Aquaculture 560 (2022) 738557

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Dietary magnesium improves the growth, antioxidant capacity, ammonia nitrogen tolerance and intestinal health of juvenile oriental river prawn *Macrobrachium nipponense*

Youqin Kong [a,*], Shanshan Wei [a], Zhili Ding [a], Changle Qi [a], Yan Liu [a], Yixiang Zhang [a], Samwel Mchele Limbu [b], Jinyun Ye [a,*]

[a] National-Local Joint Engineering Laboratory of Aquatic Animal Genetic Breeding and Nutrition, Zhejiang Provincial Key Laboratory of Aquatic Resources Conservation and Development, College of Life Science, Huzhou University, Huzhou, Zhejiang 313000, China
[b] Department of Aquaculture Technology, School of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P.O. Box 60091, Dar es Salaam, Tanzania

ARTICLE INFO

Keywords:
Macrobrachium nipponense
Magnesium requirement
Antioxidant status
Gut histology
Ammonia nitrogen

ABSTRACT

The present study evaluated the effects of magnesium (Mg) on growth, antioxidant capacity, intestinal morphology and ammonia nitrogen resistance in juvenile oriental river prawn, *Macrobrachium nipponense*. Six semipurified diets (crude protein 401.1 g kg^{-1} diet; crude lipid 88.7 g kg^{-1} diet) containing Mg levels of 1.1, 1.4, 1.6, 2.1, 2.8 or 4.5 g kg^{-1} were formulated. A total of 1080 healthy juvenile prawns (0.151 ± 0.003 g) were randomly stocked into eighteen 300 L tanks (100 × 80 × 60 cm), each tank with 60 prawns. Each experimental diet was fed to prawns in triplicate twice daily (08:00 and 17:00) for eight weeks. After the feeding trial, 20 prawns from each tank were challenged with 37 mg/L ammonia nitrogen for 96 h, and the hemolymph, hepatopancreas and muscle of the rest of the prawns were collected. Final biomass was higher in prawns fed diets containing 1.6 and 2.1 g Mg kg^{-1} compared with those fed the diet with 1.1 g kg^{-1}. Higher alkaline phosphatase (ALP) activity was obtained in the serum of prawns fed the 2.1 and 2.8 g Mg kg^{-1} diets. The highest activities of hepatopancreas superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) were observed in prawns fed 1.6–2.8 g Mg kg^{-1} of feed, and the lowest hepatopancreas malondialdehyde (MDA) concentrations and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were found in these treatment groups. Similarly, the prawns fed diets with 1.6 and 2.1 g Mg kg^{-1} had significantly lower MDA concentrations and cumulative mortality and higher antioxidant enzyme activities after ammonia nitrogen exposure. The intestinal epithelial cells were closely attached to the membrane in prawns fed the 2.1 g Mg kg^{-1} diet, but they were partly detached in those fed the 1.1 and 4.5 g Mg kg^{-1} diets. The minimum dietary Mg supplementations of juvenile *M. nipponense* were determined to be 1.6, 1.9, 2.0 and 2.2 g kg^{-1} feed, respectively, using broken-line regression analysis based on the final biomass, SOD, ALT and ALP.

1. Introduction

Magnesium (Mg) is a key dietary macromineral, which serves as a cofactor for >300 enzymatic reactions in animals and plays crucial roles in the metabolic processes of proteins, carbohydrates, and lipids (Lall, 2002; NRC, 2011). For instance, Mg is an integral component of alkaline phosphatase (ALP), which is involved in multiple metabolic processes, including vitamin metabolism and the inflammatory response (Rader, 2017). Accordingly, the activity of ALP correlates strongly with Mg content (Anderson et al., 1975; Janeway et al., 1993). More importantly, Mg helps to stimulate the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and suppress the generation of reactive oxygen species (ROS) (Fang et al., 2002; Garcia et al., 1998). Thus, Mg can affect the antioxidant capacity and immunity in terrestrial animals (Sahin et al., 2005; Shivakumar and Kumar, 1997) and aquatic organisms (Longbaf Dezfouli et al., 2019; Musharraf and Khan, 2018; Wang et al., 2011; Zafar and Khan, 2021; Zhang et al., 2016; Zhang et al., 2021). However, studies on the effects of Mg on antioxidant capacity in crustaceans are limited and have only been reported in Chinese mitten crab (*Eriocheir sinensis*) (Tian et al., 2015).

* Corresponding authors.
E-mail addresses: susankuq@zjhu.edu.cn (Y. Kong), yjy@zjhu.edu.cn (J. Ye).

https://doi.org/10.1016/j.aquaculture.2022.738557
Received 27 September 2021; Received in revised form 22 June 2022; Accepted 24 June 2022
Available online 27 June 2022
0044-8486/© 2022 Elsevier B.V. All rights reserved.

Aquatic animals can obtain Mg from water and food they ingest. Mg concentrations in the water influences the dietary Mg supplementation needed for meeting required levels in aquatic animals. Concentrations of dissolved Mg differ greatly between seawater and fresh water (Roy et al., 2010). In marine environment, crustaceans such as Kuruma prawn (*Penaeus japonicus*) (Deshimaru and Yone, 1978) and Pacific white shrimp (*Litopenaeus vannamei*) (Roy et al., 2007) do not require dietary Mg supplementation. However, the natural Mg content in fresh water is low; thus, the acquisition of Mg from the diet is a necessary and a dominant source in freshwater crustacean species such as Chinese mitten crab (Tian et al., 2015). Dietary Mg supplementation is also necessary for Pacific white shrimp reared in water at a low salinity of 2 g/L (Cheng et al., 2005). Therefore, the dietary Mg requirement can highly vary among aquatic species or for the same species in different environments. Dietary Mg deficiency in aquatic organisms has been associated with poor growth, weak carapace strength, anorexia, sluggishness and diminished Mg content in tissues (Chen and Huang, 2015; Cheng et al., 2005; Lall, 2002; Lim and Klesius, 2003; Musharraf and Khan, 2018). Therefore, determination of Mg required for each species is a prerequisite to avoid effects cause by its deficiency.

The oriental river prawn (*Macrobrachium nipponense*) is a popular and traditional aquaculture species in China and Southeast Asian countries (Guo et al., 2013; Ding et al., 2020; Ding et al., 2022). In recent years, the annual production of farmed *M. nipponense* in China has increased to $>2.0 \times 10^5$ t (Ministry of Agriculture Fisheries Bureau, 2021). The rapid development of *M. nipponense* cultivation has driven the demand for suitable artificial feed. To date, the nutritional requirements of *M. nipponense* for various minerals, including copper (Kong et al., 2014), selenium (Kong et al., 2017), zinc (Guo et al., 2013) and manganese (Ding et al., 2020), have been extensively studied. However, information on the effects of dietary Mg and its requirement for *M. nipponense* is lacking, making it difficult to formulate nutrient-balanced feed for this species.

Therefore, this study evaluated the effects of dietary Mg on the growth performance, antioxidant capacity, resistance to ammonia nitrogen stress and intestinal morphology of *M. nipponense* and thereby estimated the optimum dietary Mg required for this species. Our results provide useful information required for optimizing feed formulations for prawns to achieve efficient utilization of diets.

2. Materials and methods

2.1. Ethical statement

The use of prawns in this research was approved by the Animal Ethics Committee of Huzhou University and all experiments were conducted according to the protocols and procedures of the Laboratory Animal Management Ordinance of China.

2.2. Experimental diets

2.2.1. Diet preparation

A semipurified basal diet was formulated by using fishmeal and casein as protein sources and soybean and fish oils as lipid sources (Table 1). Magnesium sulfate (Analytical Reagent, Sinopharm, Shanghai, China) was added to the basal diet at 0, 0.3, 0.6, 1.2, 2.4 and 4.8 g Mg kg^{-1} feed with a corresponding decrease in the amount of cellulose. The process of diet preparation was as follows all of the ingredients were ground and sieved through a 60-mesh screen and then weighed and mixed according to the formulations. Subsequently, fish oil, soybean oil and double distilled water were added and mixed to create a dough-like diet. The mixture was pelleted with a screw-press pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) using a 1.5 mm die. Pellets were dried at 40 °C to decrease the moisture level below 10%. The dry pellets were stored at −20 °C until use.

