under ionic stress.

Gil-Núñez *et al*. (2020) examined the effect of varying protein sources and levels on *P. vannamei* reared at 3 ppt of salinity, finding that fish meal (FM) based diets outperformed soy meal (SM) based alternatives in terms of growth rate, protein efficiency, and survival.

These diets also contained higher levels of ash, calcium, iodine, phosphorus, and sodium, further supporting the idea that mineral-rich feeds can enhance shrimp tolerance to ionic fluctuations. Moreover, experimental data from Saraswathy *et al*. (2020) and Méndez-Martínez *et al*. (2021), demonstrated that *P. vannamei* maintained high survival and stable serum osmolality under both gradual and abrupt salinity reductions. Notably, survival was 100 % during gradual reductions down to freshwater and 95 % under abrupt reductions to 5 ppt.

Integrating mineral supplementation into shrimp diets is not only a physiological necessity but also an economically strategic approach. In systems where direct manipulation of water chemistry is not feasible, feed-based supplementation offers a practical and scalable solution (Rubio *et al*., 2012). Furthermore, designing species- and system-specific diets can increase feed efficiency, reduce environmental discharge, and enhance overall sustainability (Méndez-Martínez *et al.*, 2021; Song *et al*., 2025).

The growing interest in optimizing inland aquaculture practices has spurred investigations into the ideal ionic compositions and supplementation methods for low-salinity systems. Several studies recommend maintaining ionic ratios close to seawater to reduce energy expenditure on osmoregulation (Gil-Núñez *et al*., 2020; Saraswathy *et al*., 2020). However, the availability of mineral resources, cost, and digestibility must also be considered when implementing such strategies.

Given this context, the present study aims to evaluate the effect of dietary ion supplementation, specifically Ca^{2+}, Mg^{2+}, and K^+, on the growth performance and survival of *Penaeus vannamei* during the pre-rearing stage in well water. This developmental phase is particularly sensitive, as postlarvae transition from hatchery to grow-out environments, where nutritional and environmental factors critically influence survival and long-term performance (Lucu and Towle, 2003). The findings will contribute to the expanding body of knowledge on functional feed development and water quality management for sustainable shrimp aquaculture.

Materials and methods

Experimental Location and Design

The study was conducted in October 2023 at the Aquaculture Laboratory of the Universidad Técnica Estatal de Quevedo (UTEQ), located in Quevedo, Los Ríos, Ecuador, at an altitude of 73 meters above sea level. The geographical coordinates are 01°06'13" S latitude and 79°29'22" W longitude.

A completely randomized design (CRD) was used, consisting of four treatments: one control (T0) and three levels of ionic supplementation (T1, T2, and T3), each with three replicates. Experimental units consisted of 40-L plastic tanks, each stocked with 50 postlarvae, for a total of 600 shrimp. The experimental period lasted 28 days. Table 1 shows the concentrations of supplemented ions for each treatment. The control treatment (T0) was maintained with seawater at 34 ppt, while the other treatments used freshwater from a well.

Table 1. Ion concentrations used in each treatment.

Treatment	Ca^{2+} (mg.L^{-1})	Mg^{2+} (mg.L^{-1})	K^+ (mg.L^{-1})
T0 (control)	0.0	0.0	0.0
T1	0.1	1.2	0.4
T2	0.2	2.2	0.8
T3	0.4	4.2	1.4

Experimental Design

Postlarvae of *Penaeus vannamei*, 12 days old, were collecteed from the Biogemar S.A. hatchery, located in the province of Santa Elena, Ecuador, and transported by land in plastic bags to the experimental site. Upon arrival, shrimp were gradually acclimated by mixing the transport water with the tank water, reducing salinity at a rate of 1 ppt per hour until reaching 0 ppt, to avoid mortality due to osmotic shock.

As shown in table 2, the well water used in the treatments was previously analyzed for quality, measuring the concentrations of cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) and anions (carbonates, bicarbonates, sulfates, and chlorides) (Federation and Aph Association, 2005). Water quality parameters were monitored daily using a CCOWAY multiparameter sound, and an ATC refractometer.

Table 2. Physicochemical analysis of well water.

Parameter (mg.L^{-1})	Level
Ca^{2+}	5.24
Mg^{2+}	1.94
Na^+	8.09
K^+	2.63
CO_3	0.02
HCO_3	17.08
Cl	17.50
SO_4	2.47

Cations and anions studied in the well water used for the experiment.

