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# Artificial Seawater Use of Bittern Minerals on the Survival and Growth of Vannamei Shrimp (*Litopenaeus vannamei*) and Potential Resources

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**ABSTRACT**

Whiteleg shrimp (*Litopenaeus vannamei*) cultivation can be carried out in areas far from the sea with the RAS (Recirculating Aquaculture Systems) system using artificial seawater (ASW) added with bittern as a mineral source. This study aimed to determine the effect of artificial seawater formulation using bittern minerals on growth, survival, glucose levels, and hepatopancreas histology in whiteleg shrimp. This study used a completely randomized design (CRD) with 5 treatments and 4 replications including (P0) seawater; (P1) 18.56 grams of crossover salt + 8.4 ml/liter of bittern; (P2) 14.93 grams of crossover salt + 16.86 ml/liter of bittern; (P3) 11.53 grams of crossover salt + 25.13 ml of bittern/liter; (P4) 23 grams of crossover salt/liter. This study began with the crystallisation and characterisation of bittern, continued with mineralisation tests, and prepared artificial seawater using a combination of salt and bittern solutions. Cultivation was carried out for 20 days, and then survival, growth in length and weight, water quality, blood glucose, and histology of hepatopancreas tissue were observed. The formulation of artificial seawater treated with P1 was the best, with a survival rate of 78% and growth no different from control seawater. Therefore, artificial seawater has the potential to be used for the cultivation of whiteleg shrimp.

**Keywords**: artificial seawater, bittern mineral, *Litopenaeus vannamei*

## INTRODUCTION

Whiteleg shrimp (*Litopenaeus vannamei*) cultivation in Indonesia is a leading sector that is a priority for national aquaculture development. Shrimp production in Indonesia in the last five years was 4,736,445.42 tons with a revenue value of IDR 189,457,816,800,000.00 (KKP, 2023). Various efforts have been made to increase the productivity of whiteleg shrimp (*L. vannamei*) cultivation by improving cultivation technology from semi-intensive to intensive (Soegianto *et al.*, 2025). Whiteleg shrimp production in Indonesia can be increased again by expanding the cultivation area, not only in coastal areas but also needs to be developed in land areas (Witoko *et al.*, 2018). Opportunities for whiteleg shrimp cultivation on land have limitations regarding seawater sources and the cost of moving from the coast to the cultivation site. Therefore, artificial seawater is needed to meet the need for seawater (Pan *et al.*, 2024).

Artificial seawater production is limited by the high price of minerals (Scholz *et al.*, 2025). This can be overcome using salt production waste called bittern (Randazzo *et al.*, 2024). Waste from the salt production process, known as bittern, contains several compounds, including sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) (Jumaeri *et al.*, 2021). In addition, bittern also contains several other compounds, such as chloride (Cl), bromine (Br-), and sulfate ions ($SO_4^{2-}$) (Wajima, 2015). Bittern itself can be obtained abundantly in Indonesia. According to data published by BPS (Central Statistics Agency) and KKP (Ministry of Maritime Affairs and Fisheries) in 2022, national salt production in 2021 and 2022 was 879.9 thousand tons and 859 thousand tons, respectively, with each ton of salt production producing up to 1.9 m3 of bittern (Darwis *et al.*, 2022).

The bittern content is rich in minerals and can be used as artificial seawater (ASW), which can be used in the cultivation process (Okutsu *et al.*, 2018). Artificial seawater (ASW) is a mixture of chemical compounds consisting of mineral salts dissolved in water designed to replicate seawater by changing its components to resemble real seawater (Wawrzyniak *et al.*, 2021). Mineral content such as calcium (Ca), copper (Cu), magnesium (Mg), sodium (Na), potassium (K), and chlorine (Cl) contained in ASW can be utilised for the process of cultivating vannamei shrimp (Truong *et al.*, 2022). Sufficient mineral needs for shrimp cultivation can increase the growth and immune response of vannamei shrimp (Patrachotpakinkul *et al.*, 2021). According to research by Ilham *et al.* (2024), adding minerals can increase the growth and immune response in vannamei shrimp. In addition, adding organic Cu minerals can increase the immune response of vannamei shrimp (Yang *et al.*, 2023). This study aims to determine the optimal dose of a bittern in artificial seawater (ASW) as a medium for cultivating vannamei shrimp.