Table 1
Formulation and proximate chemical composition of the basal diet (g kg^{-1} dry weight).

Ingredients	g kg^{-1}
Casein[a]	250
Fishmeal[b]	270
Corn starch	200
Fish oil[b]	40
Soybean oil[b]	20
Vitamin mix[c]	20
Mg-free mineral mix[d]	30
Attractant[e]	30
Cholesterol	5
Choline chloride	5
Lecithin	5
Cellulose	95
Carboxymethylcellulose	30
Total	1000
Proximate composition	
Moisture	98.1
Crude protein	401.1
Crude lipid	88.7
Crude ash	72.8

[a] Sigma–Aldrich Co., Shanghai, China.
[b] Zhejiang Yixing Feed Group Co. Ltd., China.
[c] Vitamin mixture (mg 100 g^{-1} mixture): vitamin A 420000 IU, vitamin C 6000 mg, α-tocopherol acetate 2000 mg, vitamin D3 120,000 IU, vitamin K 1000 mg, vitamin B1 1000 mg, vitamin B2 1000 mg, vitamin B6 1600 mg, vitamin B12 2 mg, niacin 5000 mg, folic acid 400 mg, inositol 6000 mg, biotin 10 mg, calcium pantothenic 3500 mg, Hangzhou Minsheng Bio-Tech Co., Ltd., China.
[d] Composition of mineral mixture (g kg^{-1} diet): KCl 0.84, NaH_2PO_4 6.45, KH_2PO_4 3, $Ca(H_2PO_4)_2{\cdot}H_2O$ 7.95, $CaCO_3$ 3.15, $C_6H_{10}CaO_6{\cdot}5H_2O$ 4.95, $FeC_6H_5O_7{\cdot}5H_2O$ 0.36, $CuSO_4{\cdot}5H_2O$ 0.1055, $ZnSO_4{\cdot}7H_2O$ 0.1428, $MnSO_4{\cdot}H_2O$ 0.0321, $AlCl_3{\cdot}6H_2O$ 0.0045, $CoCl_2{\cdot}6H_2O$ 0.042, Na_2SeO_3 0.0013, KI 0.0069, cellulose 2.9649.
[e] Alanine 0.6%, glycine 0.6%, glutamic acid 0.6%, betaine 1.2%.

2.2.2. Diet Mg concentrations and chemical composition analysis

The Mg concentrations in the diets were measured using an atomic absorption spectrophotometer (AA240Duo; Agilent, California, USA) to be 1.1, 1.4, 1.6, 2.1, 2.8 and 4.5 g kg^{-1} feed. The chemical compositions of the experimental diets were analyzed using standard methods (AOAC, 2005; Table 1). Moisture was determined by the gravimetric analysis after oven drying at 105 °C to a constant weight. Crude protein was measured using the Kjeldahl method. Lipid content was determined by the ether extraction method using a Soxhlet system (2055 Soxhlet Avanti; Foss Tecator, Hoganas, Sweden). Ash content was determined via a muffle furnace at 550 °C for 6 h.

2.2.3. Diet leaching

The dietary Mg leaching test was conducted based on Coote et al. (1996) and Cheng et al. (2005) methods. Preweighed experimental diets (10 g) were placed into a 0.5 mm mesh screen and laid on the bottom of the tank without prawns. Aeration and water temperature were adjusted consistently to the experimental conditions. The diets were removed from the tanks after 0, 0.5 and 1 h and dried overnight at 45 °C. The Mg content in the dried diets was measured using an atomic absorption spectrophotometer as indicated above.

2.3. Feeding trial and sampling

Juvenile oriental river prawns were obtained from Bangda Ecological Agriculture Co., Ltd. in Huzhou (Zhejiang, China). Before the formal feeding trial, prawns were acclimatized to the laboratory conditions for two weeks. During this period, the prawns were fed a basal diet twice

daily. After the acclimatization period, 1080 healthy prawns with uniform size (0.151 ± 0.003 g) were randomly stocked into eighteen 300 L tanks (100 × 80 × 60 cm), each tank with 60 prawns in three replicates for each dietary treatment. At the beginning of the feeding trial, 60 prawns in each tank were bulk weighed to establish the initial biomass. The initial biomass of prawns in each tank was 9.06 ± 0.11 g. Each tank was supplied with five bundles of plastic water pipes, and each bundle contained 5 pipes (inner tube diameter 30 mm, length 200 mm), which served as shelters for prawns to avoid cannibalism. The prawns were fed twice daily to apparent satiation at 08:00 and 17:00 for 56 days under a natural photoperiod. Feces were removed daily by siphoning. One-third of the water in each tank was replaced daily with fresh dechlorinated water to maintain water quality. The water temperature ranged from 26 °C to 29 °C, dissolved oxygen was above 6.5 mg/L, and ammonia nitrogen was below 0.1 mg/L during the entire feeding trial period. The Mg concentration in rearing water was measured weekly by an atomic absorption spectrophotometer as described above.

At the end of the feeding trial, all the survived prawns from each tank were fasted for 24 h. Afterwards, all prawns were counted and bulk weighed to determine final biomass.

Ten prawns from each tank were anesthetized individually by immersing them in ice water (2–4 °C), and then the hemolymph was withdrawn immediately from the ventral sinus of each prawn using nonheparinized syringes. The hemolymph was stored overnight in a refrigerator at 4 °C. Serum samples were obtained following centrifugation at 4 °C and 956 ×*g* (5810R, Eppendorf, Germany) for 10 min and stored at −80 °C until analysis. Concurrently, the hepatopancreas and muscle were carefully dissected and stored at −80 °C. Three prawns from each tank in the 1.1, 2.1 and 4.5 g Mg kg^{-1} dietary treatment were randomly sampled and sacrificed as shown above for gut collection. The prawns were dissected, their guts collected and preserved separately in 4% paraformaldehyde for 24 h before histological analysis.

2.4. Sample analysis

2.4.1. Determination of survival rate and Mg content in muscle

The survival rate was calculated using the formula:

$$\text{Survival rate } (\%) = \frac{\text{Final prawn number}}{\text{Initial prawn number}} \times 100$$

Mg concentrations in muscle were measured using an atomic absorption spectrophotometer method as indicated above.

2.4.2. Serum biochemical parameter analysis

The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the serum were measured using commercial kits (Nanjing Jiancheng, China) following the manufacturer's guidelines.

2.4.3. Antioxidant enzymes activities analysis in the hepatopancreas

Crude extracts of the hepatopancreas from the prawns for assaying enzymes activities and lipid peroxidation were obtained by homogenizing the frozen tissue in precooled 0.86% (*w/v*) sodium chloride solution. The antioxidant enzymes activities, including total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) content were analyzed using specific commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) following the manufacturer's instructions. The T-AOC activity was measured according to the Fe^{3+} reduction process (Tang et al., 2005). The xanthine oxidase method was used to analyze SOD activity (Elstner and Heupel, 1976). GSH-Px activity was determined based on reduced glutathione (GSH) reduction in the reaction (Sedlak and Linday, 1968). The MDA content was measured by the thiobarbituric acid reacting substances (TBARS) assay as described by Buege and Aust (1978).

2.4.4. Histological assays

The fixed intestine tissues were dehydrated in increasing concentrations of alcohol solutions. Every dehydrated tissue was embedded in paraffin, and every paraffin was cut into continuous blocks into 5-μm slices. Then, 2 slices were stained with hematoxylin and eosin (H & E). The stained slices were scanned with a digital scanner (Pannoramic SCAN II, 3DHISTECH Ltd., Budapest, Hungary) and observed using a CaseViewer software. Changes in the intestinal epithelial cells and microvilli were noted and photographed.