Weekly measurements of Ca^{2+}, Mg^{2+}, and K^+ concentrations in each tank, were performed using colorimetric titration with a Monitor-brand water testing kit, and values were expressed in ppm (mg.L^{-1}).

Ionic Supplementation in the Feed

Three experimental diets were formulated by adding varying concentrations of Ca^{2+}, Mg^{2+}, and K^+ by spraying them directly onto the commercial feed Nicovita (0.8 mm pellet size)(table 3), based on the calculated requirements to simulate seawater-like conditions, followed by drying the feed in the shade at 26 °C for 4 hours. The ionic solutions were prepared by mixing liquid forms of the ions, and no binders were required, as the mixture did not leach into the water during feeding. Feed was offered four times per day at 8:00 a.m., 11:00 a.m., 2:00 p.m., and 5:00 p.m.

Table 3. Nutritional composition of the Nicovita 0.8 mm commercial feed used in the experiment.

Component	Value
Crude protein	35 %
Crude fat	5 %
Moisture	12 %
Ash	8 %
Crude fiber	13%
Calcium Carbonate	1.50%
Sodium Chloride	1.40%
Potassium Phosphate	0.10%
Sodium Phosphate	0.10%
Vitamin and Mineral Premix (Copper, Zinc, Magnesium, Iodine, Selenium, Iron, Vitamin A, Vitamin D_3, Vitamin E)	0.30%

The requirement for these cations and anions decreases if salinity decreases (for example, due to dilution with rainwater or freshwater input), and the concentration of ions (cations and anions) decreases. This is because the water becomes diluted, and although the types of ions present may remain the same, their quantity per liter of water is lower.

Survival and growth

During the 28-day trial, shrimp growth was recorded every 7 days, measuring weight with an analytical balance and length with a millimeter adhesive ruler. Data collection and management were facilitated using the Larvia mobile application. Additional performance indicators, including survival rate and feed conversion ratio (FCR), were also evaluated to assess the effects of Ca^{2+}, Mg^{2+} and K^{+} levels on shrimp development and productivity.

The following formulas were used (Jaffer *et al*., 2019):

Specitical Growth Rate (SGR, %/day) = [(Final weight (g) − Initial weight (g)) / Days] × 100

Survival Rate (%) = (Final number of shrimp / Initial number of shrimp) × 100

Feed Conversion Ratio (FCR) = Feed supplied (g) / Biomass gained (g)

Statistical Analysis

To determine significant differences between treatments regarding growth parameters and water quality an analysis of variance (ANOVA) was applied to the collected data. Prior to ANOVA, data were tested for normality (Shapiro Wilks) and homogeneity of variances (Levene) (Lucu and Towle, 2003). When significant differences were found (p< .05), Tukey's post hoc test was used to identify specific differences between means. Software STATISTICA 10.0 (StatSoft, Inc., USA) was used. A significance level of 5 % is reported. The linear model used was as follows:

$$Y_{ij} = \boldsymbol{\mu} + \boldsymbol{\tau_i} + \boldsymbol{\varepsilon_{ij}}$$

Where:

Y_{ij}: Observation corresponding to treatment i and repetition j

μ: Overall average of all observations

τ_i: Effect of treatment i (difference between the mean of treatment i and the overall mean)

ε_{ij}: Random error associated with observation ij, it is assumed that $\varepsilon_{ij} \sim N(0, \sigma^2)$.

Results and discussion

Physicochemical parameters in water

Physicochemical water parameters are critical determinants during the nursery phase of whiteleg shrimp (*Penaeus vannamei*), particularly when well water is used as the culture medium. In this study, temperature remained stable across treatments with no significant differences (p>0.05), and values ranged from 25.04 to 25.53 °C (table 4). These findings align with those reported by Castillo and Velásquez (2021), who stated that the optimal temperature range for this species lies between 25 and 32 °C. Thermal stability favours metabolism and growth, and prevents hepatopancreatic dysfunction, as observed at temperatures below 20 °C (Wang *et al*., 2019).

Regarding salinity, highly significant differences (F=3.20; p<0.05) were observed between the control treatment (T0) and those supplemented with ions. T0 maintained a constant salinity of 35.5 ± 0.00 ppt, while treatments T1, T2, and T3 ranged from 2.50 ppt. Although these values are markedly lower than typical seawater salinity, they showed a slight increase proportional to the dose of supplemented ions. This pattern suggests a possible ionic release from the feed into the water column, as proposed by Ordoñez-Iglesias *et al*. (2024), without producing a measurable impact on total salinity. Previous studies have shown that *P. vannamei* can tolerate a broad salinity range (0.5 to 40 ppt). However, under reduced salinity conditions, adequate ionic support is necessary to avoid physiological impairment (Saraswathy *et al*., 2020; Amir *et al*., 2021).

