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Evaluation of Different Magnesium Concentrations on the Performance of Abalone (*Haliotis squamata*) Cultured in a Recirculating Aquaculture System

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ABSTRACT

This study examined the effect of magnesium (Mg^{2+}) supplementation in a recirculating aquaculture system (RAS) on the growth performance and physiological responses of *Haliotis squamata*. Four Mg^{2+} concentrations (0, 0.125, 0.250, and 0.375 g L^{-1}) were tested over a 120-day period, using *Gracilaria gigas* as the sole feed source. The 0.250 and 0.375g L^{-1} treatments significantly enhanced specific growth rate, final body weight, and shell length of abalone ($P< 0.05$). Magnesium retention in both meat and shell tissues increased, accompanied by elevated antioxidant capacity, RNA/DNA ratio, and meat pH, particularly at 0.375g L^{-1}. However, this concentration also led to a decrease in meat protein content, alongside increases in moisture and lipid levels. A reduction in seawater Mg^{2+} concentrations confirmed the active ion uptake by abalone. This study proposes an integrated strategy that combines essential mineral regulation with macroalgal-based feeding in a closed-loop system. Such integration improves ionic balance and physiological homeostasis, thereby promoting sustainable and efficient abalone aquaculture. These findings offer a scientific basis for optimizing Mg^{2+} levels in nutrient management strategies for tropical mollusk production.

INTRODUCTION

Abalone (*Haliotis squamata*) is a marine gastropod species of high economic value due to its nutritional richness and unique flavor profile **(Ahyeong *et al.*, 2020; Ardi *et al.*, 2020; Auzoux-Bordenave *et al.*, 2020; Hadijah *et al.*, 2021)**. This species is consumed in various forms, including cooked, raw, frozen, or canned products. It is known to be a rich source of bioactive compounds such as fatty acids **(Suleria *et al.*, 2017)**, essential minerals **(Latuihamallo *et al.*, 2019)**, and phenolics, glycogen, carbohydrates, proteins,

amino acids **(Mohammadi *et al.*, 2022)**, all of which contribute to both its nutritional value and potential health benefits.

Nutritional analysis indicates that 100g of edible *H. squamata* contains approximately 20g of high-quality protein **(Shi *et al.*, 2020)**. Additionally, **Mulvaney *et al.* (2015)** reported that 100g of *H. squamata* provides 94mg of long-chain n-3 polyunsaturated fatty acids (LC-PUFAs), surpassing the levels found in beef (70mg), pork (26mg), and chicken (50mg). These nutrients play a critical role in supporting cardiovascular health, brain function, and immune response. Therefore, *H. squamata* is not only valued for its culinary appeal but also recognized as a functional food with potential health-promoting benefits **(Suleria *et al.*, 2017)**.

The global demand for *H. squamata* remains high in countries such as Japan, the United States, Colombia, and Canada **(Cook, 2014; Pratiwi *et al.*, 2024)**. However, the supply still heavily depends on wild capture. Overexploitation has led to a dramatic decline in global catch, from approximately 20,000 metric tons **(**mt**)** in the 1970s to around 4,500 mt in 2020 **(Cook, 2025)**. Although aquaculture of *H. squamata* has been developed in countries like Japan, New Zealand, and the Philippines **(Grandiosa 2020; Hadijah *et al.*, 2021)**, Indonesia still relies predominantly on wild capture **(Merdekabasuki *et al.*, 2024)**. If unmanaged, continued overharvesting may threaten natural populations **(Yusup *et al.*, 2020; Permana *et al.*, 2024)**.

Despite its economic potential, the sustainable utilization of *H. squamata* in Indonesia has yet to be fully realized **(Dwi *et al.*, 2023)**. Signs of overexploitation include decreasing harvest sizes and declining catch volumes **(Ozyurt *et al.*, 2017; Al-Ghassani *et al.*, 2022)**. One promising approach to address this issue is the development of *H. squamata* aquaculture. As an herbivorous mollusk, *H. squamata* relies on macroalgae—particularly *Gracilaria* spp.—for nutrition, both in aquaculture environments and in its natural habitat **(Li *et al.*, 2024)**. These algae are rich in carbohydrates such as agar and Floridean starch, which provide essential energy for growth and metabolic processes **(Kemp *et al.*, 2015; Prasetiyono *et al.*, 2024)**.

However, abalone aquaculture still faces technical challenges, including water quality fluctuations, net clogging, impaired water circulation, structural damage to nets, waste accumulation, and low dissolved oxygen (DO) levels **(Supriyono *et al.*, 2020)**. One solution to these challenges is the implementation of recirculating aquaculture systems (RAS)—an increasingly popular global technology that reuses water through mechanical and biological filtration **(Bregnballe, 2022; Maher *et al.*, 2023; Hutagalung *et al.*, 2024; Lembang *et al.*, 2025)**. These systems efficiently treat nitrogen compounds, particularly by reducing total ammonia nitrogen (TAN), with the aid of filter media such as bio-balls and bio-rings **(Pedreira *et al.*, 2016; Almeida *et al.*, 2019; Rahmat *et al.*, 2019)**. RAS can help maintain optimal water quality parameters for the culture of *H. squamata* larvae and juveniles.