2.5. Ammonia nitrogen challenge test

At the end of the feeding trial, 60 prawns were randomly collected from each dietary treatment (20 prawns from each tank) for ammonia nitrogen challenge. The prawns were challenged with 37 mg/L total ammonia nitrogen for 96 h. The dose for ammonia nitrogen challenge was selected based on Wang et al. (2003), who found that the 96 h LC_{50} of ammonia nitrogen was 36.6 mg/L for *M. nipponense*. A 10 g/L stock solution of ammonia nitrogen was prepared using ammonium chloride (NH_4Cl). The stock solution was used to regulate the desired concentration of ammonia nitrogen in the culture water. Ammonia nitrogen concentrations in culture water were analyzed and adjusted every 6 h by the spectrophotometric method (Solórzano, 1969).

2.6. Determination of accumulative mortality, antioxidant capacity and lipid peroxidation after ammonia nitrogen challenge

Over a period of 96 h, the cumulative mortality of prawns in each group was recorded daily. A prawn was deemed dead from ammonia nitrogen challenge when it stopped any movement and activities for at least 1 h. At the end of the ammonia nitrogen challenge, prawns in each group were collected, sacrificed as described above. Hepatopancreas were carefully dissected and stored at −80 °C for later analysis of antioxidant capacity (T-AOC, SOD and GSH-Px activities) and lipid peroxidation (MDA content) using the methods described above in the previous section.

2.7. Statistical analysis

All data obtained were tested for normality by using Shapiro Wilk test and homogeneity of variances by Levene's test. The data were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests when ANOVA detected significant differences. The results with $P < 0.05$ were considered significant. The broken-line analysis was used for the estimation of optimal dietary Mg supplementing for juvenile *M. nipponense* based on final biomass, SOD, ALT and ALP activities. All analyses were conducted using Statistical Package for Social Sciences (SPSS, 19.0 statistical software (IBM Corp., Armonk, NY, USA). All results are presented as mean ± standard deviation (SD).

3. Results

3.1. Growth performance and Mg concentration in muscle

The final biomass, survival rate, and Mg concentration in muscle of juvenile *M. nipponense* fed the experimental diets for eight weeks are shown in Fig. 1. The final biomass was significantly higher in prawns fed the 1.6 and 2.1 g Mg kg^{-1} diets than in those fed the 1.1 g Mg kg^{-1} diet ($P < 0.05$). However, no significant differences were obtained in final biomass when prawns in the 1.6 and 2.1 g Mg kg^{-1} groups were compared with those in the 1.4, 2.8 and 4.5 g Mg kg^{-1} groups ($P > 0.05$). The survival rate did not differ significantly among prawns fed the different experimental diets ($P > 0.05$). With increasing levels of dietary Mg, the Mg concentration in muscle significantly increased up to 1.6 g kg^{-1} diets and then stabilized in prawns fed higher levels of dietary Mg.

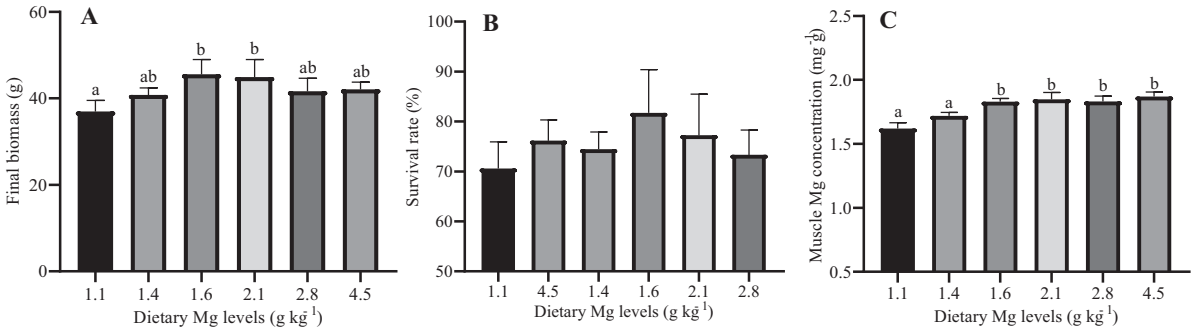


Fig. 1. The final biomass (A), survival rate (B) and muscle Mg concentration (C) of juvenile *M. nipponense* fed diets with different levels of Mg for eight weeks. Values are the mean values of 3 replicates within a treatment ± S.D. Bars with different superscript letters are statistically significant ($P < 0.05$).

The prawns fed 1.6, 2.1, 2.8 and 4.5 g kg^{-1} diets had significantly higher muscle Mg concentration than those fed the 1.1 and 1.4 g kg^{-1} diets ($P < 0.05$).

3.2. Serum biochemical parameters

Prawns fed the 2.1 and 2.8 g Mg kg^{-1} diets had significantly higher ALP activity than those fed any of the other diets ($P < 0.05$; Fig. 2). Similarly, the prawns fed the 4.5 g Mg kg^{-1} diet had significantly higher ALP activity than those fed the 1.1 and 1.4 g Mg kg^{-1} diets ($P < 0.05$). Prawns fed the 2.1 and 2.8 g Mg kg^{-1} diets had significantly lower activities of AST and ALT than those fed the 1.1 g Mg kg^{-1} diet ($P < 0.05$), but had no significant differences from those fed the 1.4, 1.6 and 4.5 g Mg kg^{-1} diets ($P > 0.05$, Fig. 2).

3.3. Antioxidant capacity in the hepatopancreas

The effects of dietary Mg on antioxidant enzymes activities in the hepatopancreas are depicted in Fig. 3. The prawns fed the 2.1 g Mg kg^{-1} diet had higher activities of T-AOC, SOD and GSH-Px than those fed the 1.1, 1.4 and 4.5 g Mg kg^{-1} diets ($P < 0.05$). However, there were no significant differences in T-AOC, SOD and GSH-Px activities between the prawns fed the 2.1 g Mg kg^{-1} diet and those fed the 1.6 and 2.8 g Mg kg^{-1} diets ($P > 0.05$). Conversely, a significantly lower MDA content was obtained in prawns fed the 1.4, 1.6, 2.1 and 2.8 g Mg kg^{-1} diets than those fed the diet with 1.1 g Mg kg^{-1} ($P < 0.05$).

3.4. Intestinal morphology

The intestinal morphology of *M. nipponense* fed the 1.1, 2.1 and 4.5 g Mg kg^{-1} diets is shown in Fig. 4. Part of the intestinal epithelial cells of the prawns fed the 1.1 g Mg kg^{-1} (Fig. 4A) and 4.5 Mg kg^{-1} diet (Fig. 4C) lost integration with the basement membrane or the cells were partly detached from it. The epithelial cells for the prawns fed the 2.1 g Mg kg^{-1} diet were closely attached to the basement membrane (Fig. 4B).

3.5. Antioxidant capacity and cumulative mortality of the prawns after ammonia nitrogen challenge

Following an ammonia nitrogen challenge, prawns fed the 2.1 g Mg kg^{-1} diet had significantly higher T-AOC, SOD and GSH-Px than those fed the diets containing 1.1 and 1.4 g Mg kg^{-1} ($P < 0.05$) but had no significant differences from those fed the 1.6 or 2.8 g Mg kg^{-1} diets ($P > 0.05$; Fig. 5). The MDA content in the hepatopancreas was significantly lower in the prawns fed the 2.1 g Mg kg^{-1} diet than in those fed the 1.1, 1.4 and 4.5 g Mg kg^{-1} diets ($P < 0.05$) but was not significantly different from that in prawns fed the 1.6 and 2.8 g Mg kg^{-1} diets ($P > 0.05$).

Cumulative mortality was significantly lower for prawns fed the 1.6 and 2.1 g Mg kg^{-1} diets than those fed the 1.1 g Mg kg^{-1} diet ($P < 0.05$), but was not significantly different from those fed the 1.4, 2.8 and 4.5 g Mg kg^{-1} diets ($P > 0.05$; Fig. 5).