## MATERIALS AND METHODS

### Crystallisation test and characteristics

The bittern solution was frozen in a freezer. The frozen solution was then crystallised in a freeze dryer at -50 °C with a pressure of 129 mbar for 36 hours. The bittern crystal salt obtained was placed in an airtight container before further analysis. The yield obtained from the crystallisation test is calculated using the following formula:

$$Yield = \frac{Weight\ of\ experimental\ product\ (Final\ weight)}{Theoretical\ product\ weight\ (\ Start\ weight)} \times 100\%$$

### Mineralization test

Bittern mineral testing employs various analytical methods, including gravimetry, atomic absorption spectrometry (AAS), argentometry, spectrophotometry, and complexometry. These methods operate on the principle that the weight of an element or compound in a sample can be accurately determined after separation and conversion into a pure, stable substance.

### Experimental procedure

This study utilised DOC 30 whiteleg shrimp acclimatised to 20 ppt salinity. During acclimatisation and experimentation, shrimp were fed using an index method (index value: 0.6). Over 20 days, survival, growth (length and weight), water quality, blood glucose, and hepatopancreas histology were monitored.

Artificial seawater was prepared by mixing salt and bitter minerals in freshwater, filtering through a 40 µm sieve, and adjusting to 20 ppt salinity (15 L volume). The artificial seawater formulation employed a Completely

Randomized Design (CRD) consisting of five treatments with four replications each P0 (15 liters of seawater); P1 (18.56 grams of coarse salt + 8.4 ml bittern/liter); P2 (14.93 grams of coarse salt + 16.86 ml bittern/liter); P3 (11.53 grams of coarse salt + 25.13 ml bittern/ liter); P4 (23 grams of coarse salt/liter).

### Hepatopancreas histology

The histological procedure was carried out according to Li *et al.* (2024) and the hepatopancreas from each treatment was taken and fixed for 24 hours. After that, the samples were dehydrated, washed, and immersed in paraffin. The samples were then sliced using a microtome with a thickness of 5 μm followed by staining with hematoxylin and eosin. After the staining process, the samples could be observed under a microscope.

### Glucose level measurement

Glucose levels were measured using the Cruz-Moreno *et al.* (2024) method. Glucose levels were measured in a microplate test. Glucose values were measured using a glucose oxidase test kit. Absorbance was measured at 500 nm in a spectrophotometer.

### Growth performance and survival rate

During the maintenance period, observations were made on the growth of vannamei shrimp, specifically weight gain and length gain, based on the formula proposed by Souza *et al.* (2019):

$$Weight\ gain = final\ weight - initial\ weight$$

$$Length\ gain = final\ length - initial\ length$$

$$Survival\ rate = \frac{Final\ fish\ number}{initial\ fish\ number} \times 100$$

### Water quality test

During the research, water quality assessments were conducted on the maintenance media, encompassing parameters such as pH, temperature, dissolved oxygen (DO), and salinity.

### Statistical analysis

Data were statistically tested using one-way analysis of variance (ANOVA) ($P < 0.05$) followed by Duncan's test to compare various treatments on several observed parameters.

## RESULT AND DISCUSSION

### Crystallisation test and characteristics

Several factors can influence Bittern's characteristics, namely the water source's chemical composition, evaporation method, and climate conditions (Bagastyo *et al.*, 2021). In addition, according to Amdouni (2009), biological influences, such as the physiological process of algae, can also be a determining factor in the characteristics and quality of Bittern.

The crystallisation test results of 100 ml of Bittern yielded 68.5 grams of bittern crystal salt. The crystallisation test results of Bittern show that Bittern's salt and mineral content is 68.5%. According to Shirazi *et al.* (2015), the bittern content in salt waste can reach 85-95%. The high value of Bittern can be influenced by the season (Femitha and Vaithayanatan, 2012). In addition, the bitter taste can also be caused by the evaporation process, which at a temperature of 34-35°C can increase the bromide content in the Bittern (Dave and Ghosh, 2005). This very high yield makes bittern water a potential source of minerals in the production of artificial seawater.

