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IMPROVING THE HEALTH OF VANNAMEI SHRIMP (*Penaeus Vanname*) MAINTAINED IN LOW-SALINITY MEDIA WITH POTASSIUM MINERAL SUPPLEMENTATION

Peningkatan Kesehatan Udang Vannamei (*Penaeus Vanname*) Yang Dipelihara Pada Media Bersalinitas Rendah Dengan Suplementasi Mineral Kalium

Andre Rachmat Scabra*, Fariq Azhar, Rangga Idris Affandi, M Sopiyan Iskandar

Aquaculture Study Program University of Mataram

Pendidikan Street, Number 37, Mataram City, Indonesia

*Corresponding email : andrescabra@unram.ac.id

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ABSTRACT

The addition of various minerals to freshwater vannamei shrimp cultivation is known to result in higher growth rates. However, growth in their natural habitat, seawater, still results in higher growth rates. This study aimed to enhance growth and analyze the immune response of L. vannamei shrimp reared in freshwater with the addition of potassium challenged with Vibrio parahaemolyticus bacteria. Information on this immune response will provide the basis for developing a potassium mineral formulation that can support shrimp health and maximize their growth. The research method used is experimental using a Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications (P1: Maintenance of seawater media (30 ppt); P2: Potassium Supplementation 0 mg/L + vibrio sp injection; P3: Potassium Supplementation 10 mg/L + vibrio sp injection; P4: Potassium Supplementation 20 mg/L + vibrio sp injection; P5: Potassium Supplementation 30 mg/L + vibrio sp injection). Based on the research that has been done, it can be concluded that the best dose of potassium addition occurs in the P5 treatment, which is 45 mg/L of Potassium. At this dose, the treatment has a significant effect on the SR value, growth, feed efficiency, and immune system (THC, DHC, AF). At P5, the survival rate was 50%, absolute weight gain 34 g, feed efficiency 57%, THC 28 × 106, AF 58%.

Keywords : freshwater media, penaeus vannamei, potassium mineral, immune system, vannamei shrimp, Vibrio parahaemolyticus bacteria

ABSTRAK

Penambahan berbagai jenis mineral pada kegiatan budidaya udang vannamei media air tawar diketahui menghasilkan pertumbuhan udang vannamei yang lebih tinggi. Namun demikian, pertumbuhan udang vannamei pada habitat aslinya, yaitu air laut, masih menghasilkan pertumbuhan yang masih lebih tinggi. Penelitian ini bertujuan untuk meningkatkan pertumbuhan dan menganalisa respon imun udang vannamei L. vannamei yang dipelihara pada media air tawar dengan penambahan mineral kalium dan diuji tantang dengan bakteri *vibrio parahaemoliticus*. Informasi tentang respon imun tersebut menjadi dasar pengembangan formulasi mineral kalium yang dapat mendukung kesehatan udang sehingga

pertumbuhannya dapat berjalan dengan maksimal. Metode penelitian yang digunakan adalah eksperimental menggunakan Rancangan Acak Lengkap (RAL) yang terdiri dari 5 perlakuan dan 3 ulangan (P1 : Pemeliharaan media air laut (30 ppt); P2 : Suplementasi Kalium 0 mg/L + injeksi vibrio sp; P3 : Suplementasi Kalium 10 mg/L + injeksi vibrio sp; P4 : Suplementasi Kalium 20 mg/L + injeksi vibrio sp; P5 : Suplementasi Kalium 30 mg/L + injeksi vibrio sp.). Berdasarkan penelitian yang telah dilakukan, dapat disimpulkan bahwa dosis penambahan kalium terbaik terjadi pada perlakuan P5 yaitu sebesar Kalium 45 mg/L. Pada dosis tersebut, perlakuan memberikan pengaruh nyata terhadap nilai SR, pertumbuhan, efisiensi pakan, dan sistem imun (THC, DHC, AF). Pada P5, nilai Survival Rate 50%, Pertumbuhan bobot mutlak 34 g, Efisiensi pakan 57%, THC 28 × 10^6, AF 58 %.

Kata kunci : bakteri *vibrio parahaemolyticus,* media air tawar, mineral kalium, *penaeus vannamei,* sistem imun, udang vannamei

INTRODUCTION

Official statistics from the Indonesian Ministry of Marine Affairs and Fisheries (MMAF), available at statistik.kkp.go.id, indicate that West Nusa Tenggara (NTB) Province recorded the highest production volume of Pacific white shrimp (*Penaeus vannamei*) in 2020, reaching 159,013.10 tons. NTB has an estimated potential aquaculture area of 27,929.5 ha, including 10,237.5 ha in Sumbawa, 4,998.5 ha in Bima, 3,500 ha in East Lombok, and up to 4,700 ha distributed across other districts in NTB. However, only approximately 4,926.5 ha (≈18%) of this potential area has been utilized.

