Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(6): 1637 – 1650 (2025)
www.ejabf.journals.ekb.eg

Optimization Potassium Enhances Osmoregulation and Growth Performance of the Pacific White Shrimp (*Litopenaeus vannamei*) in Freshwater Systems

Eulis Marlina[1*], Gregorius Nugroho Susanto[2], Abdullah Aman Damai[3], Indra Gumay Yudha[3], Qadar Hasani[3]

[1]Department of Agriculture, Faculty of Agriculture, Lampung University, Lampung, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Lampung University, Lampung, Indonesia
[3]Department of Agriculture, Faculty of Agriculture, Lampung University, Lampung, Indonesia
*Corresponding Author: eulismarlina@polinela.ac.id

ARTICLE INFO

Article History:
Received: Aug. 20, 2025
Accepted: Nov. 8, 2025
Online: Dec. 4, 2025

Keywords:
Blood glucose,
Cannibalism,
Litopenaeus vannamei
Post-larvae,
Osmoregulation,
Potassium

ABSTRACT

The role of potassium as an essential macromineral in enhancing the growth and survival of Pacific white shrimp (*Litopenaeus vannamei*, Boone 1931) in freshwater systems has been widely studied. However, its role in osmoregulation and physiological responses under freshwater conditions remains to be investigated. This study aimed to determine the optimal potassium concentration for improving growth performance, survival rate, cannibalism, osmotic gradient, and blood glucose levels in *L. vannamei*. The experiment was conducted over 56 days using post-larvae (PL 21) shrimp with an initial average length of 2.1±0.01cm and weight of 0.05 ± 0.05g. A completely randomized design (CRD) with four treatments and three replicates was employed in 12 aquaria (50 L each), and each aquarium was filled with 50 shrimps. Potassium chloride (KCl) was added at varying concentrations as follows: Treatment A (22.02 $mg{\cdot}L^{-1}$ K^+), Treatment B (44.04 $mg{\cdot}L^{-1}$ K^+), Treatment C (66.06 $mg{\cdot}L^{-1}$ K^+), and Treatment D (88.08 $mg{\cdot}L^{-1}$ K^+). The salinity was adjusted to 2 $g{\cdot}L^{-1}$ by adding sodium, magnesium, calcium, and potassium at concentrations of 608.7, 78.26, 23.18, and 22.02 $mg{\cdot}L^{-1}$, respectively. The addition of potassium to freshwater significantly affected shrimp growth, survival, feed efficiency (FE), cannibalism rate, blood glucose levels, and osmotic workload ($P<0.05$). At a potassium concentration of 66.06 $mg{\cdot}L^{-1}$, growth increased, with an average body weight (ABW) of 5.39 ± 0.346g, specific growth rate (SGR) of 5.688 ± 0.408%, feed efficiency (FE) of 72.69 ± 8.5%, and survival rate (SR) of 68.67 ± 4.16%. The osmotic gradient (GO) decreased by 0.044 ± 0.036 mOsm L^{-1} H_2O, blood glucose (BG) was 234 ± 3.8μg· g^{-1}, and cannibalism rate (CR) was 2.31 ± 2.3%. The novelty of this research lies in demonstrating the critical role of potassium in maintaining electrolyte balance and osmoregulation in shrimp. Specifically, potassium in the form of K^+ ions, supplied as potassium chloride (KCl) in the maintenance medium, can optimize nerve function and metabolism. This optimization contributes to a reduction in oxidative stress and ion imbalance, which are factors that can trigger aggressive behavior. With reduced stress, shrimp exhibit lower levels of aggression and cannibalism. Efficient metabolism and optimal ion balance allow shrimp to focus on growth and feed intake rather than engaging in cannibalistic behavior.

INTRODUCTION

The Pacific white shrimp (*Litopenaeus vannamei*, Boone) is a euryhaline osmoregulator crustacean capable of adapting to a wide range of salinities. Like other crustacean species, its life cycle is strongly influenced by salinity, which regulates the osmotic balance between intracellular and extracellular fluids **(Anggoro *et al*., 2018)**. While this species can survive in environments ranging from freshwater to seawater, the limited mineral content in freshwater systems often restricts its optimal growth and development **(Cahyanurani & Edy, 2022; Scabra *et al.*, 2023)**. Recent studies have demonstrated the feasibility of cultivating *L. vannamei* in low-salinity and even freshwater conditions **(Marlina *et al*., 2018; Ariadi *et al*., 2019)**.