In addition, the supplementation of magnesium ions (Mg^{2+}) in the culture water is expected to enhance both meat growth and shell development in *H. squamata*. Magnesium is essential for molluscan physiology and shell structure, as it is one of the primary components of the shell **(Cobo *et al.*, 2017; García-Escárzaga *et al.*, 2018)**. It acts as a cofactor in numerous enzymatic reactions, including those involved in energy metabolism, DNA and protein synthesis, and ion transport regulation **(Reddy *et al.*, 2018; Mathew & Panonnummal 2021; Yamagami *et al.*, 2021)**.

Maintaining adequate Mg^{2+} levels is thus critical for survival and development. As a divalent cation, Mg^{2+} binds with negatively charged components of cell membranes, helping to stabilize cell surface charges. In *H. squamata*, Mg^{2+} serves as a key intracellular ion involved in enzyme activation, hormone signaling, protein synthesis, and cell division **(Chen & Huang 2013; Evans *et al.*, 2021)**. Abalone species, including *H. squamata*, are known to absorb Mg^{2+} primarily through their gill epithelium **(Griffith 2017; Latuihamallo *et al.*, 2019)**.

Although limited research has been conducted on the effects of Mg^{2+} supplementation in *H. squamata*, previous studies have demonstrated the positive role of magnesium in promoting the early development of fish **(Ghobadian *et al.*, 2015)** and shrimp larvae **(Srinivasan *et al.*, 2017)**. Therefore, this study aimed to evaluate the effect of different Mg^{2+} concentrations on the growth performance of *H. squamata* cultured in a recirculating aquaculture system.

MATERIALS AND METHODS

1. Description of the study sites

This study was conducted from April to August 2023 at the Laboratory of the Indonesian National Research and Innovation Agency (BRIN), located in Gondol, Bali, at coordinates 8°09′13.4″S and 114°42′51.9″E. The laboratory is situated near stable coastal waters, providing favorable environmental conditions for marine aquaculture studies. All activities related to culture, water quality monitoring, and sample analysis were performed in this facility under controlled laboratory conditions.

2. Experimental design

The experimental procedure was adapted from **Reitz *et al.* (1960)** with slight modifications. The study employed a completely randomized design **(**CRD**)** consisting of four treatments with four replicates. The treatments involved the addition of magnesium ions (Mg^{2+}) at concentrations of 0, 0.125, 0.250, and 0.375g L^{-1}. If water volume decreased due to evaporation, water and Mg^{2+} were replenished according to the respective treatment to maintain water quality and quantity, following the approach by **Roy *et al.* (2007)**.

Juvenile abalone (*Haliotis squamata*) used in this study were obtained from the Balai Produksi Udang Unggul dan Kekerangan, Karangasem, under the supervision of the Directorate General of Aquaculture, Ministry of Marine Affairs and Fisheries (DJPB–

KKP), Bali. These were first-generation offspring (F_1) from wild-caught broodstock (F_0) spawned in December 2022, and were three months old at the beginning of the study. The shell length of the individuals ranged from 28 to 33mm, and a total of 240 individuals were used.

Culture was carried out in circular plastic basins (48cm in diameter and 28cm in height), each filled with 25 L of seawater. Every container was equipped with a polyvinyl chloride (PVC) shelter measuring 20 × 15 × 10cm and provided with continuous aeration. The stocking density was maintained at 165 individuals m^{-2}, equivalent to 15 individuals per basin. A recirculating aquaculture system (RAS) was applied to all treatments, utilizing dacron-based physical filters to ensure stable water quality.

The addition of Mg^{2+} followed the method developed by **Roy *et al.* (2007)** and **Zacarias *et al.* (2019)** for *Litopenaeus vannamei*. Water quality parameters, including temperature, pH, dissolved oxygen (DO), and salinity, were monitored every three days. In addition, alkalinity and total ammonia nitrogen (TAN) were measured monthly. Growth performance, including shell length and body weight, was assessed every 30 days throughout the experimental period. At the end of the culture period, samples were collected for magnesium concentration analysis from the abalone's meat, shell, feed, and water from the RAS. A total of 11 individuals per treatment (n = 11) were randomly selected for final sampling and laboratory analysis.

3. Chemical analysis of *H. squamata* meat and shells

Meat and shell samples of *Haliotis squamata* were collected before and after the culture period, with 11 individuals randomly selected from each treatment group. The meat was separated from the shell, rinsed with freshwater, oven-dried at 50°C for 72 hours, ground, and sieved using a 320µm mesh. The prepared samples were then analyzed for magnesium (Mg^{2+}) concentration using atomic absorption spectrophotometry (AAS) following the method of **Reitz *et al.* (1960)**. The analytical procedure involved digestion of the sample with a mixture of nitric acid (HNO_3) and sulfuric acid (H_2SO_4) in an Erlenmeyer flask, followed by heating on a hot plate. After digestion, a solution of perchloric acid–nitric acid ($HClO_4$–HNO_3) was added, and the mixture was filtered prior to Mg^{2+} determination using AAS at a wavelength of 285.2nm.

