

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

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

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### 36 **Abstract**

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

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

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### 62 **Introduction**

63 The prevalence of diseases is one of the most important limiting factors in the development of  
64 aquaculture practices (Stentiford et al., 2012). Although synthetic drugs are key to control disease  
65 outbreaks in aquaculture, their multiple negative impacts on the host, the environment, and even

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

91 After fish and molluscan shellfish Farming, crustacean farming is the most important aquaculture  
92 practice in many countries (Yearbook, 2020). The giant freshwater prawn, *M. rosenbergii*, is a  
93 particularly esteemed species in aquaculture due to its fast growth, big sizes and its habitat  
94 versatility. Although it is mainly cultured in Asian countries (Southern and South-Eastern), this  
95 species has also been introduced in many other regions and its farming is now globally widespread  
96 (Yearbook, 2020). Up to now, whether dietary supplementation of garlic extract could enhance the

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97 physiological parameters and molecular responses of giant freshwater prawn is still unknown.  
98 Thus, the purpose of the present work was to evaluate the effect of garlic extract on some  
99 physiological and biochemical parameters, immune related genes and growth performance in giant  
100 freshwater prawn.

### **101 2. Materials and methods**

#### **102 2.1. Preparation of garlic extract**

103 Fresh garlic bulbs were purchased from a local farm in Kermanshah, Iran. Peeled garlic cloves  
104 (100 g) were chopped and then blended with 200 ml distilled water for 3 min. The solution was  
105 centrifuged at 10,000 rpm for 5 min. The supernatant was removed and was passed through a  
106 Whatman filter paper (42µm). The obtained extract was kept at -20<sup>0</sup> C.

#### **107 2.2. Experimental design**

108 The *M. rosenbergii* post-larvae (PL-12, ranging from 1.0 to 1.3 cm in length and 1.1 to 1.3g) were  
109 obtained from a local commercial shrimp farm located in Ghasr-e-Shirin city (Kermanshah  
110 province, Iran). A total of 360 PL were randomly distributed in twelve (30 PL/tank) 25 L  
111 (70x40x40) aerated tanks with dechlorinated water and were acclimatized to the experimental  
112 conditions for ten days. Four groups of prawns were assigned in triplicate for 60 days. Three groups  
113 were fed with experimental diets consisting of a basal diet supplemented with garlic extract (5, 10,  
114 and 20 g/kg of garlic extract) (Breyer et al., 2015), and one group was fed with the control diet  
115 (i.e., basal diet, (Table 1). The water temperature, dissolved oxygen and pH were measured daily  
116 using aWagtech portable temperature, oxygen and pH meter (Berkshire, UK). Uneaten food and  
117 faeces were removed daily. Water was exchanged (50%) in daily manner. Shrimps were fed daily  
118 at 3% of body weight, and during the experimental period they were fed at 08:00, 13:00, 17:00  
119 and 23:00.

#### **120 2.3 Sample collection**

121 All animal experiments and animal protocols were conducted in accordance with National Ethical  
122 Framework for Animal Research in Iran. At the end of experiment, three prawns per tank (9 per  
123 treatment) were anesthetized with clove oil, 100 mg/l (Ranjit Kumar et al., 2013) and sacrificed.  
124 First, the whole prawns were weighted. Then muscle tissues from abdominal segments were

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125 immediately dissected for analysis of their proximate composition. Hepatopancreas were  
126 collected, weighed and stored at -80°C until further antioxidant enzyme and gene expression  
127 analysis (Akbari et al., 2019). Haemolymph was withdrawn from the ventral sinus of prawns at  
128 the end of experimental period using 1-ml tuberculin syringe with 24-gauge needle and were used  
129 in the analysis of different biochemical enzymes. The samples were centrifuged at 12000 g for 20  
130 min and supernatants were collected and stored at -20°C until use.

### **131 2.4 Survival, growth and nutritional indices analysis**

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

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

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

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

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

### **138 2.5. Muscle biochemical constituent**

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

### **144 2.6. Antioxidant enzyme assays**

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

### **151 2.7. Haemolymph biochemical parameters**

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

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### **159 2.8. RNA extraction**

160 For RNA extraction, samples were immediately frozen in liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$   
161 until analyzed. Biozol reagent (Bio flux; China) was used for extracting total RNA from  
162 hepatopancreas samples according to the manufacturer's instructions. Quality of RNA was  
163 estimated by electrophoresis on ethidium bromide staining and a 1.5% agarose gel. The RNA's  
164 quantity and quality was measured with a nanodrop spectrophotometer at a wavelength of 260/280  
165 nm. Samples with RNA ratios greater than 1.8 (at 260/280 nm) were used for further experiments.  
166 In accordance with the manufacturer's protocol, supprime script RT premix (2 $\times$ ) cDNA synthesis  
167 kit (GeNet BIO Inc, South Korea) was used to synthesis the first strand of cDNA (Pourmozaffar  
168 et al., 2017).