Based on designations by the Fédération Internationale de Motocyclisme (FIM) and the Dorna WorldSBK Organization (DWO), the Mandalika Circuit on Lombok Island hosts international motorcycle racing events, namely the World Superbike Championship (WSBK) and MotoGP (Grand Prix). These events are expected to substantially increase both domestic and international tourist arrivals. To accommodate this influx, ensuring the availability of safe, high-quality food in sufficient quantities should become a priority, and the production of *P. vannamei* may contribute to meeting local food demand.

The underutilization of NTB's *P. vannamei* aquaculture potential—despite national directives to increase production and the existence of a large market—highlights the need to further promote and expand this commodity. All sectors with development opportunities should receive serious attention, including the cultivation of *P. vannamei* in freshwater systems located far from coastal areas.

Freshwater culture of *P. vannamei* offers several advantages. First, *P. vannamei* generally has higher economic value than many freshwater fish commodities, such as African catfish and Nile tilapia, and freshwater farming is therefore expected to generate higher production returns. Second, certain pathogens commonly associated with seawater shrimp farming may be reduced or avoided. Several sources report that diseases such as white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) can severely constrain production and trigger crop failure in seawater-based culture; thus, freshwater rearing is expected to suppress pathogens that thrive in marine environments. Third, freshwater shrimp farming can be implemented in inland areas, using water sources such as rivers, lakes, reservoirs, or groundwater (wells).

Development of freshwater *P. vannamei* culture has been pursued since 2013. Scabra et al. (2013) reported that *P. vannamei* reared at 0 ppt salinity (freshwater) achieved survival rates up to 90%. Subsequent studies have also demonstrated strong potential for freshwater culture through supplementation with mineral sources, including calcium carbonate ($CaCO_3$) (Scabra, Cokrowati, et al., 2023), calcium oxide (CaO) (Scabra et al., 2022b), calcium hydroxide ($Ca(OH)_2$) (Scabra, Cokrowati, & Fatimah, 2023), $CaCO_3$ + magnesium sulfate (Scabra,

Marzuki, & Alhijrah, 2023), CaO + magnesium sulfate (Scabra, Diniarti, & Artiningsih, 2023), $Ca(OH)_2$ + magnesium sulfate (Scabra, Marzuki, & Rizaldi, 2023), KH_2PO_4 at 45 mg/L, and CaO + phosphorus (Scabra, Marzuki, & Yarni, 2023). Across these studies, shrimp reared in mineral-supplemented freshwater generally exhibited higher growth than shrimp reared without mineral addition, with growth trends approaching those observed under their natural seawater habitat.

This study aimed to enhance growth and evaluate the immune response of *P. vannamei* reared in freshwater supplemented with potassium minerals and subsequently challenged with *Vibrio parahaemolyticus*. Information on immune responses is expected to support the development of potassium mineral formulations that promote shrimp health and enable optimal growth under freshwater culture conditions.

METHOD

The study employed an experimental approach using a completely randomized design (CRD) with five treatments, consisting of *Penaeus vannamei* reared in freshwater media with different compositions. Each treatment was replicated three times, resulting in a total of 15 experimental units. The treatments were defined as follows:

P1: *Penaeus vannamei* reared in seawater (salinity 30 ppt).
P2: Freshwater with potassium supplementation at 0 mg/L, + injection with *Vibrio* sp.
P3: Freshwater with potassium supplementation at 10 mg/L, + injection with *Vibrio* sp.
P4: Freshwater with potassium supplementation at 20 mg/L, + injection with *Vibrio* sp.
P5: Freshwater with potassium supplementation at 30 mg/L, + injection with *Vibrio* sp.

Treatments P2–P5 also received additional minerals at identical dosages, based on previous studies: CaO at 80 mg/L (Scabra et al., 2022a), $MgSO_4$ at 40 mg/L (Scabra, Marzuki, & Rizaldi, 2023), KH_2PO_4 at 45 mg/L (Scabra, Marzuki, & Yarni, 2023), and NaCl at 1 mg/L (Ridwan et al., 2024).