4. Chemical analysis of *Gracilaria gigas*

The concentration of magnesium (Mg^{2+}) in *Gracilaria gigas* was determined by analyzing both initial and final samples from each treatment, with 78g of biomass collected per sampling point (78 g × 4 treatments). The samples were oven-dried at 50°C for 72 hours, then ground using a blender until a fine texture was achieved. The powdered samples were subsequently sieved through a 320µm mesh. Magnesium content was analyzed using atomic absorption spectrophotometry (AAS), following the method described by **Reitz *et al.* (1960)**.

5. Biochemical analysis

Random samples of *Haliotis squamata* (n = 2 individuals × 4 treatments) were collected to measure antioxidant activity following the method of **Maskar *et al.* (2015)**. Approximately, 0.5g of minced abalone meat was homogenized in 2.5mL of phosphate buffer (pH 7.0) and centrifuged at 3000 rpm for 10 minutes at 4°C. The resulting supernatant was mixed with 0.8mL of a chloroform–ethanol solution (3:5, v/v), vortexed, and recentrifuged. A volume of 1mL of the final supernatant was combined with 2.8mL of sodium carbonate buffer (pH 10.2) and 100µL of epinephrine. The absorbance was measured using a spectrophotometer at 480nm every minute for 4 minutes. The percentage inhibition and superoxide dismutase (SOD) activity were calculated using the following formulas:

$$\text{Inhibition (\%)} = (\text{Abs B} - \text{Abs S}_{4-1}) / \text{Abs B} \times 100$$

$$\text{SOD activity (U/mL)} = (\% \text{ inhibition} \times 10 / 50) \times 0.1$$

where Abs B is the absorbance of the blank, and Abs S_{4-1} is the absorbance difference between minute 4 and minute 1. The unit of SOD activity (SOD_{50}) is defined as the amount of enzyme required to cause 50% inhibition of perferryl ion oxidation.

6. Proximate analysis

The proximate composition of *H. squamata* meat and *Gracilaria gigas* was analyzed according to the standard procedures of the Association of Official Analytical Chemists **(AOAC, 1990)**. Moisture content was determined by oven-drying the samples at 105°C until a constant weight was achieved. Protein content was measured using the Kjeldahl method, which involved digestion with concentrated sulfuric acid, distillation of the released ammonia, and titration; nitrogen values were converted to protein content using a conversion factor of 6.25. Fat content was extracted using the Soxhlet method with anhydrous ether as the solvent. Crude fiber was measured via sequential digestion with 0.255 N sulfuric acid followed by 0.313 N sodium hydroxide, and the residue was dried and weighed. Ash content was quantified by incinerating the samples in a muffle furnace at 550°C for 5 hours until stable white or light-colored ash was obtained. The nitrogen-free extract (NFE), representing soluble carbohydrates, was calculated by difference using the formula:

$$\text{NFE (\%)} = 100 - (\text{moisture} + \text{ash} + \text{protein} + \text{crude fiber} + \text{fat}).$$

All analyses were performed in duplicate to ensure accuracy and reproducibility.

7. Analysis of seawater Mg^{2+} concentration

Seawater samples were collected before, during, and after the experimental period to determine magnesium concentrations based on SNI 06-6989.12 **(BSN, 2004)**. Samples were filtered through 0.45 µm membrane filters to remove suspended solids. A 10mL aliquot of the filtrate was pipetted into a 100mL volumetric flask and diluted to volume with 0.05 M nitric acid (HNO_3). The solution was homogenized, and then a second 10mL

aliquot was transferred to another 100mL flask, again diluted to the mark with 0.05 M HNO_3 and homogenized. A standard curve was prepared using Mg^{2+} standard solutions at concentrations of 0.0, 0.1, 0.2, 0.6, and 0.8ppm. Magnesium concentrations were then determined using atomic absorption spectrophotometry (AAS) at a wavelength of 285.2nm.

8. Evaluation of growth performance and feed efficiency

Growth performance parameters for *H. squamata* in the RAS system under different Mg^{2+} treatments included survival rate, specific growth rate (SGR), shell length increment, magnesium retention, and feed efficiency. Survival (%) was calculated by comparing the final and initial number of individuals. SGR (% day^{-1}) was calculated based on the logarithmic difference in body weight over the culture period. Shell length growth was assessed by the difference between final and initial shell lengths. Magnesium retention (%) was calculated by dividing the magnesium content in abalone tissue by the total magnesium consumed via feed, based on an initial meat Mg^{2+} concentration of 12.30mg kg^{-1}. Feed efficiency (%) was determined from the ratio of biomass gain to total feed administered.

9. Measurement of pH of *H. squamata* meat

The pH of abalone meat was measured using a calibrated pH meter (Hanna Instruments HI2213). A 10g sample of *H. squamata* meat was homogenized with 90mL of distilled water using a Nissei AM-3 homogenizer at 300– 500rpm. The pH was recorded once the instrument displayed a stable reading, following the protocol of **Lin *et al.* (2021)**.

10. Water quality

Water quality was assessed every two days throughout the culture period. Parameters measured included temperature, dissolved oxygen (DO), salinity, pH, alkalinity, and total ammonia nitrogen (TAN). Water quality was expected to remain stable due to the efficiency of the RAS system and the minimal residual impact of *G. gigas* feed **(Akhtar *et al.*, 2021)**.

11. Data analysis

All data are presented as mean ± standard deviation. Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. Differences between treatments were evaluated using one-way analysis of variance (ANOVA), and post hoc comparisons were performed using Duncan's multiple range test at a significance level of $P< 0.05$ **(Abdullah *et al.*, 2019)**. All statistical analyses were conducted using Microsoft Excel 2020 and SPSS version 24.0.

