Effect of garlic (*Allium sativum*) extract on growth, enzymological and biochemical responses, and immune related gene expressions in giant freshwater prawn (*Macrobrachium rosenbergii*)

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Abstract

In the current study, growth performance, biochemical constituents of muscle, activities of enzymes in the haemolymph, and expressions of immune-related genes were evaluated in the giant freshwater prawns *Macrobrachium rosenbergii* fed diets supplemented with aqueous garlic (*Allium sativum*) extract at 0, 5, 10, and 20 g/kg (w/w) for 60 days. At the end of the feeding trial, weight gain and specific growth rate were significantly improved in garlic-fed prawn groups compared with the control ($P<0.05$). Moreover, feed conversion ratio was significantly lower in the garlic-fed groups than in the control ($P < 0.05$). Activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) in the hepatopancreas, activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and levels of albumin and total protein in the hemolymph were significantly increased in the garlic treatments ($P < 0.05$). Furthermore, garlic supplemented diets improved muscle biochemical profiles, particularly contents of crude protein and total ash, and up-regulations of immune deficiency (IMD) and heat shock proteins (HSP70) gene expression ($P < 0.05$). Therefore, garlic has positive effects on growth performance and physio-biochemical responses of *M. rosenbergii*, and thus, it can be used as an additive for stress resistance and as a growth promoter in sustainable aquaculture.

Keywords: Garlic supplementation, Growth, HSP70, IMD, Prawn aquaculture

Introduction

The prevalence of diseases is one of the most important limiting factors in the development of aquaculture practices (Stentiford et al., 2012). Although synthetic drugs are key to control disease outbreaks in aquaculture, their multiple negative impacts on the host, the environment, and even human health has fueled the search of alternative more sustainable alternatives (Rico et al., 2013; Lieke et al., 2020; Reverter et al., 2020). Natural immunostimulant components, including the use of bioactive plant components, are therefore arising as promising sources to prevent and treat disease outbreaks in aquaculture (Van Hai, 2015; Pourmozaffar et al., 2019; Tamadoni Jahromi et al., 2021). Garlic (*A. sativum*) is known as an "all-healing" herb (M Abu Elala, 2016). Garlic has been used for many years as a traditional medicine and a food additive to improve human's physical health and to fight some diseases (Srivastava et al., 2012). In aquatic animals, garlic has been found to increase in growth performance through improved appetite, gastrointestinal motility, and stimulation of digestive enzymes (Lee et al., 2012). Additionally, immune responses in aquatic animals including *Oncorhynchus mykiss* (Mohebbi et al., 2012; Adineh et al., 2020), *Oreochromis niloticus* (Aly et al., 2010), *Rutilus rutilus* (Ghehdarijani et al., 2016), *Huso huso* (Gholipour Kanani et al., 2014), *Carassius auratus* (Dadgar et al., 2019) and *Litopenaeus vannamei* (Samadi et al., 2016) were enhanced following oral garlic administration. Garlic possesses wide spectrum of antimicrobial, antiviral, and antifungal activities against aquaculture-relevant pathogens such as *Aeromonas hydrophila* (Nya et al., 2009), spring viremia (Karimi Pashaki et al., 2020), *Vibrio alginolyticus*, *V. harvey*, *V. anguillarum* (Natasya-Ain et al., 2018), and *Yersinia ruckeri* (Zaefarian et al., 2017). Some of the previously reported health benefits of garlic are ascribed to their organosulphur compounds (thiosulfinates), and especially allicin (diallyl thiosulfate). For example, Breyer et al. (Breyer et al., 2015) suggested that allicin can inhibit pathogen infection (*Aeromonas salmonicida*) through improvement of host (*Oncorhynchus mykiss*) immunological parameters, including phagocytic activity, lysozyme activity and antibody production. Moreover, garlic contains other valuable ingredients such as vitamins (C, B, and A), linoleic acid, silicates, iodine salts, flavonoids, and other thiosulfinate compounds (allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate, ajoene, y-L-glutamyl-S-alkyl-L- cysteine) that might also display beneficial effects on aquatic animals (Adineh et al., 2020).

After fish and molluscan shellfish Farming, crustacean farming is the most important aquaculture practice in many countries (Yearbook, 2020). The giant freshwater prawn, *M. rosenbergii*, is a particularly esteemed species in aquaculture due to its fast growth, big sizes and its habitat versatility. Although it is mainly cultured in Asian countries (Southern and South-Eastern), this species has also been introduced in many other regions and its farming is now globally widespread (Yearbook, 2020). Up to now, whether dietary supplementation of garlic extract could enhance the physiological parameters and molecular responses of giant freshwater prawn is still unknown. Thus, the purpose of the present work was to evaluate the effect of garlic extract on some physiological and biochemical parameters, immune related genes and growth performance in giant freshwater prawn.

2. Materials and methods

2.1. Preparation of garlic extract

Fresh garlic bulbs were purchased from a local farm in Kermanshah, Iran. Peeled garlic cloves (100 g) were chopped and then blended with 200 ml distilled water for 3 min. The solution was centrifuged at 10,000 rpm for 5 min. The supernatant was removed and was passed through a Whatman filter paper (42µm). The obtained extract was kept at -20^0 C.

2.2. Experimental design

The *M. rosenbergii* post-larvae (PL-12, ranging from 1.0 to 1.3 cm in length and 1.1 to 1.3g) were obtained from a local commercial shrimp farm located in Ghasr-e-Shirin city (Kermanshah province, Iran). A total of 360 PL were randomly distributed in twelve (30 PL/tank) 25 L (70x40x40) aerated tanks with dechlorinated water and were acclimatized to the experimental conditions for ten days. Four groups of prawns were assigned in triplicate for 60 days. Three groups were fed with experimental diets consisting of a basal diet supplemented with garlic extract (5, 10, and 20 g/kg of garlic extract) (Breyer et al., 2015), and one group was fed with the control diet (i.e., basal diet, (Table 1). The water temperature, dissolved oxygen and pH were measured daily using aWagtech portable temperature, oxygen and pH meter (Berkshire, UK). Uneaten food and faeces were removed daily. Water was exchanged (50%) in daily manner. Shrimps were fed daily at 3% of body weight, and during the experimental period they were fed at 08:00, 13:00, 17:00 and 23:00.

2.3 Sample collection

All animal experiments and animal protocols were conducted in accordance with National Ethical Framework for Animal Research in Iran. At the end of experiment, three prawns per tank (9 per treatment) were anesthetized with clove oil, 100 mg/l (Ranjit Kumar et al., 2013) and sacrificed. First, the whole prawns were weighted. Then muscle tissues from abdominal segments were immediately dissected for analysis of their proximate composition. Hepatopancreas were collected, weighed and stored at -80°C until further antioxidant enzyme and gene expression analysis (Akbary et al., 2019). Haemolymph was withdrawn from the ventral sinus of prawns at the end of experimental period using 1-ml tuberculin syringe with 24-gauge needle and were used in the analysis of different biochemical enzymes. The samples were centrifuged at 12000 g for 20 min and supernatants were collected and stored at – 20º C until use.