Research Procedures

Shrimp Preparation and Rearing

This study used 15 experimental units consisting of plastic containers with a working volume of 45 L. Each container was equipped with an aeration system to maintain dissolved oxygen (DO) levels in the rearing medium. Aeration was operated continuously starting three days before shrimp stocking. The test animals were Pacific white shrimp (*Penaeus vannamei*) postlarvae at 20 days of age (PL20), obtained from the Hatchery of the Center for Superior Broodstock and Shellfish Production (BPIUUK), Karangasem Regency, Bali Province, Indonesia. Shrimp were reared for 60 days. Uneaten feed and accumulated wastes were removed daily by siphoning the bottom of each container using a hose.

Vibrio parahaemolyticus Challenge Test

The bacterial pathogen used was *Vibrio parahaemolyticus*, obtained from the Fish Health Laboratory collection at Universitas Mataram. The isolate was subcultured and purified to obtain younger and more virulent bacteria, followed by re-characterization and total plate count (TPC). The selected isolate was cultured in 25 mL of liquid seawater complete (SWC) medium for 18 h in a water shaker at 29°C. The culture was then serially diluted to a final density of 10^6 CFU/mL (Oktaviana et al., 2014). The bacterial challenge was conducted via exposure through the rearing medium (immersion), with the dose and protocol adjusted according to each treatment. After infection, shrimp were maintained for an additional 15 days before sampling for the study parameters.

Research Parameter

Survival Rate (SR)

Survival rate was calculated using the formula described by Azhar (2014), as follows:

$$SR = \frac{Nt}{N0} \times 100$$

Information:
SR : *Survival rate* (%)
Nt : Number of shrimp alive at the end of the rearing period (individuals)
No : Number of shrimp alive at the beginning of the rearing period (individuals)

Growth Rate (GR)

Absolute weight gain was calculated following the method described by Scabra et al. (2016), as follows:

$$GR = Wt - Wo$$

Information:
GR : Growth Rate (g)
Wt : Final weight (g)
Wo : Initial weight (g)

Feed efficiency (FE)

Feed efficiency was calculated following the method described by Zonneveld *et al.,* (1991):

$$EP = \frac{(Bt + Bd) - Bo}{F}$$

Information:
EP : Feed efficiency
Bt : Final absolute biomass at the end of the rearing period (g)
Bd : Total biomass of fish died during the rearing period (g)
Bo : Initial absolute biomass at the beginning of the experiment (g)
F : Total feed intake (weight of feed consumed) by the fish during the experiment (g)

Health Status (Immune Response)

Haemolymph sampling for each treatment was conducted 15 days after infection using a 1-mL syringe preloaded with 0.6 mL of anticoagulant. Haemolymph was collected from three shrimp per treatment, with 0.1 mL obtained from each individual (Hidayatullah, 2019).

a. *Total Haemocyte Count* (THC)

The collected haemolymph was used to assess immune-response parameters. Total haemocyte count (THC) was determined by placing a drop of haemolymph onto a haemocytometer chamber and covering it with a cover glass. The sample was then examined under a microscope, and haemocytes were counted using the haemocytometer grid. Observations were performed at 400× magnification, and THC was calculated using the formula described by Jannah et al. (2018), as follows:

$$THC = \frac{\sum \text{observed cell}}{\sum \text{observed box}} \times 25 \quad \frac{1}{\text{Haemocytometer volume}} \times DP$$

Information: DP = Dillution Factor

b. *Differential Haemocyte Count* (DHC)

Glass slides were immersed in methanol for 5 min and air-dried at room temperature. One drop of haemolymph was placed on the slide, spread evenly across the surface using a second slide, and allowed to dry. The smear was then refixed by immersing the slide in methanol for 10–15 min. After drying, the slide was stained with Giemsa solution for 10–15 min and air-dried. The preparation was rinsed with distilled water, dried, and examined under a microscope. Differential haemocyte count (DHC) was calculated following Jannah et al. (2018), as follows:

$$\text{DHC (hialine) (\%)} = \frac{\sum \text{Hialine cell}}{\sum \text{haemoscite observed}} \times 100$$

c. Fagocitosis activity (FA)

Phagocytic activity was measured by mixing 100 µL of haemolymph with 25 µL of a *Staphylococcus* sp. suspension in a microplate and incubating the mixture for 20 min. After incubation, a drop of the mixture was placed onto a glass slide that had been pre-treated with methanol and spread evenly to form a smear. The smear was air-dried and fixed by immersing the slide in methanol for 10–15 min, followed by Giemsa staining for 10–15 min. The stained slides were then examined under a microscope at 400× magnification. Phagocytic activity was determined as the percentage of haemocytes performing phagocytosis, following Jannah et al. (2018). The phagocytic activity (AF) was calculated as follows:

$$\text{AF} = \frac{\sum \text{Fagocite cell}}{\sum \text{amount of all phagocytic cells}} \times 100$$

Water Quality

Water quality parameters monitored during the main experimental period included temperature, pH, dissolved oxygen (DO), nitrite (NO_2^-), ammonia (NH_3), alkalinity, and calcium hardness (Ca^{2+}) (Scabra & Setyowati, 2019).