3.6. Dietary mg requirement

The optimum dietary Mg supplementation for juvenile *M. nipponense* were calculated as 1.6, 1.9, 2.0 and 2.2 g Mg kg^{-1} feed based on the broken-line regression analyses of final biomass, SOD in the hepatopancreas, and ALT and ALP in the serum, respectively (Fig. 6).

4. Discussion

The present study indicated high biomass for the prawns fed the diets containing 1.6 and 2.1 g Mg kg^{-1} diet. During our study, the Mg concentration in the rearing water ranged from 0.09 to 0.10 mmol L^{-1},

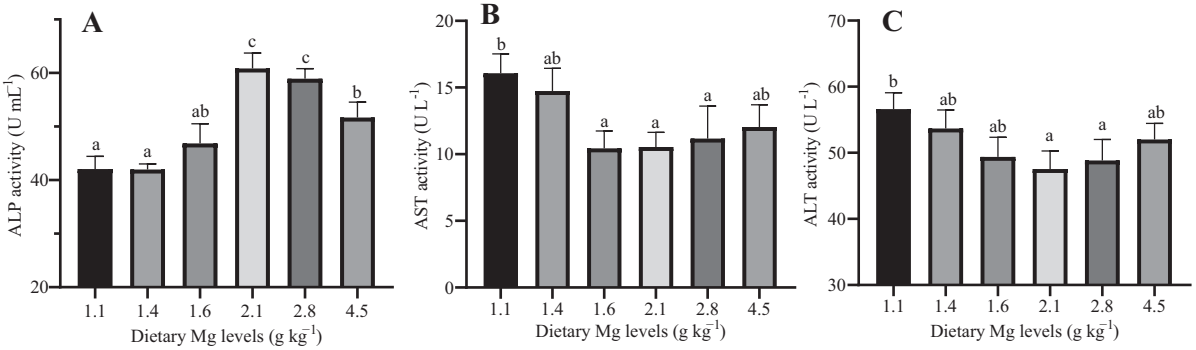


Fig. 2. Activities of (A) alkaline phosphatase (ALP), (B) aspartate aminotransferase (AST), and (C) alanine aminotransferase (ALT) in the serum of juvenile *M. nipponense* fed diets with different levels of Mg for eight weeks. Values are mean values of 3 replicates within a treatment ± S.D. Bars with different superscripts are statistically significant ($P < 0.05$).

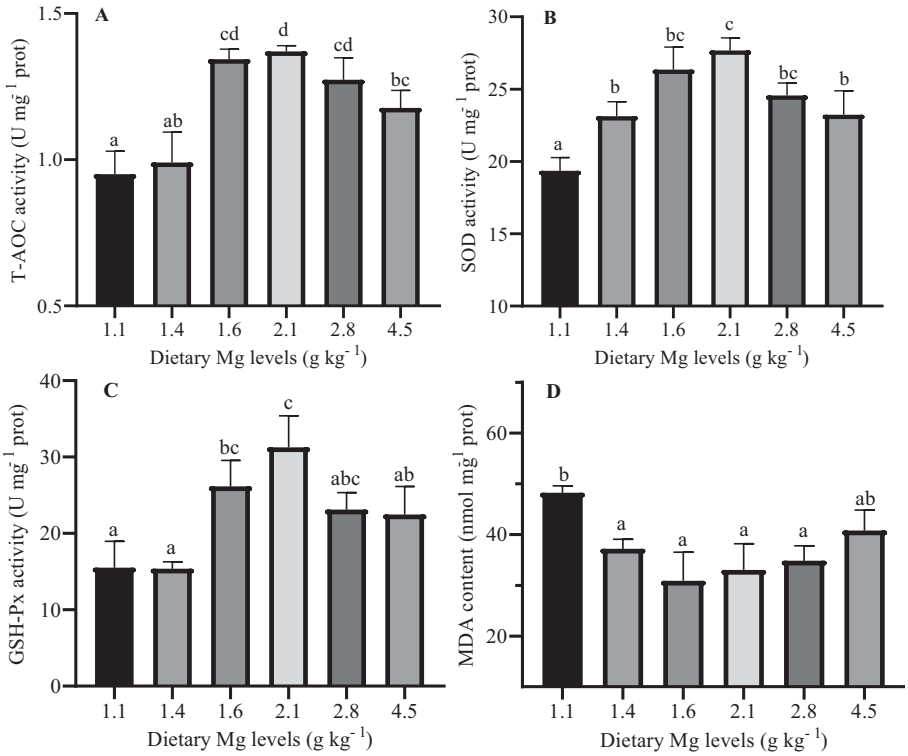


Fig. 3. The antioxidant capacity of juvenile *M. nipponense* in hepatopancreas after feeding diets with different levels of Mg for eight weeks. Activities of (A) total antioxidant capacity (T-AOC), (B) superoxide dismutase (SOD), and (C) glutathione peroxidase (GSH-Px), and (D) malondialdehyde (MDA) content. Values are mean values of 3 replicates within a treatment ± S.D. Bars with different superscripts are statistically significant ($P < 0.05$).

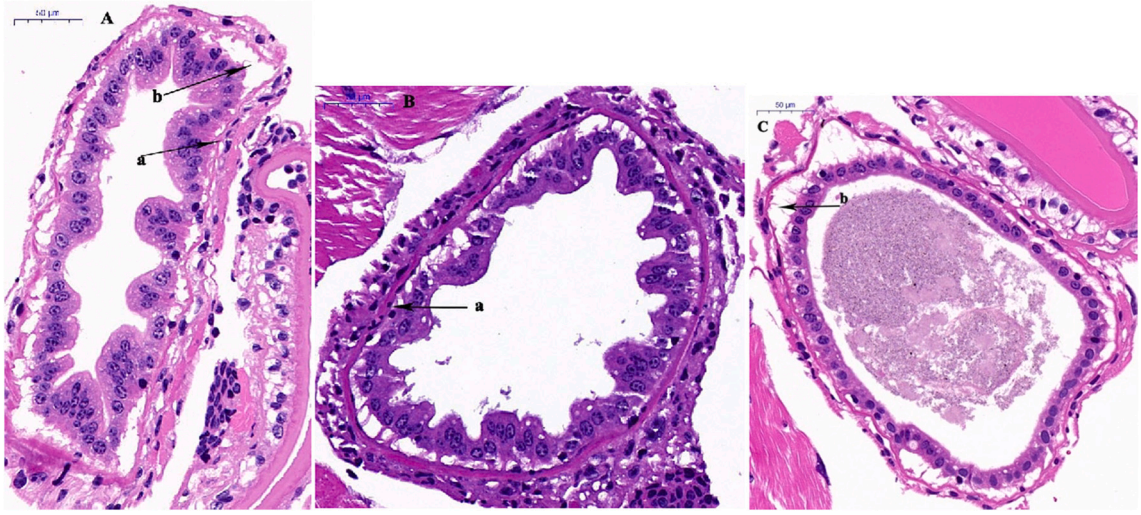


Fig. 4. Photomicrographs of intestinal tract cross-sections from *M. nipponense* fed (A) 1.1 g kg^{-1}, (B) 2.1 g kg^{-1}, or (C) 4.5 g kg^{-1} diets. Arrows point to pathological changes. Magnification 20×; scale bar 50 μm. Arrow "a" shows the intestinal epithelial cells attached closely with the basement membrane; arrow "b" shows the loose integration of the intestinal epithelial cells with the basement membrane, or the intestinal epithelial cells detached from the basement membrane.

signifying that the water combined with a diet without Mg supplementation would be insufficient to satisfy requirement for normal metabolism and growth for juvenile *M. nipponense*, similar to grass carp (*Ctenopharyngodon idella*) (Wang et al., 2011), Rohu (*Labeo rohita*) (Musharraf and Khan, 2018), and Mozambique tilapia (*Oreochromis mossambicus*) (Van der Velden et al., 1991). Hence, dietary Mg supplementation at 1.6 or 2.1 g kg^{-1} is necessary practice for improving prawn production.