### **169 2.9. Relative mRNA expression of immune-related genes**

170 The transcript expression levels were determined by Real-time Polymerase Chain Reaction (RT-  
171 PCR, Bio-Rad, USA). Table 2 exhibits the primers used for amplification of heat shock proteins  
172 (HSP70), immune deficiency (IMD), and  $\beta$ -actin (housekeeping gene). In accordance with  
173 standard protocols, 10  $\mu\text{l}$  of SYBR Green qPCR Master Mix (1 $\times$ ) (Fermentase, Lithuania), 0.2  $\mu\text{l}$   
174 of forward and reverse specific primers (100 nM), 10 ng of cDNA template, and 6.40  $\mu\text{l}$  nuclease  
175 free water to final volume of 20 ml were used (Pourmozaffar et al., 2017). PCR reaction mixtures  
176 were subjected to the following thermal profile: 95  $^{\circ}\text{C}$  for 5 min, followed by 40 cycles of 10 s at  
177 95  $^{\circ}\text{C}$ , and 10 s at 54  $^{\circ}\text{C}$  and 10 s at 72  $^{\circ}\text{C}$ . The  $2^{-\Delta\Delta\text{CT}}$  method was used in calculating the fold  
178 changes in HSP70 and IMD relative mRNA expression. The  $\beta$ -actin gene was used to normalize  
179 the expression levels of the target genes.

### **180 2.10. Statistical analysis**

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

186

### **187 3. Results**

#### **188 3.1. Growth performance**

189 Table 3 presents the growth performance of *M. rosenbergii* PL fed with garlic extracts. There was  
190 no mortality in the experiment. Final body weight for all groups was in the range 9.53- 16.43 g  
191 and significant differences were observed among the groups ( $P > 0.05$ ). Significant increase ( $P <$   
192  $0.05$ ) in FBW, WG, and SGR was observed in treated groups in a dose-dependent manner  
193 compared with control group. A significant reduction in FCR was observed in the treated groups  
194 compared to control group ( $P < 0.05$ ). Highest and lowest weight gains and SGR values were  
195 observed in the control group and 20 g/kg garlic extract respectively. Giant freshwater prawn fed  
196 with a diet containing garlic extract at 20 g/kg showed the highest FBW, WG and SGR, and the  
197 lowest FCR ( $P < 0.05$ ).

#### **198 3.2. Muscle biochemical constituent**

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

#### **207 3.3. Antioxidant enzymes**

208 The antioxidant enzyme activities of *M. rosenbergii* fed with different garlic extracts are shown in  
209 Table 5. SOD, CAT, and GSH-Px activities of prawn fed the extract-containing diets were

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210 significantly higher than those of shrimp fed the control diet ( $P < 0.05$ ). Moreover, the activity of  
211 these enzymes was highest in the 20 and 10 g/kg garlic groups, respectively, which was also  
212 significantly higher than in control and 5 g/kg garlic groups ( $P < 0.05$ ).

### 213 3.4. Haemolymph biochemical parameters

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

### 219 3.5. Related immune gene expression

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

223

## 224 4. Discussion

225 In the present study, growth parameters of *M. rosenbergii* were significantly increased when fed  
226 with a garlic extract supplemented diet. These results are in accordance with previous reports in *L.*  
227 *vannamei* (Labrador et al., 2016; Samadi et al., 2016; Kumar et al., 2019), *O. mykiss* (Nya et al.,  
228 2009; Etyemez Büyükdeveci et al., 2018; Adineh et al., 2020), *R. rutilus* (Ghehdarijani et al.,  
229 2016), *Salmo caspius* (Zaefarian et al., 2017), *Mesopotamichthys sharpeyi* (Maniat et al., 2014),  
230 *Lates calcarifer* (Talpur et al., 2012), *O. niloticus* (Metwally, 2009), *Cyprinus carpio* (Karimi  
231 Pashaki et al., 2018), and *M. rosenbergii* (Poongodi et al., 2012). As proposed, this improvement  
232 in growth performance could be related to higher digestive enzymes activities (such as lipase,  
233 protease and amylase) as well as changes in intestinal microbiota (Shanthi et al., 2012;  
234 Radhakrishnan et al., 2015; Etyemez Büyükdeveci et al., 2018). Supa-aksorn et al. (Supa-aksorn  
235 et al., 2017), reported that amylase, lipase, and trypsin activities was higher in *O. niloticus* fed with  
236 diets enriched with garlic extract (5 and 10 g/kg) than that of fish fed with a control diet. Likewise,  
237 diets supplemented with garlic extract may act as appetizers, thereby increasing feed intake  
238 (Poongodi et al., 2012; Platel et al., 2016). Labrador et al. (Labrador et al., 2016), found that Pacific