RESULTS

1. Growth [erformance and feed efficiency

The results indicated that increasing the concentration of Mg^{2+} to 0.375g L^{-1} produced the highest growth performance in *Haliotis squamata*. This was reflected in the shell length increment (ΔSL: 1525.63 ± 158.62μm), specific growth rate (SGR: 0.94 ± 0.04% day^{-1}), Mg^{2+} retention (15.32 ± 0.64%), and RNA/DNA ratio (0.83 ± 0.03). However, feed efficiency (FE) and survival rate (SR) did not differ significantly among treatments (Duncan's test, $P > 0.05$) (Table 1).

Table 1. Shell length increment (ΔSL), specific growth rate (SGR), magnesium retention, RNA/DNA ratio, feed efficiency (FE), and survival rate (SR) of *Haliotis squamata* cultured under different Mg^{2+} concentrations

Parameter	Mg^{2+} addition (g L^{-1})			
	K	0.125	0.250	0.375
(SGR %)	0.72±0.03^{a}	0.83±0.06^{b}	0.90±0.03^{c}	0.94±0.04^{c}
(ΔSL μm)	1,116.67±197.44^{a}	1,041.04±113.88^{b}	1,500.00±197.04^{b}	1,525.63±158.62^{b}
Mg^{2+} retention	13.13±1.65^{a}	15.65±0.38^{b}	16.44±0.33^{b}	15.32±0.64^{b}
RNA/DNA	0.28±0.14^{a}	0.72±0.06^{b}	0.74±0.04^{b}	0.83±0.03^{b}
EP (%)	14.144±0.76^{a}	14.903±0.83^{a}	14.376±0.89^{a}	13.258±1.53^{a}
SR	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Note: Results are presented as mean ± standard deviation (SD), with sample size indicated for each parameter. Different superscript letters within the same row indicate statistically significant differences between treatments ($P < 0.05$). Specific growth rate (SGR), mean shell length increment (ΔSL), magnesium retention, and survival rate (SR) were each measured with n = 15. The RNA/DNA ratio was assessed with n = 2 per treatment. Feed efficiency (EP) and survival rate (SR) did not show significant differences among treatments ($P > 0.05$). K = Control (no Mg^{2+} addition).

As shown in Table (1), the growth performance of *Haliotis squamata* varied significantly with increasing Mg^{2+} concentrations. The highest shell length increment (ΔSL) was observed at 0.375g L^{-1} (1525.63 ± 158.62μm), which was significantly greater than the control (1116.67 ± 197.44μm) and the 0.125g L^{-1} treatment (1041.04 ± 113.88 μm) ($P < 0.05$). Specific growth rate (SGR) also increased gradually with Mg^{2+} supplementation, reaching the maximum value of 0.94 ± 0.04% day^{-1} at 0.375g L^{-1}, compared to 0.72 ± 0.03% day^{-1} in the control.

Magnesium retention followed a similar trend, ranging from 13.13 ± 1.65% in the control to 16.44 ± 0.33% at 0.250g L^{-1}, with significantly higher values at all Mg^{2+} treatments compared to the control ($P < 0.05$). Likewise, the RNA/DNA ratio showed a marked increase under Mg^{2+} supplementation, peaking at 0.83 ± 0.03 at 0.375g L^{-1}, which was nearly threefold higher than in the control (0.28 ± 0.14).

In contrast, feed efficiency (FE) and survival rate (SR) did not differ significantly among treatments ($P > 0.05$). Feed efficiency values ranged between 13.25 ± 1.53% and 14.90 ± 0.83%, while survival remained consistently high (100%) across all treatments. These findings indicate that Mg^{2+} addition primarily enhanced growth-related parameters without compromising feed utilization or survival performance.

2. Magnesium concentration in the meat and shell of *H. squamata* and in *Gracilaria gigas*

The concentration of magnesium (Mg^{2+}) in the shell of *Haliotis squamata* was found to be higher than that in the meat and increased with the addition of Mg^{2+} to the culture water. The shell Mg^{2+} content exhibited an upward trend across treatments, with values recorded as follows: control (K): 12.519 ± 0.160 g L^{-1}; 0.125 g L^{-1}: 35.374 ± 0.399 g L^{-1}; 0.250 g L^{-1}: 29.411 ± 0.650 g L^{-1}; and 0.375 g L^{-1}: 27.209 ± 0.340 g L^{-1} (Table 2).

Table 2. Magnesium (Mg^{2+}) concentrations in the meat and shell of *Haliotis squamata* and in *Gracilaria gigas* under different treatment concentrations

Parameter	Mg^{2+} addition (g L^{-1})			
	K	0.125	0.250	0.375
Mg^{2+} concentration in meat of *H. squamata*	14.633±0.550[a]	17.383±0.167[c]	17.311±0,448[c]	15.788±0.143[b]
Mg^{2+} concentration in shell of *H. squamata*	12.519±0.160[a]	35.374±0.399[d]	29.411±0.65[c]	27.209±0.340[b]
Mg^{2+} in *G. gigas*	9.619±0.03[a]	17.873±0,02[C]	18.121±0,05[d]	17.206±0,05[b]

Note: Values are presented as mean ± standard deviation (SD) (n = 11). Different superscript letters within the same row indicate statistically significant differences between treatments ($P < 0.05$). Mg^{2+} = magnesium; K = control (no Mg^{2+} addition); G. gigas = *Gracilaria gigas*, the feed source for *H. squamata*.