The juvenile *M. nipponense* fed the diet containing 4.5 g Mg kg^{-1} supplementation decreased slightly the biomass compared with prawns fed feeds with approximately half that concentration (the 1.6 and 2.1 g kg^{-1}). This result suggests that higher dietary Mg (> 4.5 g kg^{-1}) may inhibit *M. nipponense* growth, similar to results in gibel carp (*Carassius auratus gibelio*) (Han et al., 2012), grass carp (Wang et al., 2011), and Pacific white shrimp (Cheng et al., 2005). Conversely, high dietary Mg did not inhibit growth performance in Rohu (Musharraf and Khan, 2018), soft-shelled turtle (*Pelodiscus sinensis*) (Chen and Huang, 2015), hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) reared in fresh water

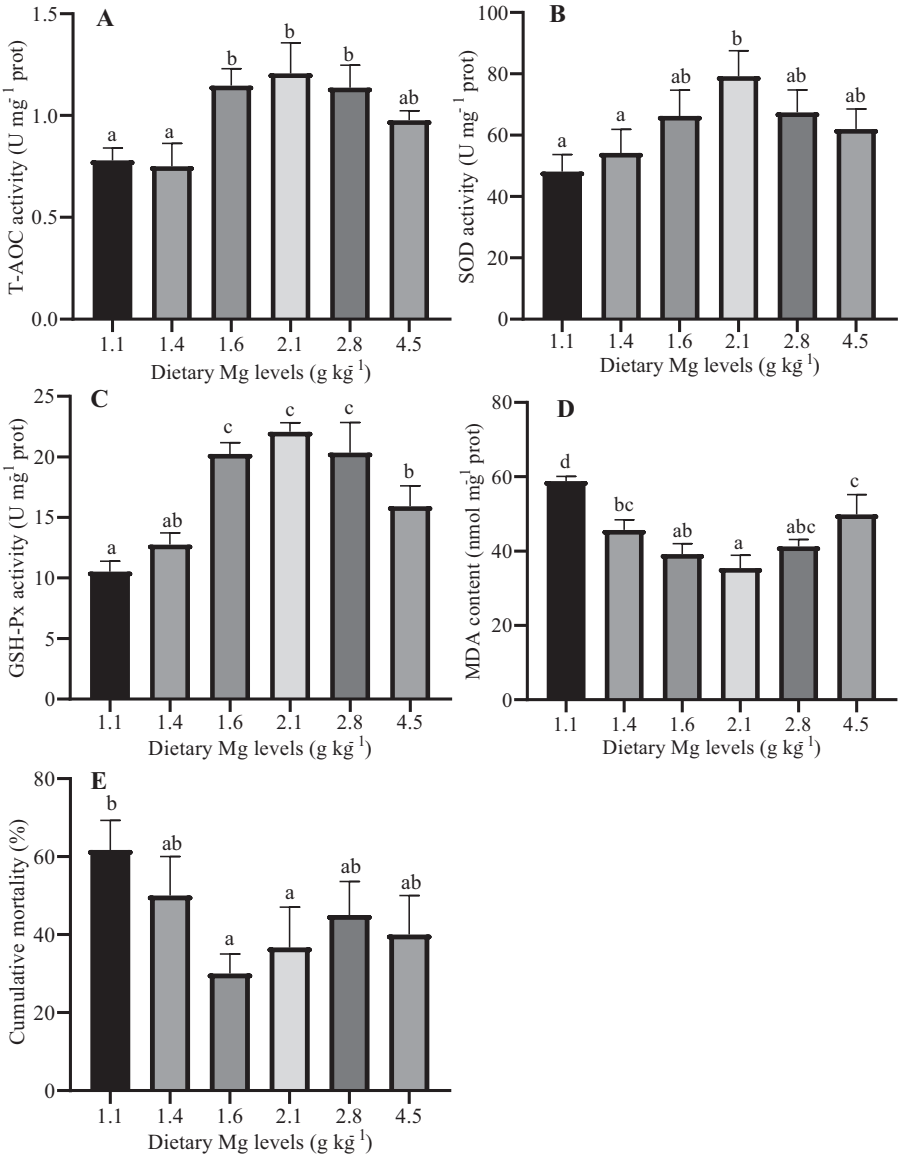


Fig. 5. The antioxidant enzyme activities (A: total antioxidant capacity (T-AOC), B: superoxide dismutase (SOD), C: glutathione peroxidase (GSH-Px)), lipid oxidation (D: malondialdehyde (MDA) content) and cumulative mortality (E) of juvenile *M. nipponense* after ammonia nitrogen challenge. Values are mean values of 3 replicates within a treatment ± S.D. Bars with different superscripts are statistically different ($P < 0.05$).

(Lin et al., 2013), and Chinese mitten crab (Tian et al., 2015). The discrepancy between findings may be related to differences in the dietary Mg concentrations tested, the distinct ability of a given species to utilize Mg, and differences in experimental conditions. These results suggest that prawn farmers can improve the growth of juvenile *M. nipponense* by dietary Mg supplementation.

In the present study, the Mg concentration in the muscle increased and then stabilized despite further increases in dietary Mg supplementation, similar to results in Rohu muscle (Musharraf and Khan, 2018). Likewise, soft-shelled turtles fed a diet supplemented with Mg levels ≥0.67 g Mg kg^{-1} gained higher muscle Mg concentrations, and the content was stable (Chen and Huang, 2015). These results suggest that a certain amount of dietary Mg is required for aquatic animals to satisfy the deposition of Mg in muscle. The stable Mg concentration in muscle with increasing dietary Mg in the juvenile prawns may be due to homeostasis after the requirement was met.

ALP (Yang et al., 2020), AST and ALT (Yeh et al., 2016) are important indicators of the general health of organisms. ALP plays key roles in multiple metabolic processes as it removes phosphate from a number of molecules, regulates animal−microbe associations (Rader et al., 2012), which is important for immunity function (Rader, 2017). The higher ALP activity in prawns fed the 2.1 and 2.8 g Mg kg^{-1} diets are attributed to Mg ability at these levels to enhance ALP enzyme function, as reported previously in juvenile hybrid tilapia (Lin et al., 2013) and soft-shelled turtles (Chen et al., 2014; Chen and Huang, 2015). However, the ability of Mg to affect ALP activity was dependent on the level used. Accordingly, the prawns fed diets with 1.1, 1.4 or 4.5 g Mg kg^{-1} showed reduced ALP activity, suggesting the existence of an optimum level somewhere in between.

AST and ALT are two key transaminases released into blood as a result of injuries in liver cells (Kim and Kang, 2016); thus, their enzyme activities increase. In our study, prawns fed the 2.1 or 2.8 g Mg kg^{-1} decreased AST and ALT activities, indicating less hepatopancreas injury (Zhang et al., 2016). But dietary Mg levels of 1.1 g kg^{-1} resulted in higher AST and ALT activities in prawns, indicating hepatopancreas injury. These results corresponded to the slow growth of the prawns fed the same dietary Mg levels, indicating that inadequate dietary Mg might have caused hepatopancreas injury, which retarded the growth