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239 white leg shrimp (*L. vannamei*) fed diets enriched with garlic powder (20, 40, and 60 g/kg) showed  
240 the highest weight gain compared to other groups. In another study, the highest growth parameters  
241 in *Tilapia zillii* fingerlings were recorded when fish fed a diet containing 20 g/kg garlic for 75 days  
242 (Jegade, 2012). Allicin in garlic can enhance growth performance through stimulating intestinal  
243 flora, improving digestive system, and enhancing energy utilization (Supa-aksorn et al., 2017).  
244 The proximate body composition is often regarded as a suitable indicator of the physiological  
245 condition of an organism (Kotiya et al., 2019). Our results showed that *M. rosenbergii* body  
246 composition was significantly affected by the oral administration of garlic extracts. The crude  
247 protein and ash of prawns fed control diet were notably ( $P < 0.05$ ) lower than treated groups fed  
248 garlic extracts. Samadi et al. (Samadi et al., 2016), showed positive effects of dietary garlic  
249 supplementation on crude protein by *L. vannamei* fed with a garlic extract at 800 mg/kg, which is  
250 consistent with results of the present study. Additionally, Adineh et al. (Sarhadi et al., 2020) found  
251 that diets enriched with garlic extracts (10 g/kg) led to markedly increased crude protein and  
252 markedly reduced crude fat in whole fish body. Similar results were documented in *O. niloticus*  
253 and *T. zillii* fed with diets containing 30 and 10-30 g/kg garlic extract, respectively (Shalaby et al.,  
254 2006; Ajiboye et al., 2016). It has been shown that garlic affects body protein metabolism caused  
255 by hormonal regulation through raising protein metabolism or stimulating hormone secretion  
256 (Srivastava et al., 2012). The fat value in this study was similar to Samadi et al. (Samadi et al.,  
257 2016), Maniat and Ghotbeddin (Maniat et al., 2014), and Shalaby et al. (Shalaby et al., 2006)  
258 reports. *M. rosenbergii* body fat decreased from 9.82 to 5.22 % with increases in dietary garlic  
259 extract levels. Garlic administration may affect whole-body fat due to hepatic activities reduction  
260 of cholesterogenic and lipogenic enzymes like fatty acid synthase, dehydrogenase and malic  
261 enzyme which are caused to reducing of carcass lipid values (Yeh et al., 2001). In addition, some  
262 organosulfur compounds in garlic such as diallyl-disulfide (allicin) and S-allyl cysteine may  
263 prevent the synthesis of cholesterol, which could also lead to lower fat levels (Samadi et al., 2016).  
264 Kim et al. (Kim et al., 2011) also showed that dietary addition of garlic oil has no notable difference  
265 in moisture level of *L. vannamei*. Overall, it seems that dietary supplementation with garlic and its  
266 derivatives (e.g., extracts, bioactive compounds) affect positively aquatic animals body  
267 composition, however these effects are dependent on the level of garlic used, cultured species, and  
268 the environmental conditions (Mahmoud et al., 2019).

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269 SOD/CAT system is the first defense mechanism against reactive oxygen species (ROS)  
270 (Mahmoud et al., 2019). In the present study, antioxidant activities were higher in prawn fed diets  
271 supplemented with garlic extract compared to control group. Prawn fed the diets containing 10 and  
272 20 g/kg garlic diets exhibited the highest SOD, CAT and GSH-Px values compared with other  
273 diets. These results are in accordance with earlier studies in Nile tilapia (*O. niloticus*) (Metwally,  
274 2009; Mahmoud et al., 2019), common carp (*C. carpio*) (Naeiji et al., 2013; Yousefi et al., 2020)  
275 and European seabass, *Dicentrarchus labrax* (Mosbah et al., 2018) and rainbow trout (*O. mykiss*)  
276 (Mohebbi et al., 2012; Adineh et al., 2020) through enhancing the endogenous antioxidant  
277 enzymatic mechanisms and counteracting the effects of ROS (Abdel-Tawwab et al., 2021). In  
278 addition, Jahanjoo et al. (Jahanjoo et al., 2018) reported that the SOD activity was notably  
279 increased in fish fed diets supplemented with 1% ginger or garlic. Garlic has been shown to  
280 activate defense mechanisms and counteract infection through the production of superoxide anions  
281 (Sahu et al., 2007). Sulphur-containing compounds (diallyl disulphide and s-allyl cysteine) and  
282 flavonoids are two important types of antioxidant components within garlic, which might be  
283 related to the higher antioxidant activities observed in prawns fed with garlic extracts (Sharma et  
284 al., 2010).