Table 1. Water quality parameters measured during the study

Parameter	Unit	Instrument/Method
Temperature	°C	Digital thermometer
Dissolved oxygen (DO)	mg L^{-1}	DO meter
pH	–	pH meter
Ammonia (NH_3)	mg L^{-1}	Spectrophotometry
Nitrite (NO_2^-)	mg L^{-1}	Spectrophotometry
Total alkalinity	mg L^{-1}	Titrimetric method
Calcium hardness (Ca^{2+})	mg L^{-1}	Titrimetric method

Data Analysis

The data were analyzed using analysis of variance (ANOVA) in SPSS at a 5% significance level to evaluate treatment effects. When significant differences were detected, mean comparisons were performed using Duncan's multiple range test (DMRT).

RESULT

Survival Rate (SR)

Based on one-way ANOVA, the treatments had a significant effect on survival rate ($p < 0.05$). Duncan's multiple range test indicated that P1 differed significantly from P2, P3, and

P4 but not from P5; P2 differed significantly from P1, P4, and P5 but not from P3; P3 differed significantly from P1 and P5 but not from P2 and P4; P4 differed significantly from P1 and P2 but not from P3 and P5; and P5 differed significantly from P2, P3, and P4 but not from P1.

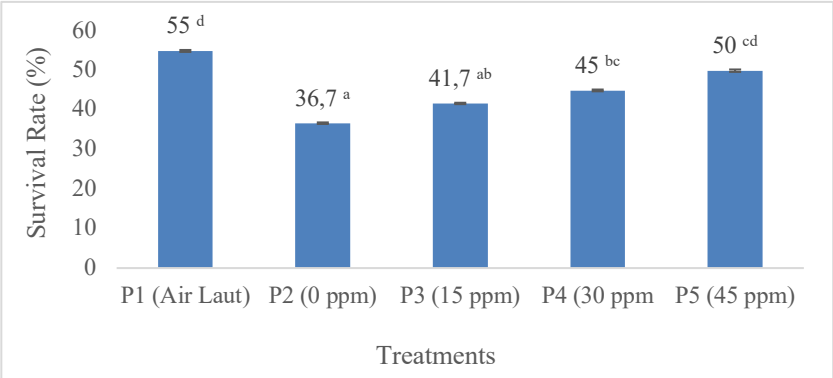

Figure 1. *Survival Rate* (SR)

Absolute Weight Growth

Based on one-way ANOVA, the treatments had a significant effect on absolute weight gain ($p < 0.05$). Duncan's multiple range test indicated that P1 differed significantly from P2, P3, and P4 but not from P5; P2 differed significantly from P1, P4, and P5 but not from P3; P3 differed significantly from P1 and P5 but not from P2 and P4; P4 differed significantly from P1 and P2 but not from P3 and P5; and P5 differed significantly from P2 and P3 but not from P1 and P4.

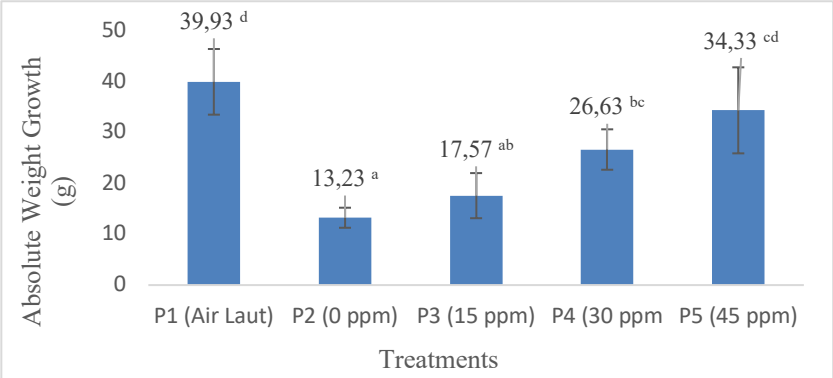

Figure 2. Absolute Weight Growth

Feed Efficiency

Based on one-way ANOVA, the treatments had a significant effect on feed efficiency ($p < 0.05$). Duncan's multiple range test indicated that P1 differed significantly from P2, P3, and P4 but not from P5; P2 differed significantly from P1, P4, and P5 but not from P3; P3 differed significantly from P1, P4, and P5 but not from P2; P4 differed significantly from P1, P2, and P3 but not from P5; whereas P5 differed significantly from P2 and P4 but not from P1 and P4.