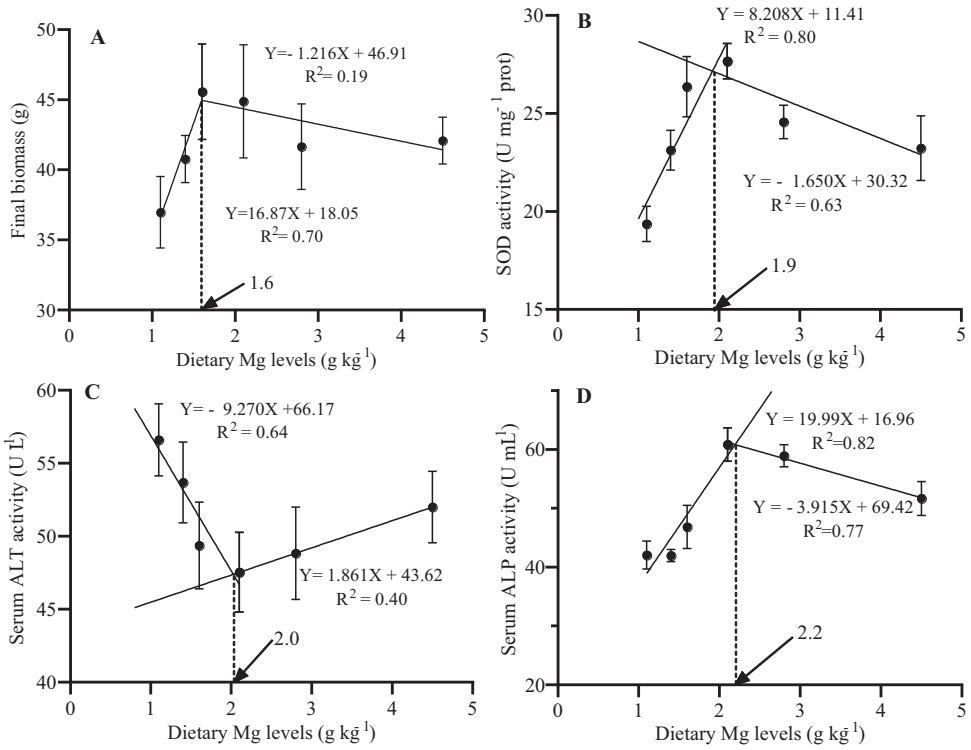


Fig. 6. Broken-line regression analysis of the relationships between the dietary Mg levels and final biomass (A), SOD activity (B) in the hepatopancreas and activities of ALT (C) and ALP (D) in the serum indicates that the optimal dietary Mg supplementation for juvenile *M. nipponense* is 1.6, 1.9, 2.0 and 2.2 g Mg kg^{-1} diet, respectively. Each point represents the mean of three replicates within a treatment.

performance in juvenile *M. nipponense*. However, the hepatopancreas injury sustained by the prawns was not fatal enough to reduce the survival rate. These results indicated that optimum dietary Mg is required by *M. nipponense* for general health maintenance. Taken together, these findings suggest that optimum dietary Mg supplementation is important for maintaining general health in *M. nipponense*.

Animals have evolved some antioxidant enzymes, such as SOD and GSH-Px for decreasing excessive ROS to maintain the balance of free radicals in cells (Fang et al., 2002). SOD is the most powerful antioxidant enzyme, which converts superoxide anion radicals to generate oxygen and hydrogen peroxide (Nozik-Grayck et al., 2005). GSH-Px catalyzes toxic peroxides to nontoxic hydroxyl compounds and promotes the decomposition of hydrogen peroxide (Czeczot et al., 2006). T-AOC measures the cumulative action of all the antioxidants present in plasma and body fluids (Ghiselli et al., 2000). In our study, the prawns fed the 1.6 and 2.1 g kg^{-1} Mg of dietary supplements increased the activities of T-AOC, SOD and GSH-Px, similar to results in rats (Shivakumar and Kumar, 1997) and grass carp (Wei et al., 2018). This result indicates that dietary Mg at 1.6 or 2.1 g kg^{-1} enhanced the antioxidant capacity of the juvenile prawns. To understand the effect of increased antioxidant capacity, we measured MDA, which is a final product of lipid peroxidation, commonly used as an indicator of endogenous oxidative damage (Dotan et al., 2004). The results showed low MDA content in prawns fed the 1.4. 1.6, 2.1 and 2.8 g Mg kg^{-1} diets, suggesting that dietary Mg supplementation in this range inhibits peroxidation in juvenile *M. nipponense*. We speculate that dietary Mg inhibit peroxidation by (i) improving antioxidant capacity by scavenging ROS (Fang et al., 2002); or (ii) attenuating the production of ROS (Garcia et al., 1998; Fang et al., 2002; Wei et al., 2018). Therefore, feeding juvenile *M. nipponense* with optimum dietary Mg increased the antioxidant capacity and reduced MDA content. However, relatively low ($\leq$ 1.1 g kg^{-1}) and high ($>$ 4.5 g kg^{-1}) dietary Mg reduced the antioxidant capacity of the prawns, similar to results in grass carp (Wang et al., 2011) and Chinese mitten crab (Tian et al., 2015). These results corresponded to the decreased growth and increased AST and ALT activities in our study. Therefore, the poorer growth observed in the *M. nipponense* fed the lowest Mg supplementation (1.1 g Mg kg^{-1}) might be a consequence of hepatopancreas injury owing to increased ROS production caused by low antioxidant capacity.

The animal gut is an independent organ and plays important roles in immune function and nutrient digestion and absorption (Tan et al., 2018). Gut integrity is often used to evaluate the health condition of animals (Bischoff, 2011). In the present study, we analyzed the intestinal morphology of *M. nipponense* fed diets containing 1.1, 2.1 and 4.5 g Mg kg^{-1}. We found altered intestinal epithelial cells, including epithelial cells detached from the basement membrane in prawns fed the diets supplemented with the lowest and highest concentrations of Mg used. This result suggested that relatively low (1.1 g kg^{-1}) or high (4.5 g kg^{-1}) dietary Mg can damage juvenile prawns gut health, similar to results in grass carp (Wei et al., 2018). We hypothesized that dietary Mg deficiency or excess may damage intestinal structure, thus weakens its ability to absorb nutrients and thereby inhibits the growth of *M. nipponense*.

Ammonia nitrogen is a common stress factor in aquaculture environments, which originates from protein metabolites of aquatic organisms and decomposition of residual feed and other organic wastes (Dong et al., 2020). Ammonia nitrogen exposure was used to understand the ability of dietary Mg supplementation to enhance the stress tolerance of the prawns. After ammonia nitrogen challenge, we found enhanced antioxidant enzyme activities, reduced MDA content and cumulative mortality in prawns fed the 1.6 and 2.1 g Mg kg^{-1} diets, similar to results in Japanese quail (*Coturnix coturnix*) (Sahin et al., 2005). These results indicate that dietary Mg supplementation enhances the ability of juvenile prawns to tolerate ammonia nitrogen stress by improving their antioxidant capacity, thereby reducing MDA content and cumulative mortality.

Our results showed decreased final biomass and ALP activity in the serum at low and high dietary Mg contents. Consequently, we evaluated the optimum dietary Mg supplementation of juvenile *M. nipponense*. The

optimum dietary Mg supplementation in juvenile *M. nipponense* for maximum growth was evaluated as 1.6 g kg^{-1} based on final biomass and 2.2 g kg^{-1} based on the ALP activity. Thus, an amount ranging from 1.6 to 2.2 g Mg kg^{-1} was deemed sufficient to increase growth and maintain health of *M. nipponense* because SOD and ALT activities were maximized in the prawns fed the 1.9 and 2.0 g Mg kg^{-1} diets. The Mg requirement of juvenile *M. nipponense* obtained in this study is lower than those of Pacific white shrimp (2.6 to 3.4 g kg^{-1}) (Cheng et al., 2005) and Chinese mitten crab (3.0 to 4.5 g kg^{-1}) (Tian et al., 2015). However, the optimum amount obtained in this study is comparatively higher than that for freshwater prawn (*M. rosenbergii*) (> 1.46 g kg^{-1}) (Srinivasan et al., 2017) and Chinese shrimp (*P. chinensis*) (1.1 g kg^{-1}) (Zhou et al., 1993). The differences in the optimum dietary Mg requirement of prawns and crabs are likely to be related to species, rearing environment, Mg source, and analysis models used (Chen and Huang, 2015; Han et al., 2012; NRC, 2011). We propose that farmers should supplement the diets of *M. nipponense* with 1.6 to 2.2 g Mg kg^{-1} to enhance the growth and health.