A significant advantage of freshwater cultivation is the reduced risk of viral and bacterial infections that commonly occur in brackish water systems. **Febriani *et al*. (2018)** reported that *L. vannamei* reared in low-salinity environments exhibited enhanced resistance to MSSV (Monodon Slow Growth Syndrome Virus) infections. However, the balance of macrominerals, particularly sodium (Na), magnesium (Mg), calcium (Ca), and potassium (K), remains critical for shrimp growth and survival in these systems **(Boyd 2018; Widigdo *et al*., 2019; Supono *et al*., 2023)**.

These essential minerals, which are abundant in seawater and brackish water, play vital roles in osmoregulation, molting, and post-molting processes **(Widigdo *et al*., 2019; Supono *et al.*, 2023)**. In freshwater systems, mineral deficiencies can lead to physiological impairments, including muscle cramps, molting difficulties, stunted growth, increased stress, and elevated mortality rates. As **Sharma *et al*. (2023)** emphasized, minerals are crucial organic components that support metabolic functions and provide structural material for bones, teeth, and exoskeletons. Among these, potassium (K) is particularly important, as it is required in substantial quantities both in water and in feed.

Anggoro *et al*. (2018) observed that extreme salinity fluctuations induce stress in shrimp, leading to high mortality rates. Under such conditions, shrimp expend excessive energy on osmoregulation, diverting resources away from growth. The primary challenge in freshwater *L. vannamei* farming is therefore maintaining adequate macromineral levels to support normal physiological functions and growth.

Previous studies have established the importance of potassium in low-salinity and freshwater systems not only for growth and survival but also for overall shrimp health **(Roy *et al*., 2007; Kaligis., 2015; Mcgraw & Scarpa, 2016)**. However, the physiological responses to potassium deficiency particularly in terms of cannibalism and blood glucose levels as stress indicators remain insufficiently explored. This study aims to address this gap by examining how potassium supplementation in freshwater systems influences cannibalism rates and stress responses in *L. vannamei*.

Therefore, this study is designed to evaluate the role of potassium in relation to stress indicators by examining the percentage of cannibalism and blood glucose as indicators of stress due to potassium deficiency.

MATERIALS AND METHODS

Shrimp collection and experimental setup

The study utilized 12 aerated aquaria (50 L capacity each), each aquarium was filled with 50 shrimps lined with black plastic to minimize external stress. Post-larval shrimp (PL21) of *Litopenaeus vannamei* with an average length of 2.1±0.01cm and weight of 0.05±0.05g were obtained from the Marine Research Center (MRC) Laboratory, Kalianda, South Lampung, Indonesia - a Technical Research and Development unit of PT Central Proteina Prima, Indonesia (Fig. 1). The macromineral used for water modification included NaCl, MgO, $CaCO_3$, and KCl.

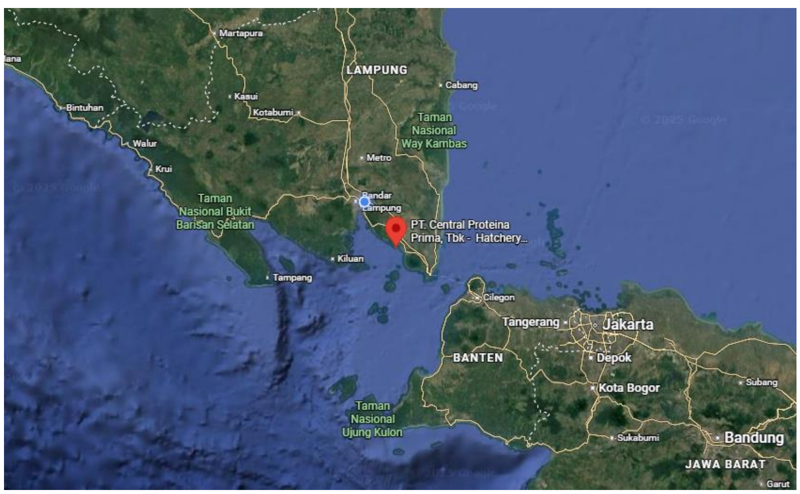

Fig. 1. Geographical location of PT Central Proteina Prima, Kalianda, South Lampung, Indonesia