2.4 Survival, growth and nutritional indices analysis

At the end of experiment, growth parameters and survival (S) were calculated as described by Zhou et al. (Zhou et al., 2007) and Akbary (Akbary et al., 2020)

Survival (S) (%) = final prawn number/initial prawn number × 100

Weight gain (WG) (%) = {(Final body weight (FBW)− Initial body weight (IBW)) / IBW} × 100

Specific growth rate (SGR%)={(ln FBW - ln IBW) / Experiment period} ×100

Food conversion ratio (FCR) = Total feed intake (g) / Total wet weight gain (g)

2.5. Muscle biochemical constituent

A standard method (AOAC, 2003) was used to measure crude protein, crude lipid, moisture, and ash contents of the prawn muscle and experimental diets. The samples were analyzed for crude protein (Kjeldahl method, Kjeltec 2100 Distillation Unit, Hoganas, Sweden), crude fat (Soxhlet extraction), moisture (24 h at 105 ºC) and ash content (4h at 550 ºC). (Jahanbakhshi et al., 2012; Akbary et al., 2018).

2.6. Antioxidant enzyme assays

Frozen hepatopancreas samples were homogenized in a phosphate buffer (0.1 mol /l, pH 7.2) on ice. Homogenates were centrifuged at 1000×g for 15 min at 4°C. After being centrifuged, the supernatant was extracted and placed in new tubes (1–1.5 mL) and then frozen at -20 °C up to the time of analysis. The activities of CAT, GSH-Px, and SOD enzymes were determined according to the Zehra and Khan (2019) procedure. Antioxidant enzyme activities were expressed in units per milligram of protein (U/mg protein).

2.7. Haemolymph biochemical parameters

The haemolymph samples were mixed with an anticoagulant solution (100 mM sodium citrate, 10 mM tris-Hcl, 250 mM sucrose, pH 7.2) (Ranjit Kumar et al., 2013). The aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities and albumin concentration were measured using detection kits (Pars Azmoon Co. Iran) and an autoanalyzer (Hitachi 917, Japan) (Tamadoni Jahromi et al., 2020). Total protein concentration was measured according to the Lowry method (Lowry et al., 1951).

2.8. RNA extraction

For RNA extraction, samples were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed. Biozol reagent (Bio flux; China) was used for extracting total RNA from hepatopancreas samples according to the manufacturer's instructions. Quality of RNA was estimated by electrophoresis on ethidium bromide staining and a 1.5% agarose gel. The RNA's quantity and quality was measured with a nanodrop spectrophotometer at a wavelength of 260/280 nm. Samples with RNA ratios greater than 1.8 (at 260/280 nm) were used for further experiments. In accordance with the manufacturer’s protocol, suprime script RT premix (2×) cDNA synthesis kit (GeNet BIO Inc, South Korea) was used to synthesis the first strand of cDNA (Pourmozaffar et al., 2017).

2.9. Relative mRNA expression of immune-related genes

The transcript expression levels were determined by Real-time Polymerase Chain Reaction (RT-PCR, Bio-Rad, USA). Table 2 exhibits the primers used for amplification of heat shock proteins (HSP70), immune deficiency (IMD), and β-actin (housekeeping gene). In accordance with standard protocols, 10 µl of SYBR Green qPCR Master Mix (1×) (Fermentase, Lithuania), 0.2 µl of forward and reverse specific primers (100 nM), 10 ng of cDNA template, and 6.40 µl nuclease free water to final volume of 20 ml were used (Pourmozaffar et al., 2017). PCR reaction mixtures were subjected to the following thermal profile: 95 ºC for 5 min, followed by 40 cycles of 10 s at 95 ºC, and 10 s at 54 ºC and 10 s at 72 °C. The $2^{-\Delta\Delta CT}$ method was used in calculating the fold changes in HSP70 and IMD relative mRNA expression. The β-actin gene was used to normalize the expression levels of the target genes.

2.10. Statistical analysis

Results were analyzed statistically using One-way analysis of variance (ANOVA) and Duncan's post hoc test was performed to determine the significant differences from each other. Normality and homogeneity were tested using the Kolmogorov–Smirnov and the Levene's tests, respectively. $P < 0.05$ was considered significant difference. Statistical analysis was performed using SPSS software (version 20).

3. Results

3.1. Growth performance

Table 3 presents the growth performance of *M. rosenbergii* PL fed with garlic extracts. There was no mortality in the experiment. Final body weight for all groups was in the range 9.53- 16.43 g and significant differences were observed among the groups ($P > 0.05$). Significant increase ($P < 0.05$) in FBW, WG, and SGR was observed in treated groups in a dose-dependent manner compared with control group. A significant reduction in FCR was observed in the treated groups compared to control group ($P < 0.05$). Highest and lowest weight gains and SGR values were observed in the control group and 20 g/kg garlic extract respectively. Giant freshwater prawn fed with a diet containing garlic extract at 20 g/kg showed the highest FBW, WG and SGR, and the lowest FCR ($P < 0.05$).

3.2. Muscle biochemical constituent

The muscle composition of prawn's carcass fed the experimental diets are presented in (Table 4). The highest concentration of protein was recorded in prawns fed with diets containing 20 g/kg garlic extract ($P < 0.05$). The lipid content decreased with increasing garlic extract levels in the diets ($P < 0.05$). The highest lipid content (9.82 ± 0.45 %) in muscle tissue was observed in the control group ($P < 0.05$). Significant increase in ash content was observed in *M. rosenbergii* fed with diets containing garlic extract ($P < 0.05$). Prawns fed with diets enriched with garlic extracts had lower average values of moisture content but no significant difference was observed among the groups ($P > 0.05$).

3.3. Antioxidant enzymes

The antioxidant enzyme activities of *M. rosenbergii* fed with different garlic extracts are shown in Table 5. SOD, CAT, and GSH-Px activities of prawn fed the extract-containing diets were significantly higher than those of shrimp fed the control diet ($P < 0.05$). Moreover, the activity of these enzymes was highest in the 20 and 10 g/kg garlic groups, respectively, which was also significantly higher than in control and 5 g/kg garlic groups ($P < 0.05$).

3.4. Haemolymph biochemical parameters

Haemolymph enzymes' activity of prawn fed with experimental diets are presented in Table 6. ALT, AST, ALP activities and total protein and albumin levels increased with the increase of garlic levels in the diets ($P < 0.05$). The highest ALT, AST, and ALP activities were observed in prawns fed with 20g/kg garlic group ($P < 0.05$). Similar trends were observed in the levels of total protein and albumin in prawn hemolymph ($P < 0.05$).