285 Hepatic enzymes are commonly used as indicators of hepatopancreas yield, because these enzymes  
286 have crucial roles in the interactions of carbohydrates, fats and amino acids (Hemre et al., 1996).  
287 Higher values of AST and ALT may be therefore regarded as indicators of alterations in the  
288 permeability of hepatopancreas tissue cells (Zeng et al., 2016). Our results showed that, ALP,  
289 ALT, and AST activities were significantly higher in the 20 g/kg garlic group than in the other  
290 groups. Similarly, Mohebbi et al. (Mohebbi et al., 2012) indicated that serum alanine  
291 aminotransferase and aspartate aminotransferase levels in rainbow trout increased after fed with  
292 40 and 50 g/kg garlic powder. Joseph et al. (Joseph et al., 1989) demonstrated that rats fed with a  
293 garlic extract at 200 g/l had significant increase in hepatic enzyme activity compare to other  
294 experimental groups after 10 days. Moreover, increase in hepatopancreas antioxidant activities  
295 were accompanied by higher activity of haemolymph AST and ALT. The observed dose-  
296 dependent increases in ALT, AST and ALP in treated shrimp could eventually suggest that  
297 hepatopancreas functions were activated. When the lesions in the hepatopancreas were not aimed  
298 to observe and in the view of the better growth, these alterations may be taken as an initial adaptive

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299 mechanism of prawns. ALT and AST increases were also observed in African catfish fed with  
300 dietary clove (Adeshina et al. 2018).

301 Total protein and albumin levels were significantly increased in the 10 and 20 g/kg garlic groups  
302 compared with other groups which are in agreement with Talpur and Ikhwanuddin (Talpur et al.,  
303 2012) and Adineh et al. (Adineh et al., 2020) results. Higher levels of total protein and albumin  
304 are often related to improved innate immunity following functional feed supplementation (Nya et  
305 al., 2009; Pourmozaffar et al., 2019). The S-allyl cysteine in garlic may play a crucial role in the  
306 function of organs related to blood cell and immune system stimulation (Ndong et al., 2011).  
307 Meanwhile, Nya and Austin (Nya et al., 2009) found that *O. mykiss* fed diets enriched with 1 and  
308 10 g/kg garlic exhibited higher serum total protein than fish fed with control diet. The enhancement  
309 of total protein level may be due to induction of antiprotease activity by garlic (Zaefarian et al.,  
310 2017).

311 Our results showed that the expression of HSP70 and IMD genes in the hepatopancreas were  
312 significantly increased in prawn fed with diets containing garlic extract compared to the control  
313 group. HSP70 act as a molecular stress protein, which can contribute to the maintenance of cellular  
314 homeostasis (Liu et al., 2016) and endogenous peroxidase activity for catalyzing the conversion  
315 of ROS (Duan et al., 2018). Consistent with our results, previous literature showed the up-  
316 regulation of related-immune genes following feeding Nile tilapia (*O. niloticus*) with diet  
317 containing *Spirulina platensis* + garlic (M Abu Elala, 2016). Moreover, Kaleo et al. (Kaleo et al.,  
318 2019) and Duan et al. (Duan et al., 2018) demonstrated that the expression of HSP70 gene in the  
319 *M. rosenbergii* and *L. vannamei* was significantly up regulated after fed diets containing M.  
320 oleifera (2.5 and 5 g/kg) and succinic acid (2.5, 5, and 10 g/kg), respectively. The up-regulation  
321 of HSP70 is related to modulating cellular anti-stress reactions (Feder et al., 1999; Wan et al.,  
322 2014). Based on the present study, there was a significant increase in expression of IMD gene  
323 when prawns fed diets containing garlic extracts. IMD contributed shrimps innate immune  
324 reactions (Li et al., 2013). The up-regulation of expression of IMD in *M. rosenbergii* may be  
325 attributed to high concentrations of garlic extract, so that the prawn's body for resisting stress might  
326 have to activate the IMD signaling pathway (Kaleo et al., 2019). As suggested, increase in IMD  
327 and HSP-70 gene expressions could also support stress management in prawns fed with garlic. It  
328 has been reported that an increase in HSP-70 gene expression in fish fed with plant-and probiotic-

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329 enriched diets was rather related to an anti-stress response (Abarike et al. 2020). Sung et al. (2012)  
330 also reported that enhancing HSP-70 synthesis could protect common carp against ammonia-  
331 related stress. Therefore in the present study, the general health of *M. rosenbergii* was found to  
332 be improved by up-regulations of IMD and HSP-70 genes.