Experimental design

A completely randomized design (CRD) was implemented, in which the tank served as the experimental unit. Four potassium treatment concentrations were tested, each with three tank replicates. Freshwater was adjusted to 2 $g \cdot L^{-1}$ salinity following **Boyd (2018)**, with baseline mineral concentrations of Na^+ 608.7 $mg \cdot L^{-1}$, Mg^{2+} 78.26 $mg \cdot L^{-1}$, Ca^{2+} 23.18 $mg \cdot L^{-1}$, and K^+ 22.02 $mg \cdot L^{-1}$. Water was renewed at a rate of 5–10% per day, and shrimp were fed three times daily (08:00, 14:00, and 20:00) at 3–5% of biomass using a commercial feed containing 30% crude protein. Treatments were established by supplementing potassium (K^+) at different concentrations (Table 1).

Table 1. Mineral concentrations ($mg \cdot L^{-1}$) across experimental treatments

Mineral	**Treatments**			
	A	**B**	**C**	**D**
Na	608.7	608.7	608.7	608.7
Mg	78.26	78.26	78.26	78.26
Ca	23.18	23.18	23.18	23.18
K	**22.02**	**44.04**	**66.06**	**88.08**

Growth parameters

Weekly measurements of 20 randomly selected shrimp per aquarium were conducted for 56 days. Growth metrics were calculated as follows:

The growth parameters were measured as per the methodologies described by **Huisman (1976)**, **Tacon (1987)**, **Effendi (2002)** and **Hseu *et al*. (2023)**.

Weight gain ($g \cdot shrimp^{-1}$) = final weight (g) – initial weight (g).

$$\text{Specific growth rate (SGR } \% \cdot \text{day}^{-1}) \ SGR = \frac{\text{Ln final wt} - \text{Ln initial wt}}{\text{days}} X100$$

$$\text{Survival rate (SR); } SR(\%) = \frac{\text{final live count}}{\text{initial stock}} X\,100$$

Cannibalism rate

Observations of the level of cannibalism are known from the number of dead shrimps based on the shape of the body which is no longer intact.

$$CR\ (\%) = CR = \frac{(\text{Initial count} - \text{Final live} - \text{Carcasses})}{\text{Initial count}} \times 100$$

$$\text{Feed efficiency; } FE\ (\%) = \frac{(\text{weight gain} + \text{mortality weight})}{\text{total feed given (g)}} \times 100\%$$

Physiological parameters

Physiological measurements were conducted following established protocols from **Wedemeyer and Yasutake (1977), Ferraris *et al*. (1986)** and **Anggoro (1992)**.

Blood glucose levels ($\mu g \cdot g^{-1}$) were measured from blood samples collected using a 1mL syringe containing heparin as an anticoagulant. Glucose concentrations were quantified spectrophotometrically using a Spektro UV-Vis Carry 100 instrument, with calculations performed according to the equation of **Wedemeyer and Yasutake (1977)**.

$$BG = \frac{\text{Absorben sample}}{\text{Absorben standard}} \text{xglucose standard}$$

The osmotic gradient was determined as the differential between shrimp hemolymph and media osmolarity.

$$GO\ (mOsm \cdot L^{-1}\ H_2O) = [P_{osm}\ \text{hemolymph} - P_{osm}\ \text{osmolarity media}]$$

Osmotic workload was calculated according to **Ferraris *et al.* (1986)** and **Anggoro (1992)**, representing the energy expenditure required for osmoregulation.

Statistical analysis

All data were analyzed using SPSS v29 with one-way ANOVA (α = 0.05); normality and homogeneity tests were performed beforehand, and if significantly different, Duncan's test was used (**Steel & Torrie, 1993**). The data were presented in tables and graphs. The physical and chemical parameters of the water were analyzed descriptively.