3.5. Related immune gene expression

Expression of the HSP70 and IMD genes was significantly up-regulated in garlic groups ($P < 0.05$) (Fig 1 and Fig 2). Moreover, the highest expression of IMD and HSP70 genes was observed in prawns fed a diet containing 20 g/kg garlic extract ($P < 0.05$).

4. Discussion

In the present study, growth parameters of *M. rosenbergii* were significantly increased when fed with a garlic extract supplemented diet. These results are in accordance with previous reports in *L. vannamei* (Labrador et al., 2016; Samadi et al., 2016; Kumar et al., 2019), *O. mykiss* (Nya et al., 2009; Etyemez Büyükdeveci et al., 2018; Adineh et al., 2020), *R. rutilus* (Ghehdarijani et al., 2016), *Salmo caspius* (Zaefarian et al., 2017), *Mesopotamichthys sharpeyi* (Maniat et al., 2014), *Lates calcarifer* (Talpur et al., 2012), *O. niloticus* (Metwally, 2009), *Cyprinus carpio* (Karimi Pashaki et al., 2018), and *M. rosenbergii* (Poongodi et al., 2012). As proposed, this improvement in growth performance could be related to higher digestive enzymes activities (such as lipase, protease and amylase) as well as changes in intestinal microbiota (Shanthi et al., 2012; Radhakrishnan et al., 2015; Etyemez Büyükdeveci et al., 2018). Supa-aksorn et al. (Supa-aksorn et al., 2017), reported that amylase, lipase, and trypsin activities was higher in *O. niloticus* fed with diets enriched with garlic extract (5 and 10 g/kg) than that of fish fed with a control diet. Likewise, diets supplemented with garlic extract may act as appetizers, thereby increasing feed intake (Poongodi et al., 2012; Platel et al., 2016). Labrador et al. (Labrador et al., 2016), found that Pacific white leg shrimp (*L. vannamei*) fed diets enriched with garlic powder (20, 40, and 60 g/kg) showed the highest weight gain compared to other groups. In another study, the highest growth parameters in *Tilapia zillii* fingerlings were recorded when fish fed a diet containing 20 g/kg garlic for 75 days (Jegede, 2012). Allicin in garlic can enhance growth performance through stimulating intestinal flora, improving digestive system, and enhancing energy utilization (Supa-aksorn et al., 2017).

The proximate body composition is often regarded as a suitable indicator of the physiological condition of an organism (Kotiya et al., 2019). Our results showed that *M. rosenbergii* body composition was significantly affected by the oral administration of garlic extracts. The crude protein and ash of prawns fed control diet were notably ($P < 0.05$) lower than treated groups fed garlic extracts. Samadi et al. (Samadi et al., 2016), showed positive effects of dietary garlic supplementation on crude protein by *L. vannamei* fed with a garlic extract at 800 mg/kg, which is consistent with results of the present study. Additionally, Adineh et al. (Sarhadi et al., 2020) found that diets enriched with garlic extracts (10 g/kg) led to markedly increased crude protein and markedly reduced crude fat in whole fish body. Similar results were documented in *O. niloticus* and *T. zillii* fed with diets containing 30 and 10-30 g/kg garlic extract, respectively (Shalaby et al., 2006; Ajiboye et al., 2016). It has been shown that garlic affects body protein metabolism caused by hormonal regulation through raising protein metabolism or stimulating hormone secretion (Srivastava et al., 2012). The fat value in this study was similar to Samadi et al. (Samadi et al., 2016), Maniat and Ghotbeddin (Maniat et al., 2014), and Shalaby et al. (Shalaby et al., 2006) reports. *M. rosenbergii* body fat decreased from 9.82 to 5.22 % with increases in dietary garlic extract levels. Garlic administration may affect whole-body fat due to hepatic activities reduction of cholesterogenic and lipogenic enzymes like fatty acid synthase, dehydrogenase and malic enzyme which are caused to reducing of carcass lipid values (Yeh et al., 2001). In addition, some organosulfur compounds in garlic such as diallyl-disulfide (allicin) and S-allyl cysteine may prevent the synthesis of cholesterol, which could also lead to lower fat levels (Samadi et al., 2016). Kim et al. (Kim et al., 2011) also showed that dietary addition of garlic oil has no notable difference in moisture level of *L. vannamei.* Overall, it seems that dietary supplementation with garlic and its derivatives (e.g., extracts, bioactive compounds) affect positively aquatic animals body composition, however these effects are dependent on the level of garlic used, cultured species, and the environmental conditions (Mahmoud et al., 2019).

SOD/CAT system is the first defense mechanism against reactive oxygen species (ROS) (Mahmoud et al., 2019). In the present study, antioxidant activities were higher in prawn fed diets supplemented with garlic extract compared to control group. Prawn fed the diets containing 10 and 20 g/kg garlic diets exhibited the highest SOD, CAT and GSH-Px values compared with other diets. These results are in accordance with earlier studies in Nile tilapia (*O. niloticus*) (Metwally, 2009; Mahmoud et al., 2019), common carp (*C. carpio)* (Naeiji et al., 2013; Yousefi et al., 2020) and European seabass, *Dicentrarchus labrax* (Mosbah et al., 2018) and rainbow trout (*O. mykiss*) (Mohebbi et al., 2012; Adineh et al., 2020) through enhancing the endogenous antioxidant enzymatic mechanisms and counteracting the effects of ROS (Abdel-Tawwab et al., 2021). In addition, Jahanjoo et al. (Jahanjoo et al., 2018) reported that the SOD activity was notably increased in fish fed diets supplemented with 1% ginger or garlic. Garlic has been shown to activate defense mechanisms and counteract infection through the production of superoxide anions (Sahu et al., 2007). Sulphur-containing compounds (diallyl disulphide and s-allyl cysteine) and flavonoids are two important types of antioxidant components within garlic, which might be related to the higher antioxidant activities observed in prawns fed with garlic extracts (Sharma et al., 2010).

Hepatic enzymes are commonly used as indicators of hepatopancreas yield, because these enzymes have crucial roles in the interactions of carbohydrates, fats and amino acids (Hemre et al., 1996). Higher values of AST and ALT may be therefore regarded as indicators of alterations in the permeability of hepatopancreas tissue cells (Zeng et al., 2016). Our results showed that, ALP, ALT, and AST activities were significantly higher in the 20 g/kg garlic group than in the other groups. Similarly, Mohebbi et al. (Mohebbi et al., 2012) indicated that serum alanine aminotransferase and aspartate aminotransferase levels in rainbow trout increased after fed with 40 and 50 g/kg garlic powder. Joseph et al. (Joseph et al., 1989) demonstrated that rats fed with a garlic extract at 200 g/l had significant increase in hepatic enzyme activity compare to other experimental groups after 10 days. Moreover, increase in hepatopancreas antioxidant activities were accompanied by higher activity of haemolymph AST and ALT. The observed dose-dependent increases in ALT, AST and ALP in treated shrimp could eventually suggest that hepatopancreas functions were activated. When the lesions in the hepatopancreas were not aimed to observe and in the view of the better growth, these alterations may be taken as an initial adaptive mechanism of prawns. ALT and AST increases were also observed in African catfish fed with dietary clove (Adeshina et al. 2018).

