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Effect of different ratios of sodium and potassium on the growth and survival rate of Pacific white shrimp (*Litopenaeus vannamei*) cultured in freshwater

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Abstract. Pacific white shrimp (*Litopenaeus vannamei*) has been successfully cultivated in low salinity and in freshwater. Some constraints that arise in the cultivation process include the deficiency of macrominerals, such as sodium, magnesium, calcium, and potassium. The presence of these macrominerals and their proportion affect their absorption by shrimp. Therefore, this study aims to determine the effect of different ratios of sodium (Na^+) and potassium (K^+) on the growth and survival rate of Pacific white shrimp reared in freshwater media. A completely randomized design (CRD) was used, with 3 treatments and 4 repetitions. The treatments were represented by different ratios of Na and K, namely: A - 17:1; B - 27:1; C - 37:1. The duration of the experiment was 35 days. The results showed that the ratio of sodium and potassium in freshwater media significantly affected ($p<0.05$) the growth and survival rate of Pacific white shrimp. B treatment gave the best growth and survival rates, namely 69.50±5.26% and 1.69 ±0.03 g, respectively, with a specific growth rate of 11.90±0.04%. Observations of water quality showed that it is in line with the standards for shrimp farming. Furthermore, *Vibrio* was not found in any treatment group at the end of the experiment.

Key Words: K^+, macromineral, Na^+, *Vibrio*.

Introduction. Pacific white shrimp (*Litopenaeus vannamei*) culture has developed rapidly in Asia, including in Indonesia (Amelia et al 2021), due to its advantages including high survival rate at high stocking density and resistance to disease attacks (Briggs et al 2004). Being a euryhaline species, it can live in a wide salinity range, between 0.5-45 ppt (Wyban & Sweeney 1991; Davis et al 2002). Furthermore, this ability enables the vannamei shrimp to be cultured in low salinity waters, far from the coast. In Indonesia, Pacific white shrimp cultivation has been successfully carried out by farmers with a survival rate of more than 80% (Aruna & Felix 2017; Supono 2021).

One of the important factors that influence the success of Pacific white shrimp culture is the macromineral content in the water (Davis et al 2005; Boyd 2018). Minerals that dominate seawater and brackish water include sodium, magnesium, calcium, and potassium. These macrominerals affect the osmoregulation and molting process in prawns. Potassium plays an important role in the post-molting process, affecting the survival rate of vannamei shrimp (Nehru et al 2018; Widigdo et al 2019). Furthermore, calcium is an important mineral needed for bone formation and the prevention of metal ions across the gills, while magnesium affects the growth and survival rates (Davis et al 2005). Boyd (2018) reported that Pacific white shrimp may die due to a lack of potassium ions (1 mg L^{-1}), but after the concentration is increased to 50 mg L^{-1}, the mortality rate is reduced. The rate of its absorption is influenced by the presence of sodium (Liu et al 2014).

Pacific white shrimp have also been successfully cultivated in freshwater (Araneda et al 2008), but the cultivation is often affected by a lack of macrominerals, leading to slow growth, molting failure, and death (Suguna 2020). The concentration and ratio of macrominerals greatly determine their absorption by shrimp (Boyd 2018). The proportion of each macromineral in an artificial system must be adjusted as its proportion in seawater (Atwood et al 2003). The absorption of sodium ions is influenced by potassium and vice versa, while the absorption of magnesium ions is influenced by the concentration of calcium ions and vice versa (Aruna & Felix 2017; Suguna 2020). Therefore, this study aimed to determine the effect of different ratios of sodium (Na^+) and potassium (K^+) ions on the growth and survival rate of Pacific white shrimp reared in freshwater media.

Material and Method

Time and place of the study. This study was carried out between April–May 2022 for 35 days at the Fisheries Laboratory, Department of Fisheries and Marine, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia.

Research design. The culture containers used had a volume of 80 L with a total of 12 units. Each container was filled with 50 L of freshwater and equipped with 3 aeration stones to supply oxygen. A completely randomized design (CRD) consisting of 3 treatments and 4 replications was used. These treatments were: A - Na^+ and K^+ at a ratio of 17:1; B - Na^+ and K^+ at a ratio of 27:1; C - Na^+ and K^+ at a ratio of 37:1.