5. Conclusions

Dietary Mg supplementation is necessary requirement for juvenile *M. nipponense* for improving growth, general health and the ability to tolerate ammonia nitrogen stress. We recommend supplementing the diets for juvenile *M. nipponense* with 1.6 to 2.2 g Mg kg^{-1} for improved growth and health based on broken-line analysis of final biomass and the activities of SOD, ALT and ALP.

CRediT authorship contribution statement

Youqin Kong: Conceptualization, Writing – original draft. **Shanshan Wei:** Investigation, Validation. **Zhili Ding:** Methodology. **Changle Qi:** Data curation. **Yan Liu:** Visualization. **Yixiang Zhang:** Software, Resources. **Samwel Mchele Limbu:** Writing – review & editing. **Jinyun Ye:** Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by grants from Zhejiang Province Public Welfare Technology Application Research Project (CN) (No. LGN21C190004, LGN21C190003), for whom we are grateful.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738557.

References

Anderson, R.A., Bosron, W.F., Kennedy, F.S., Vallee, B.L., 1975. Role of magnesium in *Escherichia coli* alkaline phosphatase. Proc. Natl. Acad. Sci. U. S. A. 72, 2989–2993. https://doi.org/10.1073/pnas.72.8.29.

AOAC, 2005. Official Methods of Analysis. Cambridge University Press, Gaithersburg, MD, USA.

Bischoff, S.C., 2011. 'Gut health': a new objective in medicine? BMC Med. 9, 24. https://doi.org/10.1186/1741-7015-9-24.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Chen, C.Y., Huang, C.H., 2015. Effects of dietary magnesium on the growth, carapace strength and tissue magnesium concentrations of soft-shelled turtle, *Pelodiscus sinensis* (Wiegmann). Aquac. Res. 46, 2116–2123. https://doi.org/10.1111/are.12367.

Chen, C.Y., Chen, S.M., Huang, C.H., 2014. Dietary magnesium requirement of soft-shelled turtles, *Pelodiscus sinensis*, fed diets containing exogenous phytate. Aquaculture 432, 80–84. https://doi.org/10.1016/j.aquaculture.2014.05.001.

Cheng, K.M., Hu, C.J., Liu, Y.N., Zheng, S.X., Qi, X.J., 2005. Dietary magnesium requirement and physiological responses of marine shrimp *Litopenaeus vannamei* reared in low salinity water. Aquac. Nutr. 11, 385–393. https://doi.org/10.1111/j.1365- 2095.2005.00364.x.

Coote, T.A., Hone, P.W., Kenyon, R., Maguire, G.B., 1996. The effect of different combinations of dietary calcium and phosphorus on the growth of juvenile *Haliotis laevigata*. Aquaculture 145, 267–279. https://doi.org/10.1016/S0044-8486(96)01303-8.

Czeczot, H., Scibior, D., Skrzycki, M., Podsiad, M., 2006. Activity of antioxidant enzymes in patients with liver cirrhosis. Wiad. Lek. 59, 762–766.

Deshimaru, O., Yone, Y., 1978. Requirement of prawn for dietary minerals. Bull. Jap. Soc. Sci. Fish. 44, 907–910. https://doi.org/10.2331/suisan.44.907.

Ding, Z., Chen, X., Kong, Y., Shao, X., Zhang, Y., Ye, J., 2020. Dietary manganese requirement and its effects on antioxidant enzyme activities, intestinal morphology and microbiota in oriental river prawn *Macrobrachium nipponense* (De Haan). Aquaculture 516, 734622. https://doi.org/10.1016/j.aquaculture.2019.734622.

Ding, Z., Xiong, Y., Zheng, J., Zhou, D., Kong, Y., Qi, C., Limbu, S.M., 2022. Modulation of growth, antioxidant status, hepatopancreas morphology, and carbohydrate metabolism mediated by alpha-lipoic acid in juvenile freshwater prawns *Macrobrachium nipponense* under two dietary carbohydrate levels. Aquaculture 546, 737314. https://doi.org/10.1016/j.aquaculture.2021.737314.

Dong, X., Liu, Q., Kan, D., Zhao, W., Guo, H., Lv, L., 2020. Effects of ammonia-N exposure on the growth, metabolizing enzymes, and metabolome of *Macrobrachium rosenbergii*. Ecotoxicol. Environ. Saf. 189, 110046 https://doi.org/10.1016/j.ecoenv.2019.110046.

Dotan, Y., Lichtenberg, D., Pinchuk, I., 2004. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog. Lipid Res. 43, 200–227. https://doi.org/10.1016/j.plipres.2003.10.001.

Elstner, E.F., Heupel, A., 1976. Inhibition of nitrite formation from hydroxyl- ammonium chloride: a simple assay for superoxide dismutase. Anal. Biochem. 70, 616–620.

Fang, Y.Z., Yang, S., Wu, G.Y., 2002. Free radicals, antioxidants, and nutrition. Nutrition 18, 872–879. https://doi.org/10.1016/S0899-9007(02)00916-4.

Garcia, L.A., DeJong, S.C., Martin, S.M., Smith, R.S., Buettner, G.R., Kerber, R.E., 1998. Magnesium reduces free radicals in an in vivo coronary occlusion-reperfusion model. J. Am. Coll. Cardiol. 32, 536–539. https://doi.org/10.1016/S0735-1097(98)00231-9.

Ghiselli, A., Serafini, M., Natella, F., Scaccini, C., 2000. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic. Biol. Med. 29, 1106–1114. https://doi.org/10.1016/S0891-5849(00)00394-4.

Guo, J.L., Chen, J.M., Sun, L.H., Shen, B.Q., Pan, Q., 2013. Dietary zinc requirement of juvenile oriental river prawn (*Macrobrachium nipponense*). Chin. J. Anim. Nutr. 25, 661–668.

Han, D., Liu, H., Liu, M., Xiao, X., Zhu, X., Yang, Y., Xie, S., 2012. Effect of dietary magnesium supplementation on the growth performance of juvenile gibel carp, *Carassius auratus gibelio*. Aquac. Nutr. 18, 512–520. https://doi.org/10.1111/j.1365-2095.2011.00910.x.

Janeway, C.M., Xu, X., Murphy, J.E., Chaidaroglou, A., Kantrowitz, E.R., 1993. Magnesium in the active site of *Escherichia coli* alkaline phosphatase is important for both structural stabilization and catalysis. Biochemistry 32, 1601–1609. https://doi.org/10.1021/bi00057a026.

Kim, J.H., Kang, J.C., 2016. Changes in hematological parameters, plasma cortisol, and acetylcholinesterase of juvenile rockfish, *Sebastes schlegelii* supplemented with the dietary ascorbic acid. Aquacult. Rep. 4, 80–85. https://doi.org/10.1016/j.aqrep.2016.07.001.

Kong, Y.Q., Ding, Z.L., Du, Z.Y., Sun, S.M., Wang, L.G., Li, E.C., Chen, L.Q., 2014. Dietary copper requirement of juvenile oriental river prawn *Macrobrachium nipponense*, and its effects on growth, antioxidant activities, and resistance to *Aeromonas hydrophila*. Isr. J. Aquacult.-Bamid. 66 (IJA_66.2014.1017).

Kong, Y., Ding, Z., Zhang, Y., Ye, J., Du, Z., 2017. Dietary selenium requirement of juvenile oriental river prawn *Macrobrachium nipponense*. Aquaculture 476, 72–78. https://doi.org/10.1016/j.aquaculture.2017.04.010.

Lall, S.P., 2002. The minerals. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, third ed. Academic Press Inc., San Diego, CA, USA, pp. 259–308.

Lim, C., Klesius, P.H., 2003. Influence of dietary levels of magnesium on growth, tissue mineral content, and resistance of channel catfish *Ictalurus punctatus* challenged with *Edwardsiellu ictaluri*. J. World Aquacult. Soc. 34, 18–28. https://doi.org/10.1111/j.1749-7345.2003.tb00035.x.

Lin, Y.H., Ku, C.Y., Shiau, S.Y., 2013. Estimation of dietary magnesium requirements of juvenile tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, reared in freshwater and seawater. Aquaculture 380-383, 47–51. https://doi.org/10.1016/j.aquaculture.2012.11.034.