Total protein and albumin levels were significantly increased in the 10 and 20 g/kg garlic groups compared with other groups which are in agreement with Talpur and Ikhwanuddin (Talpur et al., 2012) and Adineh et al. (Adineh et al., 2020) results. Higher levels of total protein and albumin are often related to improved innate immunity following functional feed supplementation (Nya et al., 2009; Pourmozaffar et al., 2019). The S-allyl cysteine in garlic may play a crucial role in the function of organs related to blood cell and immune system stimulation (Ndong et al., 2011). Meanwhile, Nya and Austin (Nya et al., 2009) found that *O. mykiss* fed diets enriched with 1 and 10 g/kg garlic exhibited higher serum total protein than fish fed with control diet. The enhancement of total protein level may be due to induction of antiprotease activity by garlic (Zaefarian et al., 2017).

Our results showed that the expression of HSP70 and IMD genes in the hepatopancreas were significantly increased in prawn fed with diets containing garlic extract compared to the control group. HSP70 act as a molecular stress protein, which can contribute to the maintenance of cellular homeostasis (Liu et al., 2016) and endogenous peroxidase activity for catalyzing the conversion of ROS (Duan et al., 2018). Consistent with our results, previous literature showed the up-regulation of related-immune genes following feeding Nile tilapia (*O. niloticus*) with diet containing *Spirulina platensis* + garlic (M Abu Elala, 2016). Moreover, Kaleo et al. (Kaleo et al., 2019) and Duan et al. (Duan et al., 2018) demonstrated that the expression of HSP70 gene in the *M. rosenbergii* and *L. vannamei* was significantly up regulated after fed diets containing M. oleifera (2.5 and 5 g/kg) and succinic acid (2.5, 5, and 10 g/kg), respectively. The up-regulation of HSP70 is related to modulating cellular anti-stress reactions (Feder et al., 1999; Wan et al., 2014). Based on the present study, there was a significant increase in expression of IMD gene when prawns fed diets containing garlic extracts. IMD contributed shrimps innate immune reactions (Li et al., 2013). The up-regulation of expression of IMD in *M. rosenbergii* may be attributed to high concentrations of garlic extract, so that the prawn's body for resisting stress might have to activate the IMD signaling pathway (Kaleo et al., 2019). As suggested, increase in IMD and HSP-70 gene expressions could also support stress management in prawns fed with garlic. It has been reported that an increase in HSP-70 gene expression in fish fed with plant-and probiotic-enriched diets was rather related to an anti-stress response (Abarike et al. 2020). Sung et al. (2012) also reported that enhancing HSP-70 synthesis could protect common carp against ammonia-related stress. Therefore in the present study, the general health of *M. rosenbergii* was found to be improved by up-regulations of IMD and HSP-70 genes.

Conclusion

In summary, our results show some potential in using low-doses of garlic in prawn aquaculture as a growth-stimulator and antioxidant promoter. In order to fully evaluate the positive/negative potential of garlic-enriched diets, further research also needs to assess whether increased HSP-70 in garlic-fed shrimp would be beneficial in stressful conditions (e.g. shrimp exposed to pathogens or to pollutants), or if in contrast garlic-treated shrimp would display exacerbated stress-responses. In our study, despite increases in ALT and AST, treated prawn displayed higher growths and higher antioxidant activities (CAT, SOD, GSH-px) suggesting that garlic enrichment, at least at low doses, could potentially be beneficial. However, with the progressive increase in garlic concentration, an important increase in the AST, ALP and ALT was observed, which could suggest the presence of hepatic toxicity. However, further research is needed to explore whether a garlic-enriched diet could cause histological changes in the hepatopancreas of *M. rosenbergii*.

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References

Abarike, E.D.; Jian, J.; Tang, J.; Cai, J.; Sakyi, E.M.; Kuebutornye, F.K.A., 2020. A mixture of

Chinese herbs and a commercial probiotic *Bacillus* species improves hepato-immunological, stress, and antioxidanat parameters, and expression of HSP70 and HIF-1a mRNA to hypoxia, cold and heat stress in Nile tilapia, *Oreochromis niloticus. Aquaculture Reports.*, **18**, 100438.

Abdel-Tawwab, M.; Khalil, R. H.; Diab, A. M.; Khallaf, M. A.; Abdel-Razek, N.; Abdel-Latif, H. M. R.; Khalifa, E., 2021: Dietary garlic and chitosan enhanced the antioxidant capacity, immunity, and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass. *Fish and Shellfish Immunology*., **113**, 35–41.

Adeshina, I.; Jenyo-Oni, A.; Emikpe, B.O.; Ajani, E.K.; Abdel-Tawwab, M., 2018. Stimulatory effect of dietary clove, Eugenia caryohyllata, bud extract on growth performance, nutrient utilization, antioxidant capacity, and tolerance of African catfish, *Clarias gariepinus* (B.), to *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society.*, **50**, 390-405.

Adineh, H.; Harsij, M.; Jafaryan, H.; Asadi, M., 2020: The effects of microencapsulated garlic (*Allium sativum*) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles. *Journal of Applied Animal Research*., **48**, 372–378.

Ajiboye, O. O.; Qari, R., 2016: Short-term evaluation of graded levels of dietary garlic powder (*Allium sativum* L.) as growth promoter on growth, survival and feed utilization of redbelly tilapia, *Tilapia zillii* reared in glass aquaria tanks. *International Journal of Marine Science*., **6**, 1–7.

Akbary, P.; Jahanbakhshi, A., 2018: Growth yield, survival, carcass quality, haematological, biochemical parameters and innate immune responses in the grey mullet (*Mugil cephalus* linneaus, 1758) fingerling induced by immunogen® prebiotic. *Journal of Applied Animal Research*., **46**, 10–16.

Akbary, P.; Jahanbakhshi, A., 2019: Nano and macro iron oxide (Fe_2O_3) as feed additives: Effects on growth, biochemical, activity of hepatic enzymes, liver histopathology and appetite-related gene transcript in goldfish (*Carassius auratus*). *Aquaculture*., **510**, 191–197.

Akbary, P.; Adeshina, I.; Jahanbakhshi, A., 2020: Growth performance, digestive enzymes, antioxidant activity and immune responses of *Litopenaeus vannamei* fed with *Jania adhaerens* J.V. supplemented diet against *Photobacterium damselae* infection. *Animal Feed Science and*

Technology., **270**, 1–31.