The freshwater used was enriched with the addition of macrominerals, namely sodium, magnesium, calcium, and potassium. The concentration of magnesium and calcium added was equivalent to a salinity of 5 ppt, namely 196 mg L^{-1} and 60 mg L^{-1} (Boyd 2018), while that of sodium and potassium was based on the treatment, as presented in Table 1. The mineral materials used include NaCl, $MgCl_2$, $CaCO_3$, and KCl.

Table 1

The mineral concentration of each treatment

Mineral	*Treatment (A)*	*Treatment (B)*	*Treatment (C)*
Na^+ (mg L^{-1})	935	935	935
Mg^+ (mg L^{-1})	196	196	196
Ca^+ (mg L^{-1})	60	60	60
K^+ (mg L^{-1})	55	34.6	25.3

The test animals were Pacific white shrimp post-larvae (PL 15), obtained from a hatchery in Kalianda, South Lampung. Each treatment unit was stocked with 50 shrimp, equivalent to 1 PL L^{-1} or 1000 PL m^{-3}. Feeding was carried out using commercial feed with a protein content of 30%, 6% fat, 3.5% fiber, and 13% ash content. The samples were fed 3 times a day at 08.00, 14.00, and 22.00 using the blind feeding method.

Study parameters. Parameters observed in Pacific white shrimp included growth rate and survival rate. Water quality was also determined, including temperature, pH, dissolved oxygen (DO), total *Vibrio* count (TVC), alkalinity, and total ammonia nitrogen (TAN). Growth rate (GR), specific growth rate, and survival rate were calculated using the formula described by Far et al (2009):

GR (g) = final body weight (g) - initial body weight (g)

SGR (%) = (Ln Wt – Ln Wo)/time period (days) x 100

SR (%) = initial stock/final population x 100

Temperature, pH, and dissolved oxygen (DO) were measured using a water quality checker every three days. Alkalinity was determined using the APHA (1992) method before stocked and TAN was determined using the phenate method at the end of the culture. Total *Vibrio* count was measured using the method described by Chau et al (2011). The method uses thiosulphate citrate bile sucrose agar (TCBS) as selective media for growing *Vibrio*.

Statistical analysis. Data on the growth and survival rates of Pacific white shrimp were analyzed using ANOVA with a 95% confidence level and when they were significantly different, further testing was carried out using Duncan's test. Subsequently, the water quality parameters were analyzed descriptively.

Results and Discussion. Data on the growth rate of Pacific white shrimp after being reared for 35 days are presented in Figure 1, while the specific growth rate is presented in Figure 2.

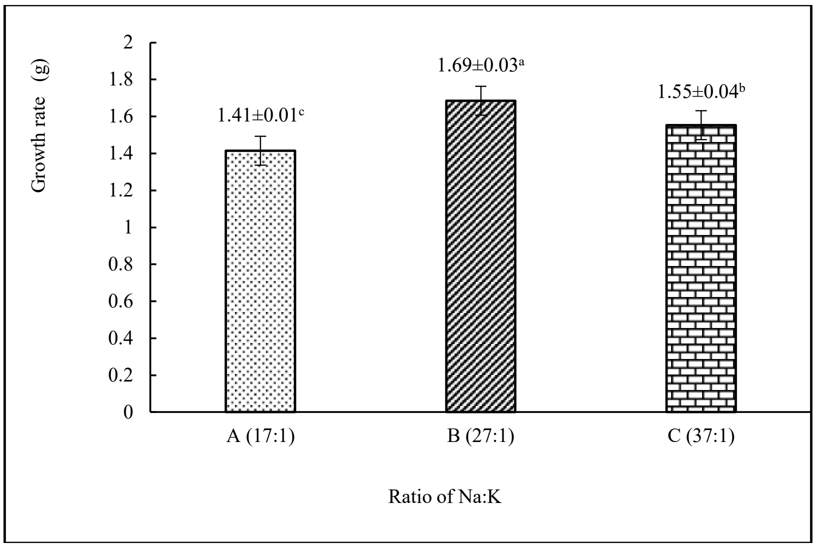

Figure 1. Growth rate of *Litopenaeus vannamei*.