### Mineralization test

The test for characterizing the salt content of bittern gave the following results (Table 1). The mineral content was analyzed using several methods, including gravimetry, atomic absorption spectrometry (AAS), argentometry, spectrophotometry, and complexometry.

**Table 1.** Crystallization test and characteristics

| Parameter | Unit | Analysis Results | Analysis Method |
|---|---|---|---|
| Moisture content | % | 23,61 | Gravimetry |
| Sodium | % Na | 30,23 | AAS |
| Potassium | % K | 0,78 | AAS |
| Chloride | % Cl | 46,60 | Argentometry |
| Sulfate | % SO4 | 11,39 | Spektrofotometry |
| Calcium | % Ca | 0,00 | Complexometry |
| Magnesium | % Mg | 10,64 | Complexometry |

**Table 2.** Artificial Seawater formulation for the production of shrimp cultivation media

| Mineral | PO | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|
| **$Na^+$ (g/L)** | 6,52 | 7,01 | 7,86 | 8,18 | 7,5 |
| **Cl (g/L** | 11,51 | 11,02 | 11,0 | 10,19 | 11,3 |
| **K (g/L)** | 0,24 | 0,037 | 0,081 | 0,120 | 0,03 |
| **Mg (g/L)** | 0,78 | 0,57 | 1,10 | 1,63 | 0,04 |
| **Ca (g/L)** | 0,24 | 0,02 | 0,02 | 0,02 | 0,02 |
| **$SO_4$ (g/L)** | 1,62 | 1,02 | 1,59 | 2,12 | 0,45 |

The results show that bittern contains 23.61% water content, 30.23% sodium, 0.78% potassium, 46.60% chloride, 11.39% sulfate, 0.00% calcium, and 10.64% magnesium. This content is based on the results of research by Randazzo *et al.* (2024) that bittern contains 60.87 g/kg sodium, 7.41 g/kg potassium, 160.11 g/kg chloride, 36.83 g/kg sulfate, 0.166 g/kg calcium, and 33.94 g/kg magnesium on a dry weight basis. The mineral content in bittern has varying values. This statement is supported by Shirazi *et al.* (2015), who stated that the mineral content in bittern can be influenced by several factors, one of which is the pH value in the evaporation process. Low pH values can reduce magnesium values. The results were used to calculate the mineral content and formulation of artificial seawater with a salinity of 20 ppt.

## Artificial seawater formulation

The composition of bittern was calculated by comparison with the yield of bittern crystal salt. The bittern formulation is in Table 2. Different bitter minerals depict the mineral content in different artificial seawaters. Differences in bittern doses in artificial seawater affect the mineral content in the maintenance media. Minerals are one of the limiting factors in the shrimp cultivation process, so they can affect the success of the cultivation process (Estrada-Mata *et al.*, 2022).

The mineral content in artificial seawater is dominated by sodium, potassium, and chloride; this is because the bittern content that has been tested is dominated by minerals such as sodium, potassium, and chloride (Table 1). Adding sodium and chloride plays an important role in increasing the survival rate of whiteleg shrimp (Estrada-Mata *et al.*, 2022; Bagheri *et al.*, 2023). The addition of sodium to shrimp can also increase trypsin activity in the shrimp hepatopancreas and increase the length and circumference of the villi in the shrimp digestive organs, so that it has an impact on shrimp digestibility and decreases the FCR value (Silva *et al.*, 2016). In addition to sodium and chloride, potassium can increase growth and survival in white leg shrimp. This is because potassium can support the

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osmoregulation process in white leg shrimp, which can increase growth. However, potassium deficiency can cause damage to shrimp gills, characterized by the release and removal of the epithelial layer from the surface of the primary and secondary lamella, which causes bleeding in the shrimp gills (Pathak *et al.*, 2024).

**The glucose level of vannamei shrimp**

The stress level in this study was measured based on the glucose levels in the hemolymph of whiteleg shrimp. The test results showed that the glucose levels of whiteleg shrimp at P0, P4, and P1 were in the normal range because they had glucose levels below 150 mg/dL for the first 6-12 hours. In contrast, glucose levels in P2 and P3 showed an increase (Figure 1). Stress in shrimp can be indicated by increased glucose levels in the hemolymph (Mahasri *et al.*, 2021). High glucose levels of over 150 mg/dl indicate stress in whiteleg shrimp (Cuzon *et al.*, 2004). Shrimp cultivated using the P2 and P3 formulations have the potential to experience high levels of stress. The level of stress in shrimp can be caused by several factors, namely disease attacks and environmental conditions, including pH, temperature, ammonia levels (Kathyayani *et al.*, 2019; Shirly-Lim *et al.*, 2024), and stocking density (Mahasri *et al.*, 2021).