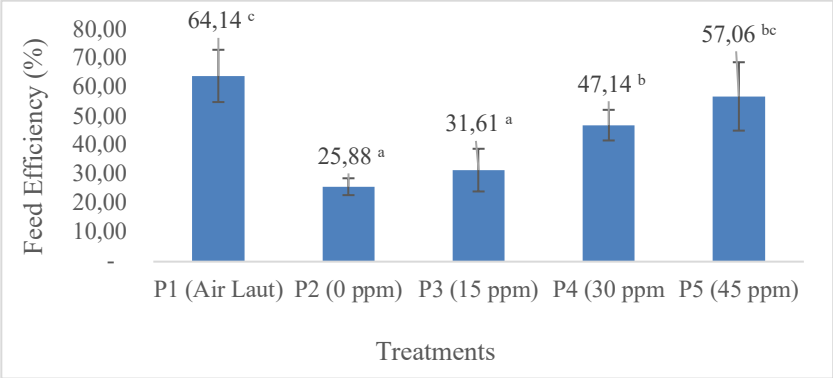

Figure 3. Feed Efficiency

Total Haemocyte Count (THC)

Total haemocyte count (THC) values are presented in Figure 4.8. As shown in the figure, THC of cultured *Penaeus vannamei* ranged from 24.16 × 10^6 to 32.19 × 10^6 cells/mL. The highest THC was recorded in P1 (control) reared in seawater. The lowest value occurred in P2 (freshwater without potassium supplementation) at 24.16 × 10^6 cells/mL, followed by P3 (freshwater + 15 mg/L potassium) at 25.43 × 10^6 cells/mL. Treatments P4 and P5 (30 and 45 mg/L potassium) yielded THC values of 27.01 × 10^6 and 28.00 × 10^6 cells/mL, respectively. One-way ANOVA indicated that potassium supplementation had no significant effect on THC ($p > 0.05$), and Duncan's post-hoc test likewise showed no significant differences among treatments, with P1 not differing from any group.

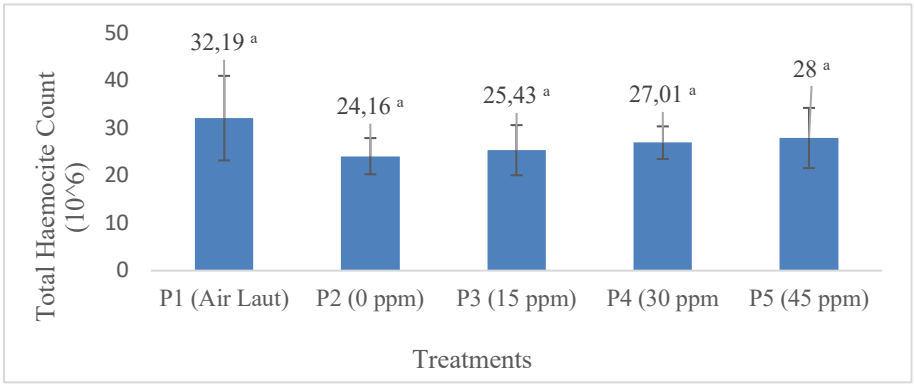

Figure 4. *Total Haemocyte Count* (THC)

Differential Haemocyte Count (DHC)

Differential haemocyte count (DHC) values are presented in Figure 4.9. DHC in *Penaeus vannamei* comprised three haemocyte types: hyaline, granulocyte, and semi-granulocyte cells. One-way ANOVA showed that potassium supplementation significantly affected the proportion of hyaline cells ($p < 0.05$), with P1 differing significantly from all treatments; P5 did not differ from P3 and P4 but differed significantly from P1 and P2. The semi-granulocyte fraction was also significantly affected ($p < 0.05$): P1 did not differ from P4 and P5 but differed significantly from P2 and P3. The highest hyaline proportion occurred in P2 (64%), followed by P3 (60%), P4 (59%), P5 (58%), and the lowest in P1 (53%). For granulocytes, the highest value was observed in P1 (29%), followed by P3 (27%), P2 (26%), and P4 (26%), with the lowest in P5 (25%). Semi-granulocytes were highest in P1 (18%), followed by P5 (17%), P4 (15%), P3 (13%), and the lowest in P2 (10%).