Longbaf Dezfouli, M., Ghaedtaheri, A., Keyvanshokooh, S., Salati, A.P., Mousavi, S.M., Pasha-Zanoosi, H., 2019. Combined or individual effects of dietary magnesium and selenium nanoparticles on growth performance, immunity, blood biochemistry and antioxidant status of Asian seabass (*Lates calcarifer*) reared in freshwater. Aquac. Nutr. 25, 1422–1430. https://doi.org/10.1111/anu.12962.

Ministry of Agriculture Fisheries Bureau, 2021. China Fishery Statistical Yearbook. Chinese Agricultural Press, Beijing.

Musharraf, M., Khan, M.A., 2018. Dietary magnesium requirement for fingerlings of Rohu (*Labeo rohita*). Aquaculture 496, 96–104. https://doi.org/10.1016/j.aquaculture. 2018.07.013.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC.

Nozik-Grayck, E., Suliman, H.B., Piantadosi, C.A., 2005. Extracellular superoxide dismutase. Int. J. Biochem. Cell Biol. 37, 2466–2471. https://doi.org/10.1016/j.biocel.2005.06.012.

Rader, B.A., 2017. Alkaline phosphatase, an unconventional immune protein. Front. Immunol. 8, 897. https://doi.org/10.3389/fimmu.2017.00897.

Rader, B.A., Kremer, N., Apicella, M.A., Goldman, W.E., McFall-Ngai, M.J., 2012. Modulation of symbiont lipid a signaling by host alkaline phosphatases in the squid-vibrio symbiosis. mBio 3. https://doi.org/10.1128/mBio. 00093-12 e00093-12.

Roy, L., Davis, D., Saoud, I., Henry, R., 2007. Supplementation of potassium, magnesium and sodium chloride in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. Aquac. Nutr. 13, 104–113. https://doi.org/10.1111/j.1365-2095.2007.00460.x.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J., Boyd, C.E., 2010. Shrimp culture in inland low salinity waters. Rev. Aquacult. 2, 191–208. https://doi.org/10.1111/j.1753-5131.2010.01036.x.

Sahin, N., Onderci, M., Sahin, K., Cikim, G., Kucuk, O., 2005. Magnesium proteinate is more protective than magnesium oxide in heat-stressed quail. J. Nutr. 135, 1732–1737. https://doi.org/10.1093/jn/135.7.1732.

Sedlak, J., Linday, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellmanx's reagent. Anal. Biochem. 25, 192–205.

Shivakumar, K., Kumar, B.P., 1997. Magnesium deficiency enhances oxidative stress and collagen synthesis in vivo in the aorta of rats. Int. J. Biochem. Cell Biol. 29, 1273–1278. https://doi.org/10.1016/S1357-2725(97)00068-X.

Solórzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799–801. https://doi.org/10.4319/lo.1969.14.5.0799.

Srinivasan, V., Bhavan, P.S., Rajkumar, G., Satgurunathan, T., Muralisankar, T., 2017. Dietary supplementation of magnesium oxide (MgO) nanoparticles for better survival and growth of the freshwater prawn *Macrobrachium rosenbergii* post-larvae. Biol. Trace Elem. Res. 177, 196–208. https://doi.org/10.1007/s12011-016-0855-4.

Tan, X.H., Sun, Z.Z., Zhou, C.P., Huang, Z., Tan, L.J., Xun, P.W., Wang, A.L., 2018. Effects of dietary dandelion extract on intestinal morphology, antioxidant status, immune function and physical barrier function of juvenile golden pompano *Trachinotus ovatus*. Fish Shellfish Immunol. 73, 197–206. https://doi.org/10.1016/j.fsi.2017.12.020.

Tang, R., Wang, T., Huang, K., 2005. Mechanisms of selenium inhibition of cell apoptosis induced by oxysterols in rat vascular smooth muscle cells. Arch. Biochem. Biophys. 441, 16–24. https://doi.org/10.1016/j.abb.2005.06.006.

Tian, W.J., Wei, J.J., Li, E.C., Yu, N., Wu, Q.Q., Chen, L.Q., 2015. Effects of magnesium supplementation on growth, body composition and antioxidant ability of juvenie Chinese mitten crab (*Eriocheir sinensis*). Chin. J. Anim. Nutr. 27, 305–312.

Van der Velden, J.A., Spanings, F.A.T., Flik, G., Zegers, C., Kolar, Z.I., Wendelaar Bonga, S.E., 1991. Growth rate and tissue magnesium concentration in adult freshwater tilapia, *Oreochromis mossambicus* (Peters), fed diets differing in magnesium content. J. Fish Biol. 39, 83–91. https://doi.org/10.1111/j.1095-8649.1991.tb04343.x.

Wang, A., Wang, W., Wang, Y., Shang, L., Liu, Y., Sun, R., 2003. Effect of dietary vitamin C supplementation on the oxygen consumption, ammonia-N excretion and Na+/K+ ATPase of *Macrobrachium nipponense* exposed to ambient ammonia. Aquaculture 220, 833–841. https://doi.org/10.1016/S0044-8486(02)00536-7.

Wang, F.B., Luo, L., Lin, S.M., Li, Y., Chen, S., Wang, Y.G., Hu, C.J., 2011. Dietary magnesium requirements of juvenile grass carp, *Ctenopharyngodon idella*. Aquac. Nutr. 17, e691–e700. https://doi.org/10.1111/j.1365-2095.2010.00829.x.

Wei, S.P., Jiang, W.D., Wu, P., Liu, Y., Zeng, Y.Y., Jiang, J., Feng, L., 2018. Dietary magnesium deficiency impaired intestinal structural integrity in grass carp (*Ctenopharyngodon idella*). Sci. Rep. 8 https://doi.org/10.1038/s41598-018-30485-8.

Yang, X.Z., Song, X.Z., Zhang, C., Pang, Y.Y., Song, Y.M., Cheng, Y.X., Zong, X., 2020. Effects of dietary melatonin on hematological immunity, antioxidant defense and antibacterial ability in the Chinese mitten crab, *Eriocheir sinensis*. Aquaculture 529, 735578. https://doi.org/10.1016/j.aquaculture.2020.735578.

Yeh, M.Y., Shih, Y.L., Chung, H.Y., Chou, J., Lu, H.F., Liu, C.H., Chung, J.G., 2016. Chitosan promotes immune responses, ameliorates glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, but enhances lactate dehydrogenase levels in normal mice in vivo. Exp. Ther. Med. 11, 1300–1306. https://doi.org/10.3892/etm.2016.3057.

Zafar, N., Khan, M.A., 2021. Effects of dietary magnesium supplementation on growth, feed utilization, nucleic acid ratio and antioxidant status of fingerling *Heteropneustes fossilis*. Anim. Feed Sci. Technol. 273, 114819 https://doi.org/10.1016/j.anifeedsci.2021.114819.

Zhang, C.X., Huang, F., Li, J., Wang, L., Song, K., Mai, K.S., 2016. Interactive effects of dietary magnesium and vitamin E on growth performance, body composition, blood parameters and antioxidant status in Japanese seabass (*Lateolabrax japonicus*) fed oxidized oil. Aquac. Nutr. 22, 708–722. https://doi.org/10.1111/anu.12393.

Zhang, Y.Y., Fan, Z., Wu, D., Li, J.N., Xu, Q.Y., Liu, H.B., Wang, L.S., 2021. Dietary magnesium requirement on dietary minerals and physiological function of juvenile hybrid sturgeon (*Acipenser schrenckii*♀ × *Acipenser baerii*♂). Aquacult. Int. 29, 1697–1709. https://doi.org/10.1007/s10499-021-00712-7.

Zhou, H.Q., Wang, Y.Q., Wang, S.C., 1993. Nutritional requirement of *Penaeus chinensis* for dietary calcium, phosphorus, magnesium, potassium and iron. J. Shanghai Fish. Univ. 2, 113–118.