Based on the study's results, increased glucose levels can be caused by the high Na: K ratio in P2 and P3. Whiteleg shrimp can grow optimally at a Na: K ratio of 27:1 (Supono *et al.*, 2023). According to Turnbull *et al.* (2021), a decrease in K value and an increase in the Na: K ratio can cause stress in shrimp. The lack of K value in the whiteleg shrimp cultivation medium can inhibit the activity of the Na-K-ATPase enzyme (Supono *et al.*, 2023). The Na-K-ATPase enzyme maintains osmolarity in shrimp by releasing three sodium ions and adding two potassium ions into the cell to maintain the balance of the cell membrane. This activity requires ATP hydrolysis as an energy source needed to move ions (Lucu and Towle, 2003).

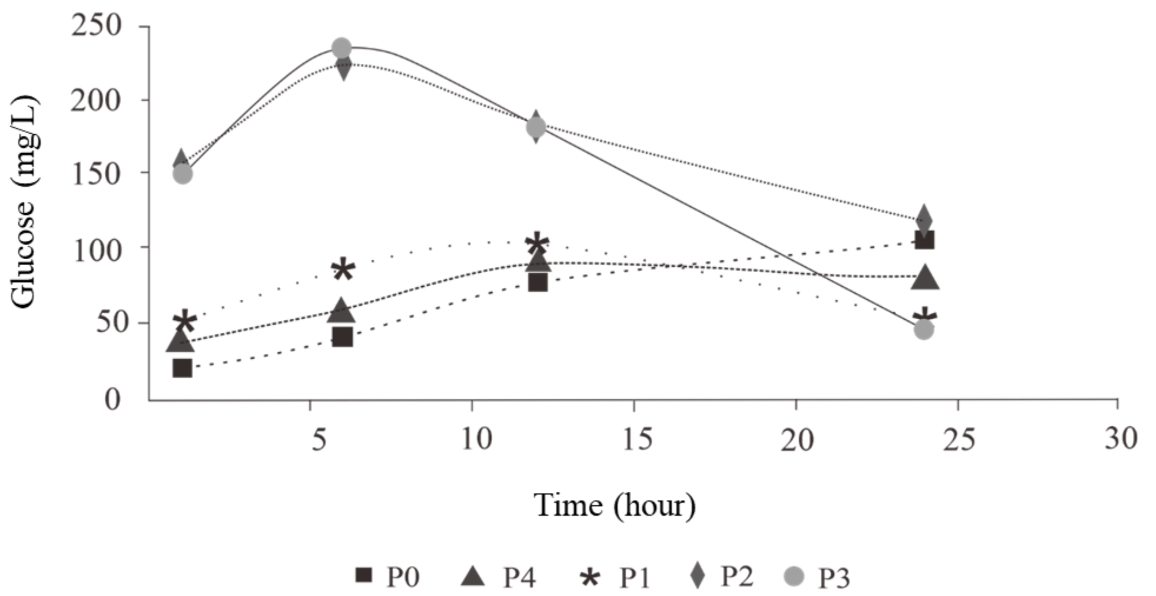

**Figure 1.** The glucose level of vannamei shrimp

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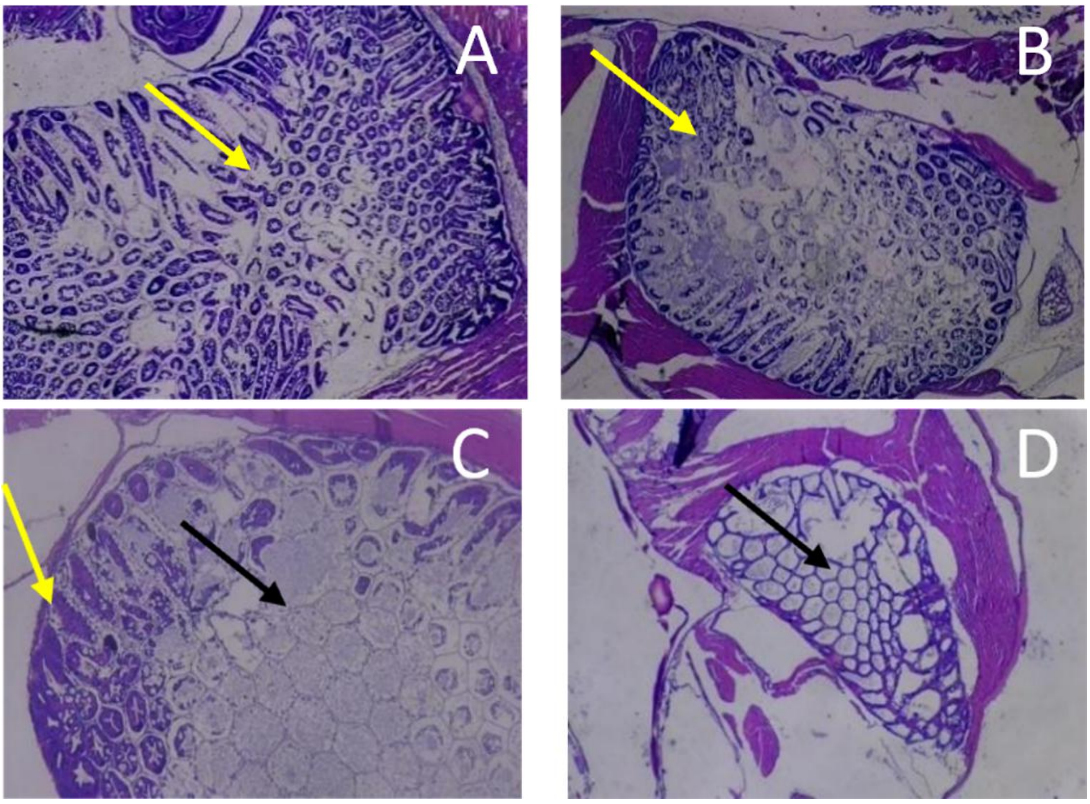
**Histopathology of the hepatopancreas**

The hepatopancreas is an important organ for crustaceans' detoxification, excretion, and metabolism (Huaping *et al.*, 2024). The results of histological analysis (Figure 2) show that P0, P1, and P4 are healthy hepatopancreas cells characterized by organelles, while in treatments P2 and P3, the hepatopancreas showed cells that did not have organelles. This is because the imbalance in mineral content causes dependence on the electrical potential of cells, which leads to disruption of coordination between cell organelles so that organelles experience a lack of nutrient supply, and lysis or damage occurs (Tulungnen *et al.*, 2017). According to Huaping *et al.* (2024), one of the conditions of unbalanced mineral content in whiteleg shrimp cultivation is characterized by the Na: K ratio. Na: K values of more than 87:1 can cause damage to B cells, characterized by changes in structure and increased alveolar volume, and some damage occurs to the structure of the liver tubules, which can reduce digestive function in whiteleg shrimp.

B cells play a role in shrimp digestion and nutrient absorption and indicate environmental stress in whiteleg shrimp (Li *et al.*, 2024). So, hepatopancreas damage in shrimp causes a decrease in optimal shrimp growth because shrimp metabolism is disrupted (Cao *et al.*, 2023). The damage mechanism begins with inflammatory cells that migrate to the infestation site and fight against the infected cells. Histopathological changes due to inflammatory cell invasion are characterized by infiltrating inflammatory cells into normal tissue. If there are damaged cells and tissues, inflammatory cells will exit the blood vessels and head to the infiltrated area so that the blood vessel tissue is found to have many vacuoles (Zhahrah *et al.*, 2016). Based on this, the use of bitter minerals to produce artificial seawater requires measurements of the mineral content to follow the needs of whiteleg shrimp.