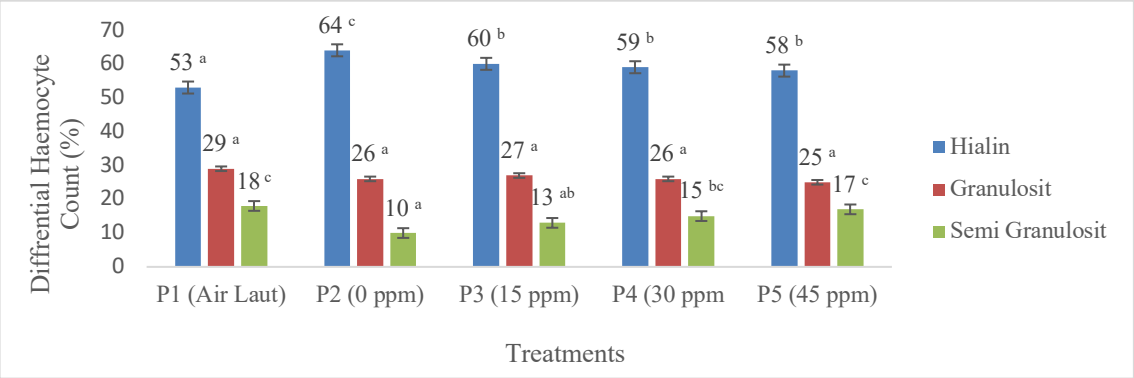

Figure 5. *Diffrential Haemocyte Count* (DHC)

Phagocytic activity (FA)

As shown in Figure 5, the phagocytic activity (AF) of cultured vannamei shrimp ranged from 52.49% to 59.38%. One-way ANOVA indicated that potassium supplementation at different doses in the rearing medium had no significant effect on AF ($p > 0.05$). The highest AF was observed in P1 (59.38%), followed by P5 (58.53%), P4 (56.32%), P3 (55.57%), and the lowest in P2 (52.49%).

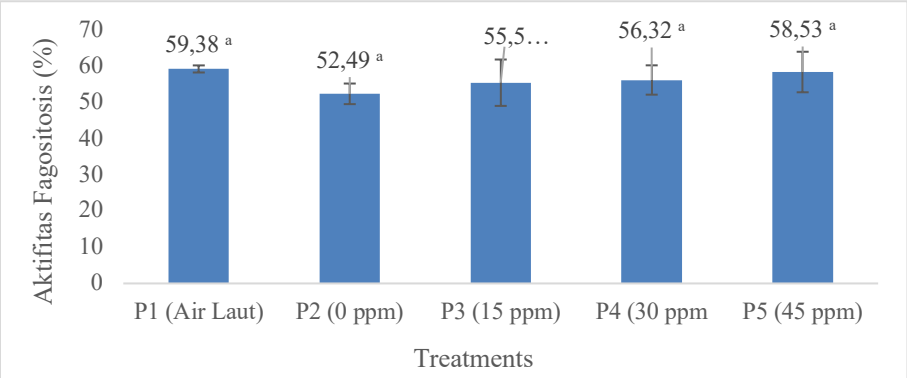

Figure 6. Phagocytic activity

Water Quality

Water quality was monitored three times during the 45-day rearing period. The measured parameters included temperature, pH, dissolved oxygen (DO), ammonia, hardness, and alkalinity. The water-quality results are presented in Table 2.

Table 2. Water Quality

No.	Parameter	Unit	Observed range	Optimal range (reference)
1	pH	–	7.1–8.5	6.5–9 (Supono, 2018)
2	Temperature	°C	26.6–30.2	25–34 °C (Putra et al., 2014)
3	Dissolved oxygen (DO)	mg/L	6.3–8.0	3–8 mg/L (Rakhfid et al., 2019)
4	Alkalinity	mg/L	94–172	75–200 mg/L (Supono, 2018)
5	Ammonia	mg/L	0.02–0.08	<0.1 mg/L (Jose, 2018)

Water quality measurements during the rearing period remained within optimal ranges. Temperature ranged from 26.6 to 30.2 °C and pH from 7.1 to 8.5. Dissolved oxygen (DO) levels were 6.3–8.0 mg/L. For chemical parameters, ammonia ranged from 0.02 to 0.08 mg/L, while alkalinity ranged from 94 to 172 mg/L.