RESULTS

Growth parameters

The experiments conducted focused on optimizing potassium in freshwater media on growth performance, osmoregulation systems and physiology of Pacific shrimp (*L. vannamei*). The results showed that external environmental factors significantly influenced the growth and physiological system of shrimp (P<0.05) (Table 2).

Table 2. Observed parameters of potassium concentration treatments on Pacific white shrimp (*Litopenaeus vannamei*) reared in freshwater media

Parameter	Treatment potassium concentrations (mg·L^{-1})			
	A (22.02 mg·L^{-1})	**B (44.04 mg·L^{-1})**	**C 66.06 mg·L^{-1})**	**D 88.08 mg·L^{-1}**
ABW	1.773±0.211^{a}	1.727±0.611^{b}	5.394±0.346^{c}	4.090±0.631^{d}
SGR	3.432 ±0.595^{a}	4.642 ±0.101^{b}	5.688 ± 0.408^{c}	5.349 ±0.368^{c}
FE	44.49±2.71^{a}	62.15±10.88^{b}	72.69±1.85^{c}	63.26 ±9.71$^{\mathbf{b}}$
SR	50.67 ± 5.03^{a}	57.33±3.05^{b}	68.67±4.16^{c}	60.00±2.00^{b}
Cannibalism	45.33±3.06^{a}	18.67±4.16^{b}	2.31±2.31^{c}	4.00±0.00^{d}
Blood glucose	400±1.0^{a}	330±5.5^{b}	234±3.8^{c}	246±4.4^{c}
GO	0.356±0.027^{a}	0.140±0.051^{b}	0.044±0.036^{b}	0.121±0.015^{b}

Notes: Different superscript letters for P< 0.05.