In terms of pH, significant differences (F=2.25; p<0.05) were detected, ranging from 7.97 in T0 to 8.5 in T1. All treatments remained within *P. vannamei* optimal range of 6.5 to 9.0 (Furtado *et al*., 2011). The higher pH values in the ion-supplemented treatments may be attributed to the slightly alkaline nature of the mineral compounds used, such as calcium carbonate and sodium bicarbonate, as reported by Furtado *et al*. (2011). This alkalinity can enhance enzymatic activity and reduce ammonia toxicity.

About dissolved oxygen, no significant differences (F=0.83; p>0.05) were detected between treatments, ranging from 3.57 to 4.67 $mg.L^{-1}$. Although these values are slightly below the optimal range of 4.0–6.6 $mg.L^{-1}$ proposed by Galkanda-Arachchige *et al*. (2020), we suggest they were sufficient to maintain essential physiological functions. Nevertheless, levels near the lower threshold may represent a risk under conditions of higher stocking density or organic load. In this context, adequate aeration and continuous monitoring are essential to ensure system stability. Overall, the physicochemical parameters observed may support the feasibility of using diets supplemented with Ca^{2+}, Mg^{2+} and K^{+} as a strategy to improve environmental quality in low-salinity systems, contributing to ionic homeostasis and improved shrimp performance (Furtado *et al*., 2011).

Highly significant differences (F=3.00, F=2.95, F=3.09, p<0.05) were also observed in the concentrations of these essential ions (Ca^{2+}, Mg^{2+} and K^{+}) between the control (T0) and treatments T1, T2, and T3, suggesting a direct response to the progressive inclusion of minerals in the feed (figure 1), respectively. These results are consistent with Galkanda-Arachchige *et al*. (2020), who demonstrated that mineral supplementation increases the concentration of key ions in the culture water, thus enhancing osmotic homeostasis.

Table 4. Physicochemical characteristics of water across experimental treatments (average of daily measurements over a 28-day period).

Variable	T0	T1	T2	T3	P
Temperature (^{0}C)	25.05±0.09a	25.53±0.10a	25.46±0.4a	25.43±0.49a	0.175
Dissolved Oxygen ($mg.L^{-1}$)	4.67±0.85a	3.57±2.66a	4.39±1.65a	4.56±2.60a	0.629
pH	7.97±0.21a	8.51±0.40c	8.43±0.70bc	8.34±0.19b	0.045
Salinity (%)	35.50±0.0a	2.50±0.02c	2.5±0.0b	2.5±0.0b	0.030

Mean values ± Standard Deviation. Different letters are significantly different (p< 0.05).

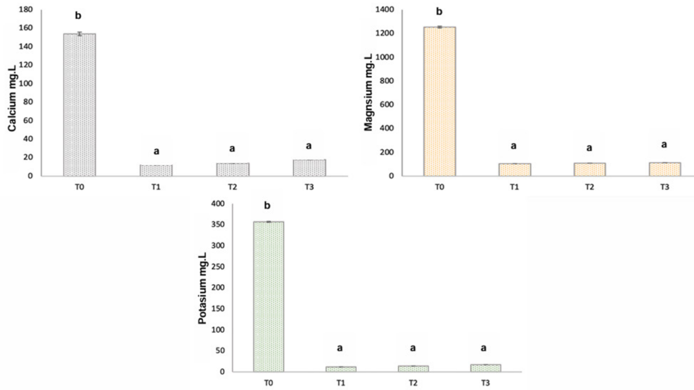

Figure 1. Ionic concentration in water across experimental treatments (average of daily measurements over a 28-day period).

In the case of calcium (Ca^{2+}), a proportional increase was observed with each supplementation level. This is particularly relevant given its structural role in exoskeleton formation and its involvement in physiological processes such as muscle contraction and hemolymph coagulation (Valenzuela-Madrigal *et al*., 2017). Similar results were reported by Amir *et al*. (2021), who observed significant improvements in survival and weight gain in shrimp cultured in brackish water following dietary Ca^{2+} supplementation. Likewise, the work of Ordoñez-Iglesias *et al*. (2024) under similar conditions demonstrated that supplementation with 0.4 $mg.L^{-1}$ of Ca^{2+} reduced necrosis incidence and supported tissue integrity.