These results indicate that the shell tissue has a greater capacity for Mg^{2+} accumulation than the soft tissue (meat) of *H. squamata*. This pattern is attributed to the role of Mg^{2+} in the biomineralization of calcium carbonate ($CaCO_3$), in which magnesium ions can replace calcium (Ca^{2+}) within the aragonite crystal structure that constitutes the shell. However, at higher external Mg^{2+} concentrations, competitive inhibition between Ca^{2+} and Mg^{2+} may occur, potentially reducing the incorporation of Mg^{2+} into the shell and resulting in a slight decline in accumulation despite increased availability.

3. Seawater Mg^{2+} concentration in the culture medium

The results showed that the addition of Mg^{2+} at various concentrations to the culture medium of *Haliotis squamata* in the recirculating aquaculture system (RAS) led to an initial increase in Mg^{2+} levels. However, a gradual decrease in Mg^{2+} concentration was observed over time due to physiological and ecological interactions (Fig. 1).

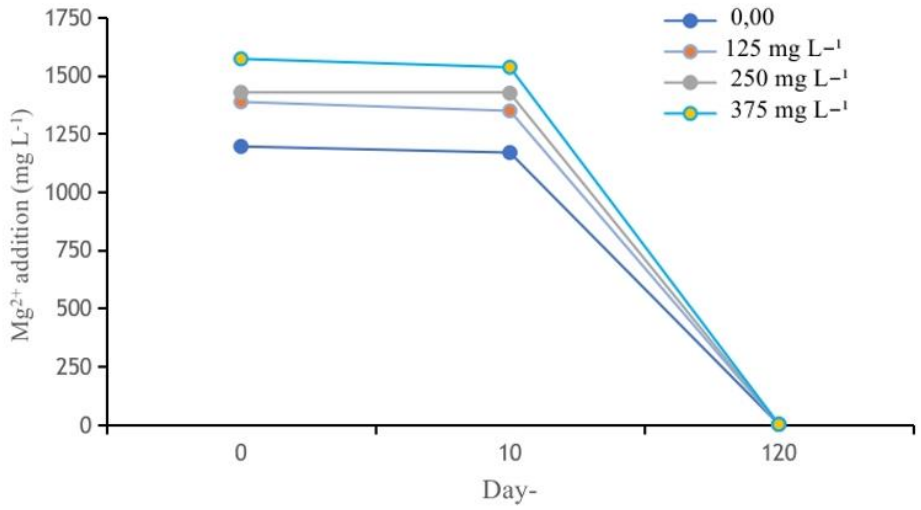

Fig. 1. Magnesium (Mg^{2+}) concentration in the culture medium of *Haliotis squamata* under different treatment concentrations over time

As illustrated in Fig. (1), the initial Mg^{2+} concentration in the culture medium of *Haliotis squamata* corresponded to the different treatment levels applied (0, 125, 250, and 375mg L^{-1}). During the first 10 days of culture, Mg^{2+} concentrations remained relatively stable across treatments, indicating effective maintenance of water quality within the recirculating aquaculture system (RAS).

A significant decline in Mg^{2+} concentration was observed thereafter, with all treatments approaching near depletion by day 120. This reduction reflects both physiological uptake by *H. squamata* for shell formation and metabolic processes, as well as potential ecological interactions within the culture medium. The highest treatment (375 mg L^{-1}) maintained elevated Mg^{2+} levels for a longer period compared to lower concentrations, suggesting a dose-dependent availability of magnesium ions.

These results indicate that while Mg^{2+} supplementation effectively increased initial ion concentrations in the culture medium, natural biological utilization and environmental dynamics gradually reduced Mg^{2+} availability over time.

4. Antioxidant status and pH of *Haliotis squamata* meat

The results showed that increasing Mg^{2+} concentrations in the recirculating aquaculture system (RAS) enhanced superoxide dismutase (SOD) enzyme activity in *Haliotis squamata*. The highest SOD activity was observed at 0.375g L^{-1}, with a value of 90.00 ± 5.00 U g^{-1}, significantly higher than the control group (69.33 ± 9.02U g^{-1}) ($P< 0.05$) (Table 3).

Table 3. Superoxide dismutase (SOD) activity and meat pH of *Haliotis squamata* cultured under different Mg^{2+} concentrations in a recirculating aquaculture system (RAS)

Parameter	Addition of Mg^{2+} concentration (g L^{-1})			
	K	0.125	0.250	0.375
SOD	69.33±9.02[a]	70.00±10.00[a]	78.33±7.64[ab]	90.00±5.00[b]
pH	6.06±0.01[a]	6.09±0.01[b]	6.10±0.01[b]	6.09±0.01[b]

Note: Values are presented as mean ± standard deviation (SD) (n = 9). Different superscript letters within the same row indicate statistically significant differences between treatments ($P < 0.05$). Mg^{2+} = magnesium; K = control (no Mg^{2+} addition); SOD = superoxide dismutase.