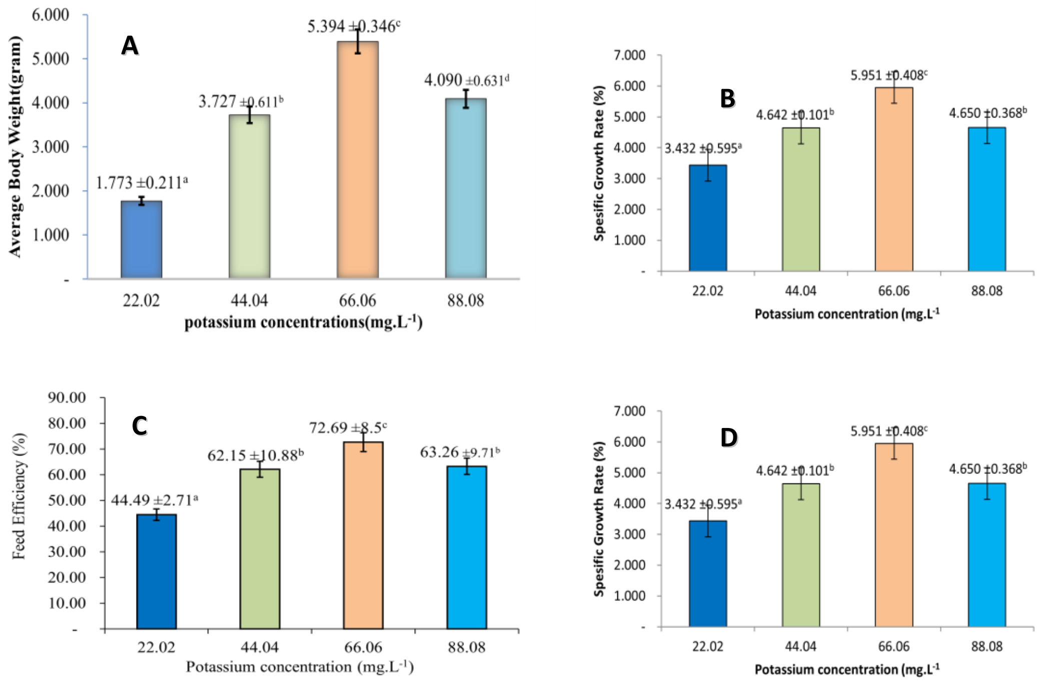

Fig. 2. Growth parameters A: ABW, B: SGR, C: FE and D: SR in each treatment

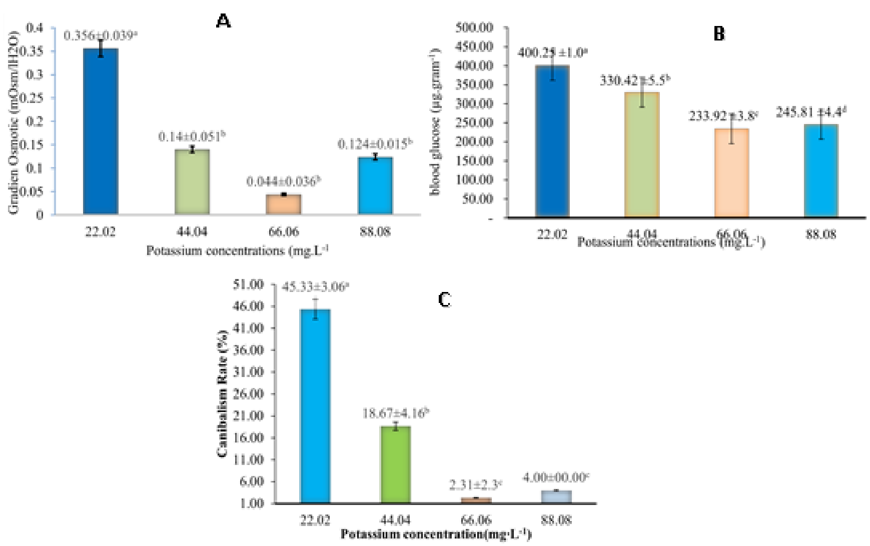

Fig. 3. Physiological parameters A (GO), B (BG), and C (CR) of vannamei shrimp in each treatment

Adding different concentrations of potassium to the water had a significant effect on the average body weight (ABW), specific growth rate (SGR), feed efficiency (FE), survival rate (SR), osmotic gradient (GO), cannibalism rate (CR), and blood glucose (BG) of Pacific shrimp ($P< 0.05$). Overall, a potassium concentration of 66.06 mg·L^{-1} was optimal, yielding the highest ABW (5.394 ± 0.346g), SGR (5.688 ± 0.408%), FE (72.69 ± 1.85%), and SR (68.67 ± 4.16%) during the 56-day cultivation period (Fig. 1 & Table 2). At the same concentration, physiological parameters were also most favorable, producing the lowest GO (0.044 ± 0.036 mOsm·L^{-1} H_2O), BG (234 ± 3.8 µg·g^{-1}), and CR (2.31 ± 2.31%) (Fig. 3 & Table 2).

Water quality was managed by removing waste and feed residues from the culture media twice daily, in the morning and evening, to maintain conditions suitable for Pacific shrimp. The results of water quality measurements over the 56-day cultivation period with the addition of macrominerals are presented in Table (3).

Table 3. Water quality parameters during maintenance

Parameter	Treatment of Potassium Concentration Addition (mg.L^{-1})				
	A (22.02)	B (44.04)	C (66.06)	D (88.08)	Pustaka
Suhu (°C)	24-30	24-29	24-29	24-29	29-30 **(Boyd, 2018)**
Ph	6.7-7.6	6.4-7.8	6.6-7.9	6.8-7.8	7-8.5 **(Supono, 2022)**
DO (mg.L^{-1})	5.6-5.8	5.4-5.8	5.6-5.9	5.4-5.9	≤ 4 **(Supono, 2022)**
Alkalinitas (mg.L^{-1} $CaCO_3$)	114.56-166.47	119.59-166.47	119.8-176.42	119.9-187.42	100-200 **(Boyd, 2018)**

DISCUSSION

The potassium mineralization process can reduce aggression and cannibalism in shrimp through several physiological and environmental mechanisms. Potassium plays an important role in maintaining electrolyte balance and osmoregulation in shrimp, which helps reduce stress. Low stress levels reduce shrimp aggression, thereby reducing the likelihood of cannibalism. At the molecular level, the Na-K-ATPase enzyme plays a vital role in transporting electrolyte fluids for ion balance within the body, through the neuroendocrine nervous system, which facilitates the osmoregulation process to maintain homeostasis **(Liu Z *et al*., 2018)**.