Discussion

Survival Rate (SR)

Survival rate (SR) represents the proportion of cultured organisms that remain alive during the rearing period. As shown in Figure 1, the highest SR occurred in P1 (seawater) at 55%, which differed significantly from all treatments except P5. The higher SR in P1 was likely due to environmental conditions being closer to the natural habitat of *Penaeus vannamei*, as the experimental shrimp originated from a marine hatchery and were reared in seawater at 30 ppt. This salinity is considered optimal for *L. vannamei* survival; the species can tolerate 1–42 ppt, with an optimum range of 15–30 ppt (Suprapto, 2005 in Sawito, 2019). Sawito (2019) also reported that the best growth and SR occurred at 30 ppt (SR 39.33%), likely because shrimp consumed more feed to meet energy demands for growth and moulting, a process that requires substantial energy.

Among freshwater treatments, the highest SR was observed in P5 (50%), which differed significantly from P2, P3, and P4. This suggests that potassium supplementation influenced shrimp survival by supporting metabolic processes and helping maintain homeostasis during osmotic stress. Potassium is an essential macromineral that works synergistically with sodium to regulate electrolyte balance, nerve impulse transmission, and energy release from proteins, lipids, and carbohydrates; Na^+/K^+-ATPase activity in crustaceans depends on K^+ and is critical for maintaining homeostasis under salinity fluctuations (Supono, 2022). In contrast, the lower SR in P2–P4 may be associated with moulting-related vulnerability and cannibalism, as freshly moulted shrimp have soft exoskeletons and release attractants that can trigger predation by conspecifics. Low K^+ levels may also impair metabolism, weaken shrimp, and accelerate mortality. Reduced potassium under low-salinity conditions has been reported to significantly decrease survival and growth of *L. vannamei* postlarvae (Scarpa, 2003 in Supono, 2022). Similarly, Anita et al. (2017) noted that low-salinity culture may disrupt synchronous moulting, increasing cannibalism and reducing survival. According to Widigdo (2013), SR is categorized as good when >70%, moderate at 50–60%, and low when <50%; thus, P2–P4 were classified as low, whereas P1 (55%) and P5 (50%) were moderate and did not differ significantly from each other.

Absolute Weight Gain

Figure 2 shows that potassium supplementation significantly affected absolute weight gain. The highest absolute weight gain occurred in P1 (seawater), likely because shrimp were reared under optimal salinity and adapted more effectively. Growth in freshwater was lower, which may be explained by reduced mineral availability at lower salinities (Nur'aisyah et al., 2017). Notably, P3 (with potassium) exhibited better growth than P2 (without potassium), consistent with potassium's role in metabolic energy release from proteins, lipids, and carbohydrates and in nerve impulse transmission (Widodo et al., 2011). Growth differences may also reflect osmoregulatory costs: in P2, energy may have been diverted from growth to osmoregulation (Rachmawati et al., 2012). Potassium is a key driver of crustacean osmoregulation via Na^+/K^+-ATPase activity during salinity fluctuations, and insufficient potassium may constrain growth (Scarpa, 2003 in Kaligis, 2016). Improved osmotic efficiency under potassium supplementation may therefore allow more energy to be allocated to growth and physiological processes (Kaligis et al., 2019).

Feed Efficiency

Feed provides protein, lipid, carbohydrate, vitamins, and minerals required for energy, growth, and reproduction. Because shrimp cannot synthesize essential nutrients such as amino acids de novo, adequate dietary protein from formulated feeds is required to support optimal

performance. Feed efficiency is defined as the ratio of shrimp biomass gain to the total feed provided during rearing (Supono et al., 2021). In Figure 3, the highest feed efficiency was recorded in P1 (seawater; 64.14%), followed by P5 (freshwater + 45 mg/L KCl; 57.06%), P4 (30 mg/L KCl; 47.14%), P3 (15 mg/L KCl; 31.61%), and the lowest in P2 (0 mg/L KCl; 25.88%). Feed efficiency is inversely related to feed conversion ratio (FCR); higher efficiency corresponds to lower FCR and better growth performance (Afrizal, 2020). Thus, lower FCR indicates that feed is utilized more effectively for biomass production.