This finding suggests that Mg^{2+} acts as a cofactor in enzymatic reactions and supports the organism's antioxidant defense system, helping to mitigate oxidative stress during culture in the RAS environment.

In addition, the addition of Mg^{2+} also influenced the meat pH of *H. squamata*, though the changes were relatively minor. The pH values increased slightly from 6.06 ± 0.01 in the control to a range of 6.09–6.10 in the Mg^{2+}-treated groups. This relative stability indicates that Mg^{2+} contributes to ionic regulation and buffering capacity, which supports acid–base homeostasis in *H. squamata* tissue.

5. Proximate composition of *Haliotis squamata* meat

The results showed that the addition of different Mg^{2+} concentrations in the recirculating aquaculture system (RAS) significantly affected the proximate composition of *Haliotis squamata* meat, including water, protein, and lipid content ($P< 0.05$) (Table 4).

Table 4. Proximate composition (% dry weight) of *Haliotis squamata* meat under different Mg^{2+} concentrations in a recirculating aquaculture system

Parameter	Mg^{2+} addition (g L^{-1})			
	K	0.125	0.250	0.375
Water content (%)	9.46±0.84[ab]	9.91±0.79[ab]	9.06±0.39[a]	10.54±0.67[b]
Protein (%)	67.58±0.38[b]	67.17±0.98[b]	66.49±0.49[b]	62.77±0.68[a]
Fat (%)	1.63±0.12[b]	2.93±0.02[c]	1.35±0.12[a]	1.79±0.19[b]

Note: Values are presented as mean ± standard deviation (SD). Different superscript letters within the same row indicate statistically significant differences between treatments ($P< 0.05$). Mg^{2+} = magnesium; K = control (no Mg^{2+} addition).

Water content increased with higher Mg^{2+} levels, reaching its highest value at 0.375g L^{-1} (10.54 ± 0.67%). This may be attributed to the strong osmotic influence of Mg^{2+} ions, which promote water retention in tissue. Conversely, the lowest water content was observed at 0.250g L^{-1} (9.06 ± 0.39%), suggesting that this concentration may represent an optimal osmotic balance for *H. squamata*.

Protein content showed a declining trend as Mg^{2+} concentration increased, from 67.58 ± 0.38% in the control group (K) to 62.77 ± 0.68% at 0.375g L^{-1}. This reduction may reflect elevated metabolic stress and increased protein catabolism at higher Mg^{2+} levels. Lipid content also varied across treatments, with a significant increase at 0.125g

L^{-1} (2.93 ± 0.02%), possibly due to enhanced lipid synthesis mediated by Mg^{2+}. However, lipid content decreased at 0.250g L^{-1} (1.35 ± 0.12%), potentially as a result of higher energy demand for osmoregulation, and increased again at 0.375g L^{-1} (1.79 ± 0.19%).

6. Water quality of *Haliotis squamata*

The results demonstrated that the addition of magnesium (Mg^{2+}) in the recirculating aquaculture system (RAS) influenced several water quality parameters in the culture of *Haliotis squamata* (Table 5).

Table 5. Water quality parameters in the culture of *Haliotis squamata* under different magnesium (Mg^{2+}) concentrations in a recirculating aquaculture system (RAS)

Parameter	Mg^{2+} addition (g L^{-1})			
	K	0.125	0.250	0.375
Temperature (°C)	26-30	25-30.3	26-30.3	25-30.3
Salinity (ppt)	35-37	35-37	35-37	35-38
pH	8.49-8.60	8.49-8.62	8.44-8.63	8.16-8.64
DO (mg L^{-1})	5.36-6.10	5.45-5.72	5.43-6.30	5.29-5.90
Alkalinity (meq L^{-1})	114-147.5	123.75-144.25	114.0-147.0	112-135.25
TAN (mg L^{-1})	0.054-0.209	0.124-0.543	0.062-0.202	0.043-0.230

Water temperature remained relatively stable within a range of 25.0– 30.3°C. Salinity increased at the highest Mg^{2+} concentration (0.375g L^{-1}), reaching 35– 38ppt. The pH fluctuated slightly between 8.16 and 8.64, while dissolved oxygen (DO) levels remained stable, ranging from 5.29 to 6.30mg L^{-1}.

Alkalinity exhibited noticeable variation across treatments, with the highest values observed at 0.125g L^{-1} (123.75–144.25 meq L^{-1}) and the lowest at 0.375g L^{-1} (112.00–135.25 meq L^{-1}). Total ammonia nitrogen (TAN) concentrations were reduced at higher Mg^{2+} levels, particularly at 0.375g L^{-1}, where values ranged from 0.043 to 0.230mg L^{-1}. These results suggest that Mg^{2+} supplementation may enhance water stability and improve nitrogen dynamics in the RAS environment.