The ABW and SGR values at the addition of potassium concentration of 22.02 mg·L^{-1} were the lowest values (1.773 ± 0.211g and 3.432 ± 0.595%) (Fig. 2A, B, and Table 2). This occurred because the shrimp carried out an osmoregulation process to adjust the physiological system due to the difference between body fluids and the environment, which required large amounts of energy. This can be seen from Fig. (3A) and Table (2) (0.356 ± 0.027 mOsmol·L^{-1} H_2O). As a result, energy was not stored for growth.

Harsa *et al.* (2020) stated that macrominerals such as Na^+, Mg^{2+}, K^+, and Ca^{2+} are involved in osmoregulation through the activation of Na^+/K^+-ATPase, allowing shrimp to conserve energy that can be redirected toward growth. Furthermore, **Aruna and Felix (2017)** highlighted the role of potassium as a vital ion in lipid, protein, and carbohydrate metabolism, functioning as a cofactor in various enzymatic and metabolic processes that contribute to improved growth and survival.

The addition of potassium to the culture medium had a significant effect on the specific growth rate (SGR) of Pacific white shrimp ($P < 0.05$) (Fig. 2A & Table 2). This indicates that potassium, as a macromineral, is absorbed through the gills and skin via semipermeable membranes and acts as a driver for Na^+/K^+-ATPase activity, which facilitates daily growth (**Supono *et al.*, 2023**). Moreover, **Aruna and Felix (2017)** emphasized that deficiencies in essential ions such as Na^+ and K^+ serve as limiting factors for shrimp growth and survival.

According to Fig. (3A), the treatment with 66.06 mg·L^{-1} potassium yielded the lowest osmotic gradient (GO), indicating greater energy conservation. This energy savings enhances feed utilization efficiency, which can then be directed toward growth (**McGraw & Scarpa, 2016**); **Scabra *et al*. (2023)** stated that under isoosmotic conditions, where shrimp body fluids are in balance with the surrounding medium, energy expenditure is minimized and metabolic processes can be focused on growth. **Truong *et al.* (2023)** also confirmed that minerals in the water can be absorbed through the integument, gills, and intestines, facilitating more efficient nutrient absorption.

The analysis of variance (Table 2) revealed that increasing potassium levels in the medium significantly affected the survival rate of Pacific shrimp. The highest survival rate (68.67 ± 4.167%) was observed in the treatment with 66.06 mg·L^{-1} potassium, whereas the lowest survival rate occurred at 22.02 mg·L^{-1} potassium (Fig. 2D).

Potassium plays an essential role in regulating body fluid electrolytes, activating Na^+/K^+-ATPase, aiding ion transport, osmoregulation, and maintaining extracellular fluid volume (**Supono *et al.*, 2023**). This enables shrimp to efficiently absorb nutrients and conserve energy for growth and survival (**Syakirin, 2018**).

The highest percentage of cannibalism (45.33 ± 3.06%) was recorded at 22.02 mg·L^{-1} potassium, while the lowest (2.31 ± 2.31%) occurred at 66.06 mg·L^{-1} potassium (Fig. 3C). The potassium mineralization process can reduce aggression and cannibalism in shrimp through several physiological and environmental mechanisms. Potassium plays an important role in maintaining electrolyte balance and osmoregulation in shrimp, which helps reduce stress. Low GO analysis shows that the expression profile of the neuroendocrine system is activated under stimulation, and monoamine neurotransmitters, including dopamine and serotonin, are released to modulate osmoregulation (Table 2). Factors contributing to cannibalism include size heterogeneity, stocking density, competition for food, molting, and environmental conditions. **Rakhmawati *et al.* (2019)** and **Zhu *et al.* (2024)** reported that cannibalism leads to deformities, mortality, and considerable economic losses in shrimp production. Potassium assumes a critical role in the post-molting phase, thereby impacting the survival rate of vannamei shrimp (**Nehru *et al.*, 2018; Widigdo *et al.*, 2019**).