### 333 **Conclusion**

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

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### 355 **References**

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

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 357 Chinese herbs and a commercial probiotic *Bacillus* species improves hepato-immunological,  
358 stress, and antioxidant parameters, and expression of HSP70 and HIF-1 $\alpha$  mRNA to hypoxia, cold  
359 and heat stress in Nile tilapia, *Oreochromis niloticus*. *Aquaculture Reports.*, **18**, 100438.
- 360 Abdel-Tawwab, M.; Khalil, R. H.; Diab, A. M.; Khallaf, M. A.; Abdel-Razek, N.; Abdel-Latif, H.  
361 M. R.; Khalifa, E., 2021: Dietary garlic and chitosan enhanced the antioxidant capacity, immunity,  
362 and modulated the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated  
363 European seabass. *Fish and Shellfish Immunology.*, **113**, 35–41.
- 364 Adeshina, I.; Jenyo-Oni, A.; Emikpe, B.O.; Ajani, E.K.; Abdel-Tawwab, M., 2018. Stimulatory  
365 effect of dietary clove, *Eugenia caryophyllata*, bud extract on growth performance, nutrient  
366 utilization, antioxidant capacity, and tolerance of African catfish, *Clarias gariepinus* (B.), to  
367 *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society.*, **50**, 390-405.
- 368 Adineh, H.; Harsij, M.; Jafaryan, H.; Asadi, M., 2020: The effects of microencapsulated garlic  
369 (*Allium sativum*) extract on growth performance, body composition, immune response and  
370 antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles. *Journal of Applied Animal*  
371 *Research.*, **48**, 372–378.
- 372 Ajiboye, O. O.; Qari, R., 2016: Short-term evaluation of graded levels of dietary garlic powder  
373 (*Allium sativum* L.) as growth promoter on growth, survival and feed utilization of redbelly tilapia,  
374 *Tilapia zillii* reared in glass aquaria tanks. *International Journal of Marine Science.*, **6**, 1–7.
- 375 Akbary, P.; Jahanbakhshi, A., 2018: Growth yield, survival, carcass quality, haematological,  
376 biochemical parameters and innate immune responses in the grey mullet (*Mugil cephalus* Linnaeus,  
377 1758) fingerling induced by immunogen<sup>®</sup> prebiotic. *Journal of Applied Animal Research.*, **46**, 10–  
378 16.
- 379 Akbary, P.; Jahanbakhshi, A., 2019: Nano and macro iron oxide (Fe<sub>2</sub>O<sub>3</sub>) as feed additives: Effects  
380 on growth, biochemical, activity of hepatic enzymes, liver histopathology and appetite-related  
381 gene transcript in goldfish (*Carassius auratus*). *Aquaculture.*, **510**, 191–197.
- 382 Akbary, P.; Adeshina, I.; Jahanbakhshi, A., 2020: Growth performance, digestive enzymes,  
383 antioxidant activity and immune responses of *Litopenaeus vannamei* fed with *Jania adhaerens*  
384 J.V. supplemented diet against *Photobacterium damsela* infection. *Animal Feed Science and*

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

385 *Technology.*, **270**, 1–31.

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

389 Breyer, K. E.; Getchell, R. G.; Cornwell, E. R.; Wooster, G. A.; Ketola, H. G.; Bowser, P. R.,  
390 2015: Efficacy of an extract from garlic, *Allium sativum*, against infection with the *Furunculosis*  
391 *bacterium*, *Aeromonas salmonicida*, in rainbow trout, *Oncorhynchus mykiss*. *Journal of the World*  
392 *Aquaculture Society.*, **46**, 273–282.