DISCUSSION

Magnesium (Mg^{2+}) plays a crucial role in calcium carbonate ($CaCO_3$) crystallization and biomineralization processes. It influences the morphology and polymorphism of $CaCO_3$ crystals, where higher Mg/Ca ratios tend to promote the formation of aragonite and calcite with higher Mg^{2+} incorporation **(Zhang *et al.*, 2018; Zou *et al.*, 2019)**. Mg^{2+} ions can interact directly with $CaCO_3$ crystals, altering their morphology, and indirectly affect biomineralization by modifying protein conformation and structure **(Zhang *et al.*, 2016)**. The presence of optimal Mg^{2+} concentrations in recirculating aquaculture systems (RAS) supports proper calcium regulation and deposition, thereby enhancing shell growth. **Al-Subiai *et al.* (2025)** demonstrated that Mg/Ca ratios above 1.07 and 2.12 significantly improved the growth performance of *Litopenaeus vannamei* during hatchery and grow-out phases. Dietary supplementation of Mg^{2+} has also been shown to reduce oxidative stress and enhance the growth of *Penaeus vannamei* cultured in inland saline

water, with magnesium–amino acid complexes proving more effective than magnesium citrate **(Panmei *et al.*, 2023)**. Similarly, **Zhang *et al.* (2016)** reported that dietary Mg^{2+} at 520mg kg^{-1} improved growth and muscle lipid content in Japanese grouper (*Lateolabrax japonicus*). In a biofloc system under low salinity conditions, *L. vannamei* larvae exhibited optimal growth at an initial Mg^{2+} concentration of 167.0mg L^{-1} **(Zacarias *et al.*, 2019)**.

Optimal Mg^{2+} concentrations also positively affect multiple growth parameters, including shell length increment, specific growth rate **(**SGR**)**, magnesium retention efficiency, and RNA/DNA ratio—an indicator of cellular health and metabolic activity **(Gao *et al.*, 2023; Hassan *et al.*, 2023)**. Studies by **Gao *et al.* (2019)** and **Irawati *et al.* (2024)** highlighted the role of Mg^{2+} in supporting metabolic processes, enhancing growth, and improving nutrient utilization efficiency in *H. squamata*. Adequate Mg^{2+} levels are essential for energy production and protein synthesis. However, excessive Mg^{2+} concentrations can disrupt metabolic functions due to increased ion excretion required to maintain homeostasis. When Mg^{2+} levels exceed optimal thresholds, *H. squamata* is at risk of hypermagnesemia, which may impair growth by elevating physiological stress and reducing feed conversion efficiency. Under such conditions, more energy is diverted toward osmoregulation rather than growth, leading to decreased SGR, feed efficiency, and RNA/DNA ratio **(Sánchez-Saavedra *et al.*, 2015)**. **Bansemer *et al.* (2015)** and **Arta *et al.* (2021)** also reported that excess Mg^{2+} can interfere with nutrient absorption and reduce metabolic efficiency, resulting in growth retardation and lower survival rates. Elevated Mg^{2+} retention has been closely linked with higher RNA/DNA ratios in several aquatic species, including *H. squamata*. Additionally, Mg^{2+} contributes to chlorophyll synthesis in macroalgae, the primary dietary source for *H. squamata*. Therefore, consumption of Mg^{2+}-enriched macroalgae-based diets can enhance feed conversion efficiency and positively influence the growth performance of *H. squamata*.

The shell of *Haliotis squamata* is primarily composed of calcium carbonate **(**$CaCO_3$**)**, which is formed through biomineralization processes involving magnesium ions **(**Mg^{2+}**)**. The presence of Mg^{2+} plays a crucial role in maintaining the structural integrity and mechanical properties of the shell, and fluctuations in its concentration can significantly influence these physical characteristics. Studies have shown that minerals such as Mg^{2+} contribute to the crystallization patterns of $CaCO_3$, thereby affecting shell strength and resistance to environmental stressors **(Xie *et al.*, 2016)**. **Ju *et al.* (2016)** also reported that under optimal Mg^{2+} concentrations, *H. squamata* exhibited improved shell development, which may enhance its survival and performance in aquaculture systems.

Furthermore, *H. squamata* is sensitive to dietary Mg^{2+} levels, where increases in this mineral are associated with enhanced shell hardness and greater resistance to predation **(Hanif *et al.*, 2023)**. Beyond its effects on shell formation, Mg^{2+} concentration also influences meat quality. Elevated mineral availability promotes the absorption of amino acids and fatty acids, which are positively correlated with meat quality **(Latuihamallo *et***

***al.*, 2015; Prasetiyono *et al.*, 2023)**. As a herbivorous species, *H. squamata* depends heavily on the nutritional quality of macroalgae in its diet. The interaction between Mg^{2+} availability and the composition of *Gracilaria gigas*, the primary feed in this study, may influence nutrient bioavailability and assimilation efficiency in *H. squamata*. Moreover, the nutritional content of *G. gigas* itself is influenced by the concentration of Mg^{2+} in the surrounding water, where appropriate Mg^{2+} levels have been shown to improve the nutritional profile of macroalgae **(Sari *et al.*, 2024)**.

The shell of *Haliotis squamata* is primarily composed of calcium carbonate ($CaCO_3$), which is formed through biomineralization processes involving magnesium ions (Mg^{2+}). The presence of Mg^{2+} plays a crucial role in maintaining the structural integrity and mechanical properties of the shell, and fluctuations in its concentration can significantly influence these physical characteristics. Studies have shown that minerals such as Mg^{2+} contribute to the crystallization patterns of $CaCO_3$, thereby affecting shell strength and resistance to environmental stressors **(Xie *et al.*, 2016)**. **Ju *et al.* (2016)** also reported that under optimal Mg^{2+} concentrations, *H. squamata* exhibited improved shell development, which may enhance its survival and performance in aquaculture systems.