In crustaceans, cannibalism is often driven by aggressive behavior associated with decreased serotonin and increased cortisol levels in the brain. **Safitrah *et al.* (2020)** suggested that adequate intake of the amino acid tryptophan, which binds serotonin, can suppress shrimp aggression, particularly during molting. In addition to nutritional factors, environmental minerals such as potassium influence physiological and behavioral responses in shrimp. Potassium, which acts as a neural conductor, plays a significant role in both cellular and behavioral regulation. **Li *et al.* (2019)** explained that increased aggressiveness in crustaceans is associated with environmental stress and salinity fluctuations. They also noted that maintaining ion homeostasis under isoosmotic conditions stimulates dopamine and serotonin release in the brain, thereby reducing aggressiveness.

Nutrient utilization in shrimp is closely related to energy demands for physiological functions. The study showed that potassium macromineral supplementation improves feed utilization efficiency (Fig. 2C). Physiologically, blood glucose levels serve as stress indicators in shrimp. The analysis of variance revealed that potassium supplementation significantly affected shrimp blood glucose levels ($P < 0.05$) (Table 2). Glucose levels decreased with increasing potassium concentrations across treatments, indicating a homeostatic state was achieved (Fig. 3B).

Macrominerals that regulate osmolarity include essential cations such as Mg^{2+}, Ca^{2+}, K^+, as well as SO_4^{2-}, Na^+, and Cl^-. These ions contribute to the osmotic pressure of hemolymph by regulating semipermeable membranes and stabilizing osmotic loads caused by salinity variations. Magnesium stabilizes ATP activity in Na^+/K^+-ATPase function, while potassium ions act as intracellular electrolytes critical for maintaining osmoregulatory balance (**Anggoro *et al.*, 2018**).

Potassium supplementation in freshwater media or shrimp feed can increase aquaculture productivity without having to increase chemical inputs that damage the environment. By optimizing this mineral requirement, shrimp farming can be more efficient and environmentally friendly, supporting the sustainable aspects of fisheries. This approach also reduces the risk of farming failure due to osmotic stress and mineral deficiencies, thereby helping to maintain the balance of the aquaculture ecosystem.

The addition of 100 $mg \cdot L^{-1}$ potassium can increase the growth and survival rate of vannamei shrimp at a salinity of 5 $g \cdot L^{-1}$. Thus, the addition of potassium as an essential mineral in shrimp life is highly necessary (**Supono *et al.*, 2020**). Several studies reported that potassium requirements for *Litopenaeus vannamei* in low-salinity or freshwater conditions typically range from 50 to 80 $mg \cdot L^{-1}$, indicating that our results fall within the reported optimal range. In addition, we included a more detailed discussion on potential environmental interactions—such as water hardness, calcium–magnesium balance, and overall ionic composition—that may influence K^+ uptake and osmoregulatory performance. Additionally, we discussed possible dietary interactions, including the role of dietary K^+ and other minerals that might compensate or interact with waterborne potassium. These revisions provide a more comprehensive context for interpreting the optimal K^+ concentration in our study. Potassium deficiency can lead to molting failure, muscle cramping, and high mortality. As an essential ion, potassium supports growth, survival, and osmoregulatory functions in both fish and crustaceans. It also plays a vital role in lipid, protein, and carbohydrate metabolism by acting as a cofactor in numerous enzymatic and metabolic processes (**Aruna & Felix, 2017; Veeranjaneyulu & Krishnaveni, 2018**).

During the maintenance process, water quality management was carried out by siphoning every morning and evening. The media water quality parameters (temperature, pH, ammonia, alkalinity, DO) were still within the tolerable range for shrimp growth (Table 3). Based on the research results, the temperature ranged from 24–29 °C, pH 7–9, ammonia 0.05 $mg \cdot L^{-1}$, alkalinity 114.56–187.42 $mg \cdot L^{-1}$ $CaCO_3$, and DO 5.6–5.9 $mg \cdot L^{-1}$. The data are still in accordance with **Boyd *et al.* (2018)** and **Supono (2022)**, where the range of water quality parameters is suitable for the growth of vannamei shrimp.

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CONCLUSION

Supplementation of 66.06 $mg \cdot L^{-1}$ K^+ in freshwater media improves growth performance, stabilizes osmoregulatory function, and reduces stress responses, providing a clear and actionable guideline for inland shrimp culture

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