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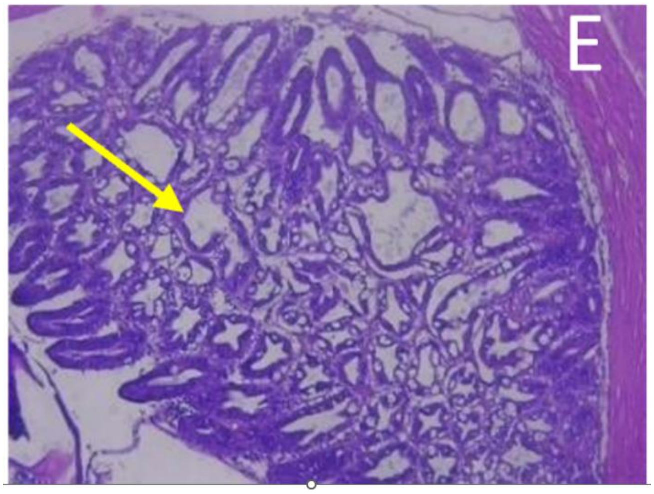


**Figure 2.** Results of shrimp hepatopancreas test at 100x magnification (black arrow lysed cells and yellow arrow intact cells). Description (A) P0: seawater (B) P1: 18.56 grams coarse salt + 8.4 ml bittern/liter (C) P2: 14.93 grams coarse salt + 16.86 ml bittern/liter (D) P3: 11.53 grams coarse salt + 25.13 ml bittern/liter (E) P4: 23 grams coarse salt/liter.

### Survival and growth of vannamei shrimp

Based on the research results, the formulation of artificial seawater with several formulations affects the survival and growth of whiteleg shrimp. The survival values at P0 and P1 have the highest values and show a decrease along with the increase in the dose of bittern used. These results are directly proportional to the growth of whiteleg shrimp, which shows the highest value at P0, and there is a decrease in growth along with the increase in the dose of bittern used. This decrease can be caused by the high concentration of bittern, which causes an increase in magnesium and sulfate minerals, which have toxic properties at high concentrations.

The potassium, magnesium, and sulfate concentrations can reduce the survival rate of whiteleg shrimp (Sharma *et al.*, 2023). In addition, the high Na: K ratio in this study can cause a decrease in the growth and survival of whiteleg shrimp. This statement is supported by Supono *et al.* (2023), who stated that the Na: K value above 27:1 can affect the activity of the Na-K-ATPase enzyme, so that it can cause a decrease in the survival value and growth of whiteleg shrimp. High Na: K values can cause an increase in Na-K-ATPase values, which indicates stress caused by the osmoregulation process. An increase in Na-K-ATPase values can absorb more energy from whiteleg shrimp to survive, so that it can reduce growth and cause death (Li *et al.*, 2011; Gao *et al.*, 2016).

### Water quality test

Water quality parameters are critical to know to support the growth of whiteleg shrimp (Destarianto *et al.*, 2023). Based on the research results, the average water quality values, namely DO, salinity, pH, and temperature, are optimal for the growth of whiteleg shrimp. The average DO, temperature, pH, and salinity values during the research period were 7.5 mg/l, 28.7°C, 8.5, and 20 ppt, respectively. These values are good values for the whiteleg shrimp cultivation process. The optimal DO and temperature values in the cultivation process are > 4 mg/l and 29 - 33 °C, respectively (Musa *et al.*, 2020). The optimal pH and salinity values in the shrimp cultivation process range from 6.5 - 8.5 and 5 - 34 ppt, respectively (Kasnir *et al.*, 2014). Good water quality management can maintain quality according to farming standards and increase pond productivity (Fuady *et al.*, 2014).

## CONCLUSION

Artificial seawater formulation with mineral bittern affects the survival and growth of *L. vannamei*. Formulation P1 (18.56 g coarse salt + 8.4 ml bittern (per liter)) is the best formulation with an SR of 78%, which is not significantly different from the seawater control supported by normal blood glucose tests below 150 mg/L and healthy hepatopancreas tests characterized by cells containing organelles. Artificial seawater has the potential to be used for the cultivation of the *L. vannamei* RAS system in areas far from the coast.

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## AUTHORS CONTRIBUTIONS

The contributions of each author are as follows: RSRS, MNF, RTPH, KIN, and NPL; collected data; processed results; drafted the manuscript; and designed tables and graphs. ASM and MA developed the main conceptual ideas and critically revised the article. All authors discussed the study's results and contributed to the final manuscript.

## CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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