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

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

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

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

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

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

411 Ghehdarijani, M. S.; Hajimoradloo, A.; Ghorbani, R.; Roohi, Z., 2016: The effects of garlic-

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 412 supplemented diets on skin mucosal immune responses, stress resistance and growth performance  
413 of the Caspian roach (*Rutilus rutilus*) fry. *Fish and Shellfish Immunology.*, **49**, 79–83.
- 414 Gholipour Kanani, H.; Nobahar, Z.; Kakoolaki, S.; Jafarian, H., 2014: Effect of ginger and garlic-  
415 supplemented diet on growth performance, some hematological parameters and immune responses  
416 in juvenile *Huso huso*. *Fish Physiology and Biochemistry.*, **40**, 481–490.
- 417 Hemre, G. I.; Waagbø, R.; Hjeltnes, B.; Aksnes, A., 1996: Effect of gelatinized wheat and maize  
418 in diets for large Atlantic salmon (*Salmo salar* L.) on glycogen retention, plasma glucose and fish  
419 health. *Aquaculture Nutrition.*, **2**, 33–39.
- 420 Jahanbakhshi, A.; Imanpuor, M.; Taghizadeh, V.; Shabani, A., 2012: Effects of replacing fish meal  
421 with plant protein (sesame oil cake and corn gluten) on growth performance, survival and carcass  
422 quality of juvenile beluga (*Huso huso*). *World Journal of Fish and Marine Sciences.*, **4**, 422–425.
- 423 Jahanjoo, V.; Yahyavi, M.; Akrami, R.; Bahri, A. H., 2018: Influence of adding garlic (*Allium*  
424 *sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the  
425 growth performance, haematoimmunological parameters and disease resistance to  
426 *Photobacterium damsela* in sobaity sea bream. *Turkish Journal of Fisheries and Aquatic*  
427 *Sciences.*, **18**, 633–645.
- 428 Jegede, T., 2012: Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and  
429 survival of *Tilapia zillii* (Gervais 1852) fingerlings. *Journal of Agricultural Science.*, **4**, 269–274.
- 430 Joseph, P. K.; Rao, K. R.; Sundaresh, C. S., 1989: Toxic effects of garlic extract and garlic oil in  
431 rats. *Indian journal of experimental biology.*, **27**, 977–979.
- 432 Kaleo, I. V.; Gao, Q.; Liu, B.; Sun, C.; Zhou, Q.; Zhang, H.; Shan, F.; Xiong, Z.; Bo, L.; Song, C.,  
433 2019: Effects of *Moringa oleifera* leaf extract on growth performance, physiological and immune  
434 response, and related immune gene expression of *Macrobrachium rosenbergii* with *Vibrio*  
435 *anguillarum* and ammonia stress. *Fish and Shellfish Immunology.*, **89**, 603–613.
- 436 Karimi Pashaki, A.; Zorriehzahra, S. M. J.; Ghasemi, M.; Sharif Rohani, M.; Hosseini, S. ., 2018:  
437 Effects of dietary garlic extract on some blood, immunity and growth parameters of common carp  
438 fingerlings (*Cyprinus carpio*). *Iranian Journal of Aquatic Animal Health.*, **4**, 28–39.

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 439 Karimi Pashaki, A.; Ghasemi, M.; Zorriehzahra, M. J.; Sharif Rohani, M.; Hosseini, S. M., 2020:  
440 Effects of dietary garlic (*Allium sativum*) extract on survival rate, blood and immune parameters  
441 changes and disease resistance of common carp (*Cyprinus carpio carpio* Linnaeus, 1758) against  
442 spring viremia of carp (SVC). *Iranian Journal of Fisheries Sciences.*, **19**, 1024–1039.
- 443 Kim, J. D.; Nhut, T. M.; Hai, T. N.; Ra, C. S., 2011: Effect of dietary essential oils on growth, feed  
444 utilization and meat yields of white leg shrimp *L. vannamei*. *Asian-Australasian Journal of Animal  
445 Sciences.*, **24**, 1136–1141.
- 446 Kotiya, A. S.; Vadher, K. H.; Bhatt, A. J.; Dave, T., 2019: Comparison of proximate composition  
447 level in *Litopenaeus vannamei* cultured in various stocking density during summer crop in  
448 province of Gujarat states in India. *Journal of Entomology and Zoology Studies.*, **7**, 59–72.
- 449 Kumar, R. M.; Sekhara Rao, A. C.; Daggula, N.; Guguloth, G.; Das, B. Y.; Indhuri, A. K., 2019:  
450 Growth promoter effect of ginger, garlic and fenugreek on Pacific white leg shrimp (*Litopenaeus  
451 vannamei*). *International Journal of Current Microbiology and Applied Sciences.*, **8**, 2993–3001.
- 452 Labrador, J. R. P.; Guinares, R. C.; Hontiveros, G. J. S., 2016: Effect of garlic powder-  
453 supplemented diets on the growth and survival of Pacific white leg shrimp (*Litopenaeus  
454 vannamei*). *Cogent Food & Agriculture.*, **2**, 1–8.
- 455 Lee, J. Y.; Gao, Y., 2012: Review of the application of garlic, *Allium sativum*, in aquaculture.  
456 *Journal of the World Aquaculture Society.*, **43**, 447–458.
- 457 Li, F.; Xiang, J., 2013: Recent advances in researches on the innate immunity of shrimp in China.  
458 *Developmental and Comparative Immunology.*, **39**, 11–26.
- 459 Lieke, T.; Meinelt, T.; Hoseinifar, S. H.; Pan, B.; Straus, D. L.; Steinberg, C. E. W., 2020:  
460 Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases.  
461 *Reviews in Aquaculture.*, **12**, 943–965.
- 462 Liu, F.; Cheng, B.; Guo, Q.; Shi, H.; Gou, L.; Lu, Y.; Wang, J.; Shen, W.; Yan, S.; Wu, M., 2016:  
463 Effects of indigowoad root (*Radix Isatidis*) on the immune responses and HSP70 gene expression  
464 of medicinal leeches (*Poecilobdella manillensis*) under *Proteus mirabilis* infection. *Aquaculture.*,  
465 **454**, 44–55.



## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 466 Lowry, O. .; Rosebrough, N. . .; Farr, A. L.; Randall, R. . ., 1951: Protein measurement with the  
467 Folin phenol reagent. *The Journal of biological chemistry.*, **193**, 265–275.
- 468 M Abu Elala, N., 2016: Effects of dietary supplementation of *Spirulina platensis* and garlic on the  
469 growth performance and expression levels of immune-related genes in Nile tilapia (*Oreochromis*  
470 *niloticus*). *Journal of Aquaculture Research & Development.*, **07**, 1–10.
- 471 Mahmoud, R.; Aziza, A.; Marghani, B.; Eltaysh, R., 2019: Influence of ginger and garlic  
472 supplementation on growth performance, whole body composition and oxidative stress in the  
473 muscles of Nile tilapia (*O. niloticus*). *Advances in Animal and Veterinary Sciences.*, **7**, 397–404.
- 474 Maniat, M.; Ghotbeddin, N., 2014: Effect of garlic on growth performance and body composition  
475 of benni fish (*Mesopotamichthys sharpeyi*). *International Journal of Biosciences (IJB).*, **5**, 269–  
476 277.
- 477 Metwally, M. A. A., 2009: Effects of garlic (*Allium sativum*) on some antioxidant activities in  
478 tilapia nilotica (*Oreochromis niloticus*). *World Journal of Fish and Marine Sciences.*, **1**, 56–64.
- 479 Mohebbi, A.; Nematollahi, A.; Dorcheh, E. E.; Asad, F. G., 2012: Influence of dietary garlic  
480 (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*  
481 *Research.*, **43**, 1184–1193.
- 482 Mosbah, A.; Guerbej, H.; Boussetta, H.; Bouraoui, Z.; Banni, M., 2018: Protective effects of  
483 dietary garlic powder against cadmium-induced toxicity in sea bass liver: A chemical,  
484 biochemical, and transcriptomic approach. *Biological trace element research.*, **183**, 370–378.
- 485 Naeiji, N.; Shahsavani, D.; Baghshani, H., 2013: Effect of dietary garlic supplementation on lipid  
486 peroxidation and protein oxidation biomarkers of tissues as well as some serum biochemical  
487 parameters in common carp *Cyprinus carpio*. *Fisheries Science.*, **79**, 699–705.
- 488 Natasya-Ain, R.; Eirna-Liza, N.; Jasmin, M. Y.; Karim, M., 2018: Antibacterial activity of garlic  
489 extracts on fish pathogenic bacteria. *Journal of Environmental Biology.*, **39**, 808–812.
- 490 Ndong, D.; Fall, J., 2011: The effect of garlic (*Allium sativum*) on growth and immune responses  
491 of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). *Journal of Clinical Immunology*  
492 *and Immunopathology Research.*, **3**, 1–9.

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 493 Nya, E. J.; Austin, B., 2009: Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila*  
494 infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases.*, **32**, 963–  
495 970.
- 496 Platel, K.; Srinivasan, K., 2016: Influence of dietary spices or their active principles on pancreatic  
497 digestive enzymes in albino rats Influence of dietary spices and their active principles on pancreatic  
498 digestive enzymes in albino rats. *Nahrung.*, **44**, 42–46.
- 499 Poongodi, R.; Saravana Bhavan, P.; Muralisankar, T.; Radhakrishnan, S., 2012: Growth promoting  
500 potential of garlic, ginger, turmeric and fenugreek on the freshwater prawn *Macrobrachium*  
501 *rosenbergii*. *International Journal of Pharma and Bio Sciences.*, **3**, 914–926.
- 502 Pourmozaffar, S.; Hajimoradloo, A.; Miandare, H. K., 2017: Dietary effect of apple cider vinegar  
503 and propionic acid on immune related transcriptional responses and growth performance in white  
504 shrimp, *Litopenaeus vannamei*. *Fish and Shellfish Immunology.*, **60**, 65–71.
- 505 Pourmozaffar, S.; Hajimoradloo, A.; Paknejad, H.; Rameshi, H., 2019: Effect of dietary  
506 supplementation with apple cider vinegar and propionic acid on hemolymph chemistry, intestinal  
507 microbiota and histological structure of hepatopancreas in white shrimp, *Litopenaeus vannamei*.  
508 *Fish and Shellfish Immunology.*, **86**, 900–905.
- 509 Radhakrishnan, S.; Saravana Bhavan, P.; Seenivasan, C.; Muralisankar, T.; Shanthi, R., 2015:  
510 Effects of native medicinal herbs (*Alternanthera sessilis*, *Eclipta alba* and *Cissus quadrangularis*)  
511 on growth performance, digestive enzymes and biochemical constituents of the monsoon river  
512 prawn *Macrobrachium malcolmsonii*. *Aquaculture Nutrition.*, **21**, 496–506.
- 513 Ranjit Kumar, N.; Raman, R. P.; Jadhao, S. B.; Brahmchari, R. K.; Kumar, K.; Dash, G., 2013:  
514 Effect of dietary supplementation of *Bacillus licheniformis* on gut microbiota, growth and immune  
515 response in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture*  
516 *International.*, **21**, 387–403.
- 517 Reverter, M.; Sarter, S.; Caruso, D.; Avarre, J. C.; Combe, M.; Pepey, E.; Pouyaud, L.; Vega-  
518 Heredia, S.; De Verdal, H.; Gozlan, R. E., 2020: Aquaculture at the crossroads of global warming  
519 and antimicrobial resistance. *Nature communications.*, **11**, 1–8.