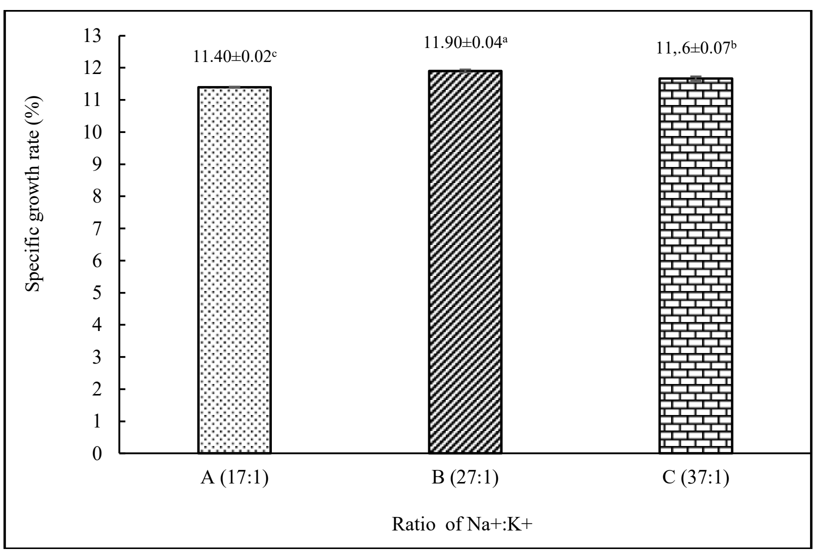

Figure 2. Specific growth rate of *Litopenaeus vannamei*.

The ANOVA test result showed that the different ratios of Na and K significantly affected ($p<0.05$) the growth of Pacific white shrimp. Further analysis revealed that treatment A produced significantly different results from treatments B and C. The best growth of Pacific white shrimp occurred in treatment B, with an average weight of 1.69±0.03 g, while the lowest was recorded in A, with an average weight of 1.41±0.01 g. The survival rates for Pacific white shrimp are presented in Figure 3.

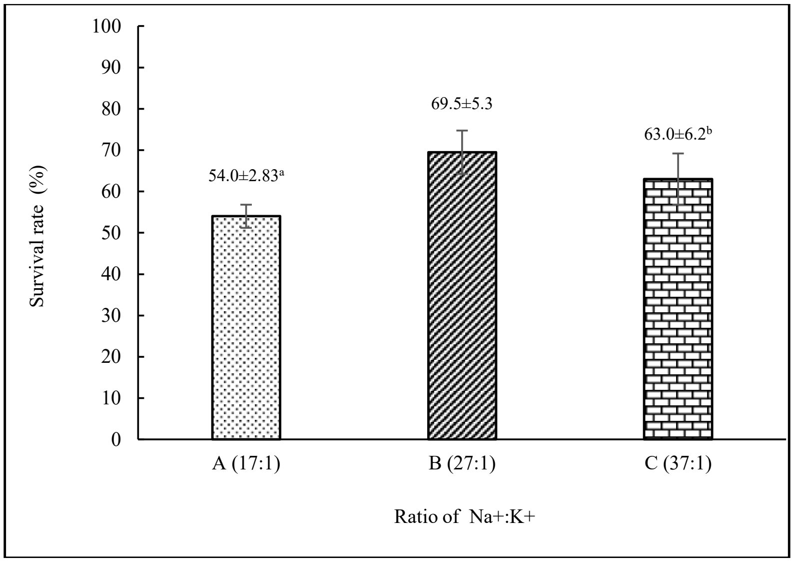

Figure 3. Survival rate of *Litopenaeus vannamei*.

ANOVA result showed that different ratios of Na and K ions significantly affected ($p<0.05$) the survival rate of Pacific white shrimp. Further analysis showed that treatment A produced significantly different survival rate was significantly different from treatments B and C, while survival rates in treatments B and C were not significantly different. The highest survival rate of Pacific white shrimp occurred in treatment B, namely 69.5±5.3%, while the lowest, 54.0±2.8% was recorded in treatment A.

The observation data for water quality, including temperature, pH, DO, alkalinity, TAN, and abundance of *Vibrio* are presented in Table 2. Furthermore, the water quality of the media during culture was still in line with the standard levels for Pacific white shrimp cultivation.