Total Haemocyte Count (THC)

Haemocytes are central to the shrimp immune system and play key roles in phagocytosis and other defense mechanisms. THC is commonly used as an indicator of immune status because haemocytes contribute to recognition of foreign particles, phagocytosis, cytotoxicity, encapsulation, wound healing, and activation of the prophenoloxidase (proPO) system (Johansson et al., 2000). In this study, the lowest THC occurred in P2 (24.16 × 10^6 cells/mL), whereas the highest was observed in P1 (32.19 × 10^6 cells/mL). Statistical analysis indicated that THC did not differ significantly among treatments. Generally, higher haemocyte levels reflect better health and higher resistance to pathogens. Yeh et al. (2009) reported normal THC values in healthy *L. vannamei* on the order of 10^7 cells/mL. Increased haemocytes, particularly granular cells, may enhance proPO activation and phenoloxidase activity, improving defense against pathogens; conversely, reduced haemocyte counts have been associated with acute and potentially lethal infections (Febriani et al., 2013).

Differential Haemocyte Count (DHC)

DHC analysis identified three haemocyte types: hyaline, granulocyte, and semi-granulocyte cells, which are commonly used as indicators of immune modulation. In this study, hyaline cells constituted 53–64% of total haemocytes, granulocytes 25–29%, and semi-granulocytes 10–18%, all within reported normal ranges for *L. vannamei* (Darwantin et al., 2016). Typically, all haemocyte types can contribute to phagocytosis; however, hyaline cells are often recruited early, followed by granulocytes and semi-granulocytes. Hyaline cells can be activated by opsonin factors produced following proPO-to-PO activation in granular cells, enabling phagocytosis of foreign material (Johansson, 1995 in Darwantin, 2016). Granulocytes are often considered particularly important in shrimp immune defense.

Phagocytic Activity (AF)

Phagocytic activity is a key cellular indicator of innate immune response and represents an early defense mechanism against microbial invasion (Suleman et al., 2019). As shown in Figure 6, phagocytic activity did not differ significantly between the control and mineral-supplemented treatments. The highest AF values were observed in P1 (59.38%), followed by P5 (58.53%), P4 (56.32%), P3 (55.57%), and the lowest in P2 (52.49%). The absence of significant differences may be related to the lack of a specific immunostimulant, as shrimp lack immunological memory and rely on stimulation of non-specific defenses. Immunostimulants are therefore widely applied because they can enhance non-specific immunity without adverse effects (Hidayat et al., 2017). Compounds such as alkaloids and flavonoids may act as antioxidants and immunomodulators by inducing cytokine-mediated responses during pathogen invasion and tissue regeneration (Hanifah, 2020).

Water Quality

Water quality is a critical determinant of shrimp survival, health, and growth; therefore, effective water-quality management is a key driver of production success (Makmur et al., 2018). In this study, temperature, dissolved oxygen (DO), pH, alkalinity, and ammonia were monitored over 45 days and remained within suitable ranges for *Penaeus vannamei*. Water temperature

ranged from 26.6 to 30.2°C, which falls within the recommended range for shrimp culture (Putra, 2014; Makmur et al., 2018). DO concentrations were 6.3–8.0 mg/L, indicating adequate oxygen availability to support shrimp activity, feeding, and growth, and consistent with optimal values reported for *L. vannamei* (Rakhfid et al., 2019). The pH ranged from 7.1 to 8.5, which is considered favorable for shrimp culture and within the recommended range of 6.5–9 (Supono, 2018); extreme pH values can alter chemical reactions in the water and biochemical processes in shrimp and may reduce productivity (Anisa et al., 2021). Ammonia concentrations remained low (0.047–0.082 mg/L) and below the commonly accepted threshold (<0.1 mg/L), suggesting minimal toxic risk during culture (Jose, 2023). Total alkalinity ranged from 94 to 172 mg/L, which is within the recommended range for shrimp ponds (75–200 mg/L) and supports buffering capacity and pH stability; overly low or excessive alkalinity may disrupt moulting frequency (Supono, 2018).

CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on the present study, the optimal potassium supplementation level was observed in treatment P5 (45 mg/L). At this dose, the treatment significantly affected survival rate, growth, feed efficiency, and immune parameters (THC, DHC, and AF). In P5, survival rate reached 50%, absolute weight gain was 34 g, feed efficiency was 57%, THC was 28 × 10^6 cells/mL, and AF was 58%.

Suggestions

For future work, we recommend evaluating higher potassium doses to determine the optimal level of potassium supplementation in freshwater culture media and to further improve shrimp survival.

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