Aly, S. M.; Mohamed, M. F., 2010: *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). *Journal of Animal Physiology and Animal Nutrition*., **94**, 31–39.

Breyer, K. E.; Getchell, R. G.; Cornwell, E. R.; Wooster, G. A.; Ketola, H. G.; Bowser, P. R., 2015: Efficacy of an extract from garlic, *Allium sativum*, against infection with the *Furunculosis bacterium*, *Aeromonas salmonicida*, in rainbow trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*., **46**, 273–282.

Chumpol, S.; Kantachote, D.; Nitoda, T.; Kanzaki, H., 2017: The roles of probiotic purple nonsulfur bacteria to control water quality and prevent acute hepatopancreatic necrosis disease (AHPND) for enhancement growth with higher survival in white shrimp (*Litopenaeus vannamei*) during cultivation. *Aquaculture*., **473**, 327–336.

Dadgar, S.; Seidgar, M.; Nekuiefard, A.; Valipour, A. R.; Sharifian, M.; Hafezieh, M., 2019: Oral administration of garlic powder (*Allium sativum*) on growth performance and survival rate of *Carassius auratus* fingerlings. *Iranian Journal of Fisheries Sciences*., **18**, 71–82.

Duan, Y.; Wang, Y.; Zhang, J.; Sun, Y.; Wang, J., 2018: Dietary effects of succinic acid on the growth, digestive enzymes, immune response and resistance to ammonia stress of *Litopenaeus vannamei*. *Fish and Shellfish Immunology*., **78**, 10–17.

Eryalcin, K. M., 2018: Impact of garlic and curcumin on the hepatic histology and cytochrome P450 gene expression of aflatoxicosis *Oreochromis niloticus* using RT-PCR. *Turkish Journal of Fisheries and Aquatic Sciences*., **18**, 81–90.

Etyemez Büyükdeveci, M.; Balcázar, J. L.; Demirkale, İ.; Dikel, S., 2018: Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*., **486**, 170–174.

Feder, M. E.; Hofmann, G. E., 1999: Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annual Review of Physiology*., **61**, 243–282.

Ghehdarijani, M. S.; Hajimoradloo, A.; Ghorbani, R.; Roohi, Z., 2016: The effects of garlic-supplemented diets on skin mucosal immune responses, stress resistance and growth performance of the Caspian roach (*Rutilus rutilus*) fry. *Fish and Shellfish Immunology*., **49**, 79–83.

Gholipour Kanani, H.; Nobahar, Z.; Kakoolaki, S.; Jafarian, H., 2014: Effect of ginger and garlic-supplemented diet on growth performance, some hematological parameters and immune responses in juvenile *Huso huso*. *Fish Physiology and Biochemistry*., **40**, 481–490.

Hemre, G. I.; Waagbø, R.; Hjeltnes, B.; Aksnes, A., 1996: Effect of gelatinized wheat and maize in diets for large Atlantic salmon (*Salmo salar* L.) on glycogen retention, plasma glucose and fish health. *Aquaculture Nutrition*., **2**, 33–39.

Jahanbakhshi, A.; Imanpuor, M.; Taghizadeh, V.; Shabani, A., 2012: Effects of replacing fish meal with plant protein (sesame oil cake and corn gluten) on growth performance, survival and carcass quality of juvenile beluga (*Huso huso*). *World Journal of Fish and Marine Sciences*., **4**, 422–425.

Jahanjoo, V.; Yahyavi, M.; Akrami, R.; Bahri, A. H., 2018: Influence of adding garlic (*Allium sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haematoImmunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream. *Turkish Journal of Fisheries and Aquatic Sciences*., **18**, 633–645.

Jegede, T., 2012: Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and survival of *Tilapia zillii* (Gervais 1852) fingerlings. *Journal of Agricultural Science*., **4**, 269–274.

Joseph, P. K.; Rao, K. R.; Sundaresh, C. S., 1989: Toxic effects of garlic extract and garlic oil in rats. *Indian journal of experimental biology*., **27**, 977–979.

Kaleo, I. V.; Gao, Q.; Liu, B.; Sun, C.; Zhou, Q.; Zhang, H.; Shan, F.; Xiong, Z.; Bo, L.; Song, C., 2019: Effects of *Moringa oleifera* leaf extract on growth performance, physiological and immune response, and related immune gene expression of *Macrobrachium rosenbergii* with *Vibrio anguillarum* and ammonia stress. *Fish and Shellfish Immunology*., **89**, 603–613.

Karimi Pashaki, A.; Zorriehzahra, S. M. J.; Ghasemi, M.; Sharif Rohani, M.; Hosseini, S. ., 2018: Effects of dietary garlic extract on some blood, immunity and growth parameters of common carp fingerlings (*Cyprinus carpio*). *Iranian Journal of Aquatic Animal Health*., **4**, 28–39.

Karimi Pashaki, A.; Ghasemi, M.; Zorriehzahra, M. J.; Sharif Rohani, M.; Hosseini, S. M., 2020: Effects of dietary garlic (*Allium sativum*) extract on survival rate, blood and immune parameters changes and disease resistance of common carp (*Cyprinus carpio* carpio Linnaeus, 1758) against spring viremia of carp (SVC). *Iranian Journal of Fisheries Sciences*., **19**, 1024–1039.

Kim, J. D.; Nhut, T. M.; Hai, T. N.; Ra, C. S., 2011: Effect of dietary essential oils on growth, feed utilization and meat yields of white leg shrimp L. vannamei. *Asian-Australasian Journal of Animal Sciences*., **24**, 1136–1141.

Kotiya, A. S.; Vadher, K. H.; Bhatt, A. J.; Dave, T., 2019: Comparison of proximate composition level in *Litopenaeus vannamei* cultured in various stocking density during summer crop in province of Gujarat states in India. *Journal of Entomology and Zoology Studies*., **7**, 59–72.

Kumar, R. M.; Sekhara Rao, A. C.; Daggula, N.; Guguloth, G.; Das, B. Y.; Indhuri, A. K., 2019: Growth promoter effect of ginger, garlic and fenugreek on Pacific white leg shrimp (*Litopenaeus vannamei*). *International Journal of Current Microbiology and Applied Sciences*., **8**, 2993–3001.

Labrador, J. R. P.; Guinares, R. C.; Hontiveros, G. J. S., 2016: Effect of garlic powder-supplemented diets on the growth and survival of Pacific white leg shrimp (*Litopenaeus vannamei*). *Cogent Food & Agriculture*., **2**, 1–8.

Lee, J. Y.; Gao, Y., 2012: Review of the application of garlic, *Allium sativum*, in aquaculture. *Journal of the World Aquaculture Society*., **43**, 447–458.

Li, F.; Xiang, J., 2013: Recent advances in researches on the innate immunity of shrimp in China. *Developmental and Comparative Immunology*., **39**, 11–26.