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This decline is primarily attributed to the active uptake of Mg^{2+} by *H. squamata* for essential physiological processes, such as osmoregulation, enzyme activation, and metabolic regulation. Magnesium plays critical roles in aquatic organisms, including facilitating muscle contraction, regulating enzymatic activity, and transmitting nerve impulses. These biological functions require a steady Mg^{2+} supply, which is assimilated into the tissues of *H. squamata*, thereby reducing the ion concentration in the surrounding water **(Nurussalam *et al.*, 2017; Sari *et al.*, 2024)**. Additionally, efficient feed utilization and nutrient absorption may further contribute to the decline of Mg^{2+} levels in the aquatic environment **(Latuihamallo *et al.*, 2015)**.

Nurussalam *et al.* (2017) demonstrated that adequate availability of inorganic ions, such as magnesium (Mg^{2+}), can enhance the antioxidant capacity of aquatic organisms by minimizing oxidative damage. Higher Mg^{2+} concentrations may activate a range of

metabolic processes that influence antioxidant status, thereby increasing the activity and effectiveness of antioxidant enzymes. In addition, water pH is a critical factor in maintaining the physiological health of aquatic species. In recirculating aquaculture systems (RAS), pH balance can be affected by the presence of Mg^{2+}, which plays a role in buffering water chemistry.

A stable pH is essential for the proper physiological functioning of *Haliotis squamata*, and it also supports the efficiency of biological filtration in the RAS by enhancing the activity of beneficial microbial communities **(Xiao *et al.*, 2019; Fiesta *et al.*, 2024)**. Elevated Mg^{2+} concentrations may increase the buffering capacity of the culture system, helping to maintain pH stability and promoting a more favorable environment for the growth and health of *H. squamata*.

The proximate composition of *Haliotis squamata* meat showed that dietary magnesium supplementation in the recirculating aquaculture system **(**RAS**)** influenced the biochemical profile, particularly protein, fat, and moisture content. Variations in proximate composition across treatments suggest that Mg^{2+} plays an important role in metabolic regulation and nutrient deposition. Magnesium is a cofactor in numerous enzymatic reactions involved in protein synthesis, lipid metabolism, and energy production, which ultimately affect flesh quality and nutritional value of aquaculture species **(Lin *et al.*, 2008)**.

Increased dietary Mg^{2+} has been reported to enhance lipid metabolism and modulate the balance between fat and protein deposition in aquatic organisms. This is consistent with findings in fish and shellfish, where mineral supplementation contributes to improved nutrient utilization efficiency and muscle biochemical characteristics **(Liu *et al.*, 2017)**. Furthermore, water content and flesh quality are closely linked to mineral balance, as ionic regulation affects osmotic stability and muscle structure **(Lorenzo *et al.*, 2019)**.

Overall, the results indicate that Mg^{2+} supplementation not only enhances physiological resilience, as observed in antioxidant response, but also contributes to favorable nutritional composition of abalone meat. This highlights the potential application of Mg^{2+} as a functional additive in sustainable aquaculture practices, where both animal health and product quality are critical considerations **(Prabhu *et al.*, 2016)**.

Water quality plays a critical role in the success of abalone culture, as physicochemical stability strongly influences growth, metabolism, and survival. The results of this study demonstrated that magnesium (Mg^{2+}) supplementation in the recirculating aquaculture system (RAS) contributed to maintaining water quality within optimal ranges for *Haliotis squamata*. Temperature and salinity were relatively stable, which is essential for sustaining metabolic activity and osmotic regulation in abalone **(Gordon *et al.*, 2006)**.

The slight variations in pH and dissolved oxygen (DO) indicate that Mg^{2+} addition did not negatively impact buffering capacity or oxygen availability, both of which are

crucial for shell formation and aerobic metabolism. Alkalinity dynamics across treatments suggest that Mg^{2+} may interact with carbonate chemistry, thereby supporting shell calcification processes and overall mineral balance in the culture medium **(Hartmann *et al*., 2023)**.

Interestingly, the reduction of total ammonia nitrogen (TAN) with increasing Mg^{2+} concentration suggests a positive effect of magnesium supplementation on nitrogen dynamics in the RAS. This may be attributed to improved microbial activity in biofilters and enhanced ion exchange processes, which collectively reduce toxic nitrogenous compounds and promote a more stable rearing environment **(Ende *et al*., 2024)**.

Overall, these findings highlight the dual role of Mg^{2+} in abalone aquaculture: supporting physiological requirements of the cultured species and contributing to improved environmental stability within intensive systems. Such interactions are particularly important for sustainable production in RAS, where maintaining water quality is a prerequisite for optimizing growth performance and animal welfare **(Badiola *et al*., 2012)**.

CONCLUSION

Magnesium supplementation in RAS offers a promising strategy to enhance the growth, health, and product quality of *Haliotis squamata*, while simultaneously improving water quality and system sustainability. The optimal dosage of 0.375g L^{-1} Mg^{2+} provides a benchmark for future mineral management practices in mollusk aquaculture. These insights contribute to the broader goal of sustainable coastal resource utilization and align with the principles of eco-friendly aquaculture development.

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