Table 2

Water quality during the study

Parameter	*Day of culture*	*Treatment* A (17:1)	B (27:1)	C (37:1)	*Standards*	*References*
Temperature (°C)		24.7 –26.8	24.7 –26.5	24.6 –26.2	23–30	Wyban & Sweeney (1991)
pH		7.6 –8.4	7.6 –8.4	7.7 –8.4	7.4-8.9	Wyban & Sweeney (1991)
DO (mg L^{-1})		4.47 –6.45	4.51 –6.56	4.27 –6.50	4-5	Wyban & Sweeney (1991)
Alkalinity (mg L^{-1})		107.0	108.5	117.5	>80	Cuéllar-Anjel et al (2010)
TVC (CFU mL^{-1})	1	-	-	-	<10^2	Cuéllar-Anjel et al (2010)
	35	-	-	-		
Ammonia (mg L^{-1})	24	0.009	0.007	0.004	<0.1	Cuéllar-Anjel et al (2010)
	35	0.004	0.004	0.007		

Note: DO - dissolved oxygen; TVC - total *Vibrio* count.

Based on the results using the freshwater medium, the Na^+:K^+ ratio of 27:1 produced the best growth and survival rates for Pacific white shrimp. This indicates that the absorption of K ions by the animal is influenced by the presence of Na ions. Similar results were also obtained by Suguna (2020), who conducted a study on Pacific white shrimp in brackish water. K minerals work together with Na in regulating the balance of fluid electrolytes in the body, where potassium is the main intracellular cation that is important in the activation of Na^+ K^+ -ATPase. It is also involved in ion transport, osmoregulation processes, and regulation of extracellular volume. Lack of potassium can cause slow growth and increased mortality (Boyd 2018). The addition of sodium in the rearing media can optimize the osmoregulation system (Mantel & Farmer 1983). These findings are consistent those of Boyd (2018), confirming that the ideal ratio of Na^+ and K^+ ions in normal seawater (34.5 ppt) is 27:1, and it is suitable for shrimp life. The main cation equivalents (macrominerals) contained in seawater can be estimated using normal and lower seawater salinity, or freshwater maintenance media. Antony et al (2015) revealed that the best Na^+:K^+ ratio for *Penaeus monodon* in brackish water ranged from 25-45.1. Meanwhile, Liu et al (2014) stated that ratios ranging from 23:1 to 33:1 can increase the growth and survival rate of Pacific white shrimp reared in low salinity.

Sodium has a very important function in the shrimp, playing a role in osmoregulation, namely ion exchange with the environment (Huong et al 2010). Potassium is an essential mineral in the body, helping in the activation of Na^+K^+-ATPase (Mantel & Farmer 1983; Shiau & Hsieh 2001). It is also the principal intracellular cation, while sodium is the major extracellular cation and chloride is the major extracellular anion (Shiau & Hsieh 2001).

***Vibrio* presence**. One of the main causes of loss in *L. vannamei* farming is disease attack, both viral (Ramos-Carreño et al 2014) and bacterial, especially with *Vibrio* (Kharisma & Manan 2012). White feces syndrome can attack the animal, causing failure in its cultivation (Hou et al 2018; Arisa et al 2021). Furthermore, *Vibrio parahaemolyticus* is the causal organism for white feces syndrome (WFS) (Limsuwan 2010; Supono et al 2019) and acute hepatopancreatic necrosis disease (AHPND) (Sriurairatana et al 2014). In this study, *Vibrio* was not detected on TCBS media at the end of the experiment, namely after 35 days. Its growth was inhibited in fresh water media, but the possibility can still exist within a longer rearing period, even though the density level is low. This finding is in line with Reed & Francis-Floyd (2002), who noted that *Vibrio* sp. can occur in shrimp originating from seawater and estuaries, and sometimes in freshwater. Although the possibility is minimal, early prevention must be carried out along with maintenance of optimal water quality to reduce its density levels.

Conclusions. Different ratios of sodium (Na^+) and potassium (K^+) in freshwater media affected the growth and survival rate of Pacific white shrimp (*L. vannamei*) reared for 35 days. The best ratio of Na^+ and K^+ was 27:1, which gave an absolute weight growth value, growth rate, and survival rate of 1.69 ±0.03 g, 11.9±0.1%, and 69.5±5. 3%, respectively.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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