Lieke, T.; Meinelt, T.; Hoseinifar, S. H.; Pan, B.; Straus, D. L.; Steinberg, C. E. W., 2020: Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Reviews in Aquaculture*., **12**, 943–965.

Liu, F.; Cheng, B.; Guo, Q.; Shi, H.; Gou, L.; Lu, Y.; Wang, J.; Shen, W.; Yan, S.; Wu, M., 2016: Effects of indigowoad root (*Radix Isatidis*) on the immune responses and HSP70 gene expression of medicinal leeches (*Poecilobdella manillensis*) under Proteus mirabilis infection. *Aquaculture*., **454**, 44–55.

Lowry, O. .; Rosebrough, N. . .; Farr, A. L.; Randall, R. . ., 1951: Protein measurement with the Folin phenol reagent. *The Journal of biological chemistry*., **193**, 265–275.

M Abu Elala, N., 2016: Effects of dietary supplementation of *Spirulina platensis* and garlic on the growth performance and expression levels of immune-related genes in Nile tilapia (*Oreochromis niloticus*). *Journal of Aquaculture Research & Development*., **07**, 1–10.

Mahmoud, R.; Aziza, A.; Marghani, B.; Eltaysh, R., 2019: Influence of ginger and garlic supplementation on growth performance, whole body composition and oxidative stress in the muscles of Nile tilapia (*O. niloticus*). *Advances in Animal and Veterinary Sciences*., **7**, 397–404.

Maniat, M.; Ghotbeddin, N., 2014: Effect of garlic on growth performance and body composition of benni fish (*Mesopotamichthys sharpeyi*). *International Journal of Biosciences (IJB)*., **5**, 269–277.

Metwally, M. A. A., 2009: Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*). *World Journal of Fish and Marine Sciences*., **1**, 56–64.

Mohebbi, A.; Nematollahi, A.; Dorcheh, E. E.; Asad, F. G., 2012: Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*., **43**, 1184–1193.

Mosbah, A.; Guerbej, H.; Boussetta, H.; Bouraoui, Z.; Banni, M., 2018: Protective effects of dietary garlic powder against cadmium-induced toxicity in sea bass liver: A chemical, biochemical, and transcriptomic approach. *Biological trace element research*., **183**, 370–378.

Naeiji, N.; Shahsavani, D.; Baghshani, H., 2013: Effect of dietary garlic supplementation on lipid peroxidation and protein oxidation biomarkers of tissues as well as some serum biochemical parameters in common carp *Cyprinus carpio*. *Fisheries Science*., **79**, 699–705.

Natasya-Ain, R.; Eirna-Liza, N.; Jasmin, M. Y.; Karim, M., 2018: Antibacterial activity of garlic extracts on fish pathogenic bacteria. *Journal of Environmental Biology*., **39**, 808–812.

Ndong, D.; Fall, J., 2011: The effect of garlic (*Allium sativum*) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). *Journal of Clinical Immunology and Immunopathology Research*., **3**, 1–9.

Nya, E. J.; Austin, B., 2009: Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*., **32**, 963–970.

Platel, K.; Srinivasan, K., 2016: Influence of dietary spices or their active principles on pancreatic digestive enzymes in albino rats Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung*., **44**, 42–46.

Poongodi, R.; Saravana Bhavan, P.; Muralisankar, T.; Radhakrishnan, S., 2012: Growth promoting potential of garlic, ginger, turmeric and fenugreek on the freshwater prawn *Macrobrachium rosenbergii*. *International Journal of Pharma and Bio Sciences*., **3**, 914–926.

Pourmozaffar, S.; Hajimoradloo, A.; Miandare, H. K., 2017: Dietary effect of apple cider vinegar and propionic acid on immune related transcriptional responses and growth performance in white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology*., **60**, 65–71.

Pourmozaffar, S.; Hajimoradloo, A.; Paknejad, H.; Rameshi, H., 2019: Effect of dietary supplementation with apple cider vinegar and propionic acid on hemolymph chemistry, intestinal microbiota and histological structure of hepatopancreas in white shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology*., **86**, 900–905.

Radhakrishnan, S.; Saravana Bhavan, P.; Seenivasan, C.; Muralisankar, T.; Shanthi, R., 2015: Effects of native medicinal herbs (*Alternanthera sessilis*, *Eclipta alba* and *Cissus quadrangularis*) on growth performance, digestive enzymes and biochemical constituents of the monsoon river prawn *Macrobrachium malcolmsonii*. *Aquaculture Nutrition*., **21**, 496–506.

Ranjit Kumar, N.; Raman, R. P.; Jadhao, S. B.; Brahmchari, R. K.; Kumar, K.; Dash, G., 2013: Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture International*., **21**, 387–403.

Reverter, M.; Sarter, S.; Caruso, D.; Avarre, J. C.; Combe, M.; Pepey, E.; Pouyaud, L.; Vega-Heredia, S.; De Verdal, H.; Gozlan, R. E., 2020: Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature communications*., **11**, 1–8.

Rico, A.; Phu, T. M.; Satapornvanit, K.; Min, J.; Shahabuddin, A. M.; Henriksson, P. J. G.; Murray, F. J.; Little, D. C.; Dalsgaard, A.; den Brink, P. J., 2013: Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. *Aquaculture*., **412**, 231–243.

Sahu, S.; Das, B. K.; Mishra, B. K.; Pradhan, J.; Sarangi, N., 2007: Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology*., **23**, 80–86.

Samadi, L.; Vanguee, N.; Mousavi, S. M.; Zakeri, M., 2016: Effect of dietary garlic extract on growth, feeding parameters, hematological indices and body composition of *Litopenaeus vannamei*. *Journal of the Persian Gulf*., **7**, 29–41.

Sarhadi, I.; Alizadeh, E.; Ahmadifar, E.; Adineh, H.; Dawood, M. A. O., 2020: Skin mucosal, serum immunity and antioxidant capacity of common carp (*Cyprinus carpio*) fed Artemisia (*Artemisia annua*). *Annals of Animal Science*., **20**, 1011–1027.

Shalaby, A. M.; Khattab, Y. A.; Abdel Rahman, A. M., 2006: Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of nile tilapia (*Oreochromis niloticus*). *Journal of Venomous Animals and Toxins Including Tropical Diseases*., **12**, 172–201.

Shanthi, R.; Saravana Bhavan, P.; Radhakrishnan, S., 2012: Influence of medicinal herbs, *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba* on growth, digestive enzymes, biochemical constituents and protein profile of the freshwater prawn *Macrobrachium rosenbergii*. *Bio Technology*., **42**, 6478–6484.

Sharma, V.; Sharma, A.; Kansal, L., 2010: The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. *Food and Chemical Toxicology*., **48**, 928–936.