Magnesium (Mg^{2+}) levels also increased with supplementation. This ion plays a critical role in enzymatic processes, neuromuscular regulation, and osmoregulation (Abdelrahman *et al*., 2023). Studies by Galkanda-Arachchige *et al*. (2020) suggest that increasing Mg^{2+} levels in water or feed improves weight gain and survival rates, especially in low-salinity environments. The present findings reinforce this view, as treatments with higher Mg^{2+} levels (T2 and T3) demonstrated better zootechnical performance compared to T1 and the control.

As for potassium (K^{+}), a clear upward trend was observed in the supplemented treatments, highlighting its importance in Na^{+}/K^{+}-ATPase activity, which is key to transmembrane ionic regulation (Lucu and Towle, 2003). Previous studies have linked potassium deficiencies to osmotic dysfunction, reduced appetite, and oxidative stress (Rubio *et al*., 2012; Saraswathy *et al*., 2020). This experiment validates those findings, as treatments with higher dietary K^{+} levels achieved improved survival rates and growth. Experimental evidence from Amir *et al*. (2021) further supports these outcomes, showing that increased dietary K^{+} improve feed conversion ratio (FCR) and body mass in shrimp reared in brackish systems.

Taken together, these results demonstrate that the strategic incorporation of Ca^{2+}, Mg^{2+} and K^{+} into shrimp diets cultured in well water not only improves the ionic quality of the environment but also actively contributes to the animals' physiological and health performance. This strategy is particularly relevant in contexts where direct water manipulation is limited or costly, positioning functional diets as an effective and scalable tool (Gil-Núñez *et al*., 2020).

The cations and ions present in the feed can improve the properties of well water. They can improve conductivity by increasing ionic concentrations. Excessive calcium and magnesium ions can negatively increase water hardness. They can also negatively affect the increase in ammonium and nitrite levels in the water body if biofiltration is lacking, which contrasts with the increase or decrease in pH (Saraswathy *et al*., 2020).

Zootechnical Parameters

The results of this study revealed significant differences ($p< 0.05$) among treatments in terms of survival, growth, and feed efficiency (table 5). Beginning on day seven, significant differences in length and weight, were observed between treatments, suggesting that ionic supplementation had a positive effect on somatic growth of *Penaeus vannamei* postlarvae. Treatment T1 consistently recorded the highest weight and length values, while T0 showed the lowest, suggesting that a diet without ionic supplementation was insufficient to meet the growth requirements. These findings align with those reported by Gil-Núñez *et al*. (2020), who demonstrated that supplementation with specific mineral salts can significantly improve growth performance in low-salinity systems. The consistency in standard deviations across replicates further supports the reliability of the results obtained.

Table 5. Zootechnical parameters of the beds under the experimental treatments.

Sampling	Variable	T0	T1	T2	T3	P
Initial	Weight (mg)	6.99±0.10a	6.99±0.11a	6.99±0.09a	6.99±0.12a	0.077
	Length (mm)	9.12±0.15a	9.12±0.16a	9.12±0.18a	9.12±0.16a	0.096
7 days	Weight (mg)	85.77±1.036a	99.75±2.63c	97.84±0.86c	92.48±0.46b	0.018
	Length (mm)	11.74±0.58a	13.12±0.60c	12.80±0.60b	12.32±0.45b	0.029
14 days	Weight (mg)	190.21±0.98a	202.61±2.29b	199.22±1.66b	190.59±1.26a	0.022
	Length (mm)	17.05±0.56a	18.59±0.35b	18.01±0.22b	17.37±0.37a	0.001
21 days	Weight (mg)	411.48±1.04a	478.32±2.87d	466.74±3.76c	454.32±1.91b	0.039
	Length (mm)	22.82±0.48a	23.71±1.11b	22.97±1.13a	22.62±0.83a	0.039
28 days	Weight (mg)	721.77±1.22a	795.23±9.47d	775.13±6.78c	758.70±7.34b	0.035
	Length (mm)	26.7±0.74a	27.58±2.42b	27.64±1.14b	26.95±1.11a	0.228
Specific Growth Rate		0.68±0.10[a]	1.03±0.08c	0.83±0.03b	0.83±0.02b	0.001
Survival Rate (%)		100.00±0.07c	51.33±1.20a	60.67±4.60b	94.67±1.21c	0.001
Feed Conversion Rate		1.186±0.10a	1.027±0.07b	1.074±0.04b	1.091±0.01b	0.468

Mean values ± Standard Deviation. Different letters are significantly different ($p<0.05$)..