Srivastava, S.; Pathak, P. H., 2012: Garlic (*Allium sativum*) extract supplementation alters the glycogen deposition in liver and protein metabolism in gonads of female Albino rats. *International Journal of Pharmaceutical Sciences and Drug Research*., **4**, 126–129.

Stentiford, G. D.; Neil, D. M.; Peeler, E. J.; Shields, J. D.; Small, H. J.; Flegel, T. W.; Vlak, J. M.; Jones, B.; Morado, F.; Moss, S.; others, 2012: Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. *Journal of invertebrate pathology*., **110**, 141–157.

Sung, Y.Y.; Roberts, R. J.; Bossier, P., 2012. Enhancement of Hsp70 synthesis protects common carp, *Cyprinus carpio* L., against lethal ammonia toxicity. *Journal of Fish Diseases*., **35**, 563-568.

Supa-aksorn, M.; Rungruangsak-Torrissen, K.; Tongsiri, S.; Rojtinnakorn, J., 2017: Garlic extract product enhancing growth performance, digestive and immune system in Nile tilapia (*Oreochromis niloticus*). *The JSFS 85th Anniversary-Commemorative International Symposium "Fisheries Science for Future Generations"*., pp. 1–2.

Talpur, A. D.; Ikhwanuddin, M., 2012: Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture*., **364**–**365**, 6–12.

Tamadoni Jahromi, S.; Pourmozaffar, S.; Rameshi, H.; Gozari, M.; Nahavandi, R., 2020: Evaluation of hemolymph biochemical properties, clearance rate, bacterial microbiota and expression of HSP genes of gulf pearl oyster *Pinctada radiata* in response to salinity changes. *Fisheries Science*., **86**, 1055–1065.

Tamadoni Jahromi, S.; Pourmozaffar, S.; Jahanbakhshi, A.; Rameshi, H.; Gozari, M.; Khodadadi, M.; Sohrabipour, J.; Behzadi, S.; Bazrkar, N.; Nahavandi, R.; Zahedi, M. R.; Moezzi, M., 2021: Effect of different levels of dietary *Sargassum cristaefolium* on growth performance, hematological parameters, histological structure of hepatopancreas and intestinal microbiota of *Litopenaeus vannamei*. *Aquaculture*., **533**, 1–17.

Van Hai, N., 2015: The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*., **446**, 88–96.

Wan, J.; Ge, X.; Liu, B.; Xie, J.; Cui, S.; Zhou, M.; Xia, S.; Chen, R., 2014: Effect of dietary vitamin C on non-specific immunity and mRNA expression of three heat shock proteins (HSPs) in juvenile *Megalobrama amblycephala* under pH stress. *Aquaculture*., **434**, 325–333.

Yearbook, F. A. O., 2020: *Fishery and Aquaculture Statistics 2018; FAO: Rome, Italy, 2020*.

Yeh, Y. yan; Liu, L., 2001: Recent advances on the nutritional effects associated with the use of garlic as a supplement cholesterol-lowering effect of garlic extracts and organosulfur compounds : human and animal studies 1. *American Journal of clinical Nutrition*., **166**, 989–993.

Yousefi, M.; Vatnikov, Y. A.; Kulikov, E. V.; Plushikov, V. G.; Drukovsky, S. G.; Hoseinifar, S. H.; Van Doan, H., 2020: The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. *Aquaculture*., **526**, 735400.

Zaefarian, A.; Yeganeh, S.; Adhami, B., 2017: Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo caspius*) and resistance against *Yersinia ruckeri* infection. *Aquaculture International*., **25**, 1987–1996.

Zehra, S.; Khan, M. A., 2019: Effects of different levels of dietary cyanocobalamin on growth, liver cyanocobalamin concentration, antioxidant capacity, intestinal enzymes and non-specific immune response for optimum inclusion in the commercial feeds of fingerling *Channa punctatus* (Bl. *Aquaculture*., **511**, 734272.

Zeng, S. L.; Long, W. Q.; Tian, L. X.; Xie, S. W.; Chen, Y. J.; Yang, H. J.; Liang, G. Y.; Liu, Y. J., 2016: Effects of dietary aflatoxin B_1 on growth performance, body composition, haematological parameters and histopathology of juvenile Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Nutrition*., **22**, 1152–1159.

Zhou, X. Q.; Zhao, C. R.; Lin, Y., 2007: Compare the effect of diet supplementation with uncoated or coated lysine on juvenile Jian Carp (*Cyprinus carpio* Var. Jian). *Aquaculture Nutrition*., **13**, 457–461.

Table 1. Composition (g/kg) and proximate analysis of the experimental diets

	Treatments			
Ingredients	Control (Garlic-Free)	Diet 1 (5 g/kg Garlic)	Diet 2 (10 g/kg Garlic)	Diet 3 (20 g/kg Garlic)
Fish meal	460	460	460	460
Soy bean meal	260	260	260	260
Wheat bran	150	150	150	150
Corn flour	90	85	80	70
Garlic powder	0	5	10	20
Vegetable oil	30	30	30	3
Vit– min premix[a]	10	10	10	10
Proximate analysis				

Crude protein	39.3	40.1	40.2	40.3
Crude fat	10.50	10.35	10.62	10.42
Crude fiber	4.5	4.8	4.2	4.6
Ash	13.3	13.7	14.1	14.3

[a]Vitamins and mineral mixes: Vitamin A (10,000,000 IU), Vitamin D3 (3,000,000 IU), Vitamin E (10,000 IU), Vitamin B1 (400 mg), Vitamin B2 (1,200 mg), Vitamin B6 (1,200 mg), coated Vitamin C (25,000 mg), Folic acid (600 mg), Niacin (6,000 mg), Calcium pantothenate (10,000 mg), Biotin (20,000 mcg), Choline Chloride (10,000 mg), Iron (12,000 mg), Copper (1,200 mg), Iodine (400 mg), Manganese (5,000 mg), Zinc (6,000 mg), Cobalt (20 mg), Selenium (20 mg)

Table 2. Primers used for the real-time PCR assay.

Gene	qPCR primers, forward/reverse	Length (bp)	Amplicon (bp)	Sequence source
IMD*	CGACCACATTCTCCTCCTCCC	21	184	KR827675.1
	TTCAGTGCATCCACGTCCCTC	21		
HSP70	TGACAAGGGTCGCCTCAGTA	20	158	EU884290.2
	CATTATCTTGTTGCGATCCTC	21		
β-actin	TCCGTAAGGACCTGTATGCC	20	96	AY651918.2
	TCGGGAGGTGCGATGATTTT	20		

Table 3. Growth performance of prawn fed with experimental diets.

parameters	Treatments			
	Control (Garlic-Free)	Diet 1 (5 g/kg Garlic)	Diet 2 (10 g/kg Garlic)	Diet 3 (20 g/kg Garlic)
Mean initial body weight (g)	1.12 ± 0.02	1.10 ± 0.03	1.13 ± 0.01	1.12 ± 0.02
Mean final body weight (g)	9.51 ± 0.12^c	12.93 ± 0.31^b	13.15 ± 0.26^b	16.43 ± 0.41^a
Weight gain(g)	8.39 ± 0.11^c	11.83 ± 0.31^b	12.02 ± 0.47^b	15.31 ± 0.22^a
SGR[1]	4.73 ± 0.33^c	5.78 ± 0.32^b	5.82 ± 0.27^b	6.93 ± 0.31^a
FCR[2]	2.12 ± 0.01^c	1.76 ± 0.02^b	1.73 ± 0.01^b	1.42 ± 0.03^a

Data are mean values of nine replicates expressed as mean ±SE. Data with different superscripts show significant differences ($P < 0.05$).
[1]Specific growth rate
[2]Feed conversion ratio

Table 4. Muscle biochemical constituent of shrimp fed with experimental diets.

parameters	Treatments			
	Control (Garlic-Free)	Diet 1 (5 g/kg Garlic)	Diet 2 (10 g/kg Garlic)	Diet 3 (20 g/kg Garlic)
Crude protein	13.2 ± 0.21[c]	15.6 ± 0.11[b]	16.1 ± 0.34[b]	18.5 ± 0.42[a]
Fat	9.82 ± 0.45[a]	7.62 ± 0.52[b]	7.41 ± 0.22[b]	5.22 ± 0.17[c]
Ash	3.82 ± 0.04[c]	4.46 ± 0.01[b]	4.58 ± 0.06[b]	5.24 ± 0.05[a]
Moisture	65.86 ± 0.18	65.82 ± 0.10	65.62± 0.15	65.59 ± 0.12

Table 5. Effect of garlic extract on hepatopancreas antioxidant capacity of *M. rosenbergii*after 60 days.

Parameters	Treatments			
	Control (Garlic-Free)	Diet 1 (5 g/kg Garlic)	Diet 2 (10 g/kg Garlic)	Diet 3 (20 g/kg Garlic)
CAT(U/mg protein)	27.4 ± 1.32[c]	33.5 ± 1.31[b]	38.7 ± 1.62[a]	39.2 ± 1.39[a]
SOD (U/mg protein)	87.5 ± 1.42[c]	98.2 ± 1.83[b]	109.4 ± 1.59[a]	110.3 ± 1.42[a]
GSH-Px (U/mg protein)	25.3 ± 1.15[c]	30.3 ± 1.25[b]	35.1 ± 1.19[a]	36.8 ± 1.61[a]

Data are mean values of nine replicates expressed as mean ±SE. Data with different superscripts show significant differences ($P < 0.05$).

Table 6. Effect of garlic extract on haemolymph biochemical parameters of *M. rosenbergii*after 60 days.

Parameters	Treatments			
	Control (Garlic-Free)	Diet 1 (5 g/kg Garlic)	Diet 2 (10 g/kg Garlic)	Diet 3 (20 g/kg Garlic)
ALT (U/ml)	105.2 ± 1.24[c]	113.3 ± 1.41[b]	115.8 ± 1.67[b]	133.2 ± 1.59[a]
AST (U/ml)	60.1 ± 1.42[c]	71.7 ± 1.10[b]	73.6 ± 1.32[b]	83.7 ± 1.70[a]
ALP (U/ml)	192.2 ± 2.56[c]	231.8 ± 3.17[b]	233.5 ± 2.72[b]	265.3 ± 3.27[a]
Total protein (g/l)	23.3 ± 1.15[c]	29.7 ± 1.28[b]	38.8 ± 1.34[a]	39.7 ± 1.41[a]
Albumin (g/l)	3.3 ± 0.12[c]	4.6 ± 0.22[b]	5.7 ± 0.17[a]	5.9 ± 0.27[a]

Data are mean values of nine replicates expressed as mean ±SE. Data with different superscripts show significant differences (P< 0.05).

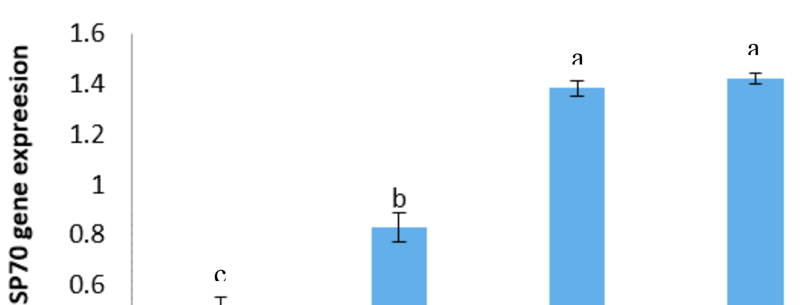

Fig 1. The relative gene expression of HSP70 in hepatopancreas of *M. rosenbergii* fed experimental diets. Data are expressed as the mean SD. Different lowercase letters indicate statistically significant differences between groups ($P < 0.05$).

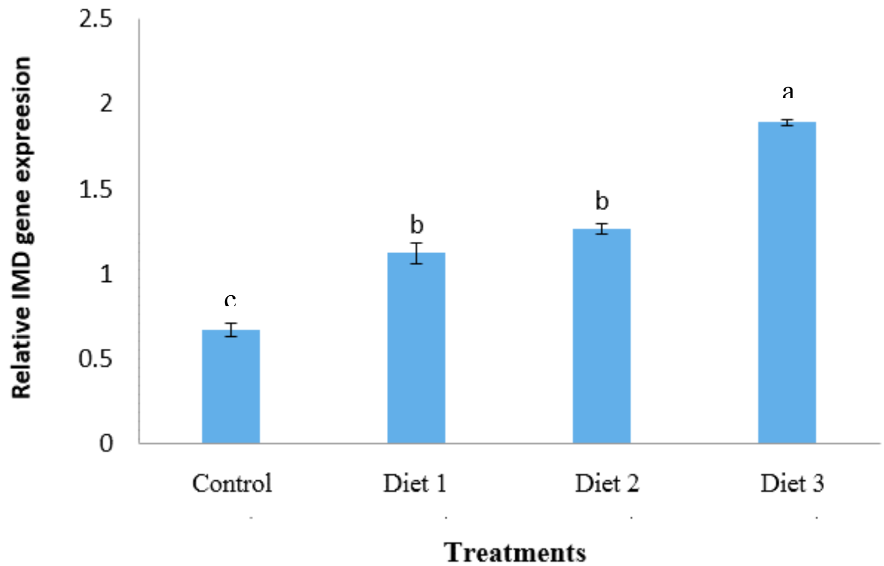

Fig 2. The relative gene expression of IMD in hepatopancreas of *M. rosenbergii* fed experimental diets. Data are expressed as the mean SD. Different lowercase letters indicate statistically significant differences between groups (P < 0.05)