From a physiological standpoint, the improvement in growth observed in the supplemented treatments may be attributed to enhanced osmoregulatory capacity, facilitated by the adequate availability of essential ions such as Ca^{2+}, Mg^{2+}, and K^{+}. These cations play a central role in cellular metabolism, tissue structural stability, and the molting process in crustaceans (Juniarti *et al*., 2022). Calcium, in particular, is fundamental for exoskeleton formation and ecdysis, while magnesium is involved in enzymatic functions and osmotic homeostasis (Juniarti *et al*., 2022). The importance of a balanced ionic ratio has been emphasized in multiple studies, including Galkanda-Arachchige *et al*. (2020), who reported significant improvements in weight gain and survival of *L. vannamei* when magnesium levels were adjusted in the culture medium.

On the other hand, despite the overall trend of increasing length and weight, body length did not always show significant differences at certain sampling points (e.g., day 28), which may be attributed to density-related factors or differential physiological responses to the type and concentration of supplemented ions. In this context, authors such as Amir *et al*. (2021) observed that longitudinal growth may be more strongly influenced by specific factors such as the calcium-to-phosphorus ratio or bicarbonate profile, whereas weight gain appears to respond more directly to dietary mineral supplementation. These findings reinforce the notion that growth in nursery systems under low salinity conditions is highly dependent on ionic bioavailability and well-designed nutritional strategies.

Furthermore, treatment T0, which used seawater (34 ppt), exhibited the highest specific growth rate (1.03 %), reflecting the natural ionic balance advantage provided by the marine environment ($F=2.67$; $p<0.05$). However, treatment T2, which included dietary supplementation with 0.2 $mg.L^{-1}$ of Ca^{2+}, 2.2 $mg.L^{-1}$ of Mg^{2+}, and 0.8 $mg.L^{-1}$ of K^{+}, achieved a growth rate of 0.91 %, closely approaching that of the marine control. This suggests that proper mineral formulation can partially compensate for the ionic deficiencies of well water (Amir *et al*. 2021).

These findings are consistent with Galkanda-Arachchige *et al*. (2020), who demonstrated that adequate concentrations of Mg^{2+} in low-salinity water significantly improved growth and feed conversion efficiency in shrimp. Studies as Amir *et al*. (2021), reported that dietary inclusion of mineral salts and phosphorus for *L. vannamei* reared in brackish water increased average body weight and survival while reducing FCR—observations that align with our results. Treatment T3, which received the highest supplementation levels (0.4 $mg.L^{-1}$ Ca^{2+}, 4.2 $mg.L^{-1}$ Mg^{2+} and 1.4 $mg.L^{-1}$ K^{+}), showed slightly lower growth (0.83 %) than T2 but achieved the highest survival rate (94.5 %), suggesting that this combination may modulate physiological stress-resistance mechanisms, even if it does not maximize growth.

Regarding feed conversion, T1 exhibited the best FCR (1.027), despite having the lowest growth rate (0.68 %). This outcome could be explained by a lower metabolic rate associated with reduced mineral inclusion, resulting in a decoupling between intake and biomass gain. Studies such as those by Ordoñez-Iglesias *et al*. (2024) and Valenzuela-Madrigal *et al*. (2017) have also emphasized that a well-balanced ionic profile in the diet not only improves nutrient digestibility and metabolism but also contributes to osmotic stability and the prevention of morphophysiological disorders during the nursery phase. In this context, treatment T3 represents a promising compromise between feed efficiency, growth performance, and survival, serving as a model for inland shrimp farming systems using low-ionic well water.

Conclusions

Dietary supplementation with essential ions (Ca^{2+}, Mg^{2+}, and K^{+}) in pre-nursery systems of *Penaeus vannamei* using well water significantly improves shrimp growth and survival. The treatment with the highest ionic dosage (T3) achieved a survival rate of 94.5 % and a favorable feed conversion ratio (FCR = 1.091), indicating that the addition of these minerals to the diet can compensate for the ionic deficiencies of well water. This strategy represents a viable alternative to optimize production in inland aquaculture systems with ionic limitations.

The survival of *P. vannamei* juveniles was higher in T1 being the best with 100 % because the juveniles did not go through high stress, since they were kept all the time in salt water (33 ppt), followed by T3 with 94.5 % survival thanks to the greater amount of ions available in the food, which allowed their stress to not be very high allowing the juveniles not to be greatly affected by the ionic imbalance present in the well water of the culture, thus ensuring greater survival, being the opposite in T1 and T2 that obtained survival rates of 51 % and 60 % respectively due to having lower amounts of Ca^{+2}, Mg^{+2} and K^{+} ions.

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