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 520 Rico, A.; Phu, T. M.; Satapornvanit, K.; Min, J.; Shahabuddin, A. M.; Henriksson, P. J. G.; Murray,  
521 F. J.; Little, D. C.; Dalsgaard, A.; den Brink, P. J., 2013: Use of veterinary medicines, feed  
522 additives and probiotics in four major internationally traded aquaculture species farmed in Asia.  
523 *Aquaculture.*, **412**, 231–243.
- 524 Sahu, S.; Das, B. K.; Mishra, B. K.; Pradhan, J.; Sarangi, N., 2007: Effect of *Allium sativum* on  
525 the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of*  
526 *Applied Ichthyology.*, **23**, 80–86.
- 527 Samadi, L.; Vanguee, N.; Mousavi, S. M.; Zakeri, M., 2016: Effect of dietary garlic extract on  
528 growth, feeding parameters, hematological indices and body composition of *Litopenaeus*  
529 *vannamei*. *Journal of the Persian Gulf.*, **7**, 29–41.
- 530 Sarhadi, I.; Alizadeh, E.; Ahmadifar, E.; Adineh, H.; Dawood, M. A. O., 2020: Skin mucosal,  
531 serum immunity and antioxidant capacity of common carp (*Cyprinus carpio*) fed Artemisia  
532 (*Artemisia annua*). *Annals of Animal Science.*, **20**, 1011–1027.
- 533 Shalaby, A. M.; Khattab, Y. A.; Abdel Rahman, A. M., 2006: Effects of garlic (*Allium sativum*)  
534 and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia  
535 (*Oreochromis niloticus*). *Journal of Venomous Animals and Toxins Including Tropical Diseases.*,  
536 **12**, 172–201.
- 537 Shanthi, R.; Saravana Bhavan, P.; Radhakrishnan, S., 2012: Influence of medicinal herbs,  
538 *Andrographis paniculata*, *Cissus quadrangularis* and *Eclipta alba* on growth, digestive enzymes,  
539 biochemical constituents and protein profile of the freshwater prawn *Macrobrachium rosenbergii*.  
540 *Bio Technology.*, **42**, 6478–6484.
- 541 Sharma, V.; Sharma, A.; Kansal, L., 2010: The effect of oral administration of *Allium sativum*  
542 extracts on lead nitrate induced toxicity in male mice. *Food and Chemical Toxicology.*, **48**, 928–  
543 936.
- 544 Srivastava, S.; Pathak, P. H., 2012: Garlic (*Allium sativum*) extract supplementation alters the  
545 glycogen deposition in liver and protein metabolism in gonads of female Albino rats. *International*  
546 *Journal of Pharmaceutical Sciences and Drug Research.*, **4**, 126–129.

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

- 547 Stentiford, G. D.; Neil, D. M.; Peeler, E. J.; Shields, J. D.; Small, H. J.; Flegel, T. W.; Vlak, J. M.;  
548 Jones, B.; Morado, F.; Moss, S.; others, 2012: Disease will limit future food supply from the global  
549 crustacean fishery and aquaculture sectors. *Journal of invertebrate pathology.*, **110**, 141–157.
- 550 Sung, Y.Y.; Roberts, R. J.; Bossier, P., 2012. Enhancement of Hsp70 synthesis protects common  
551 carp, *Cyprinus carpio* L., against lethal ammonia toxicity. *Journal of Fish Diseases.*, **35**, 563-568.
- 552 Supa-aksorn, M.; Rungruangsak-Torrissen, K.; Tongsir, S.; Rojtinnakorn, J., 2017: Garlic extract  
553 product enhancing growth performance, digestive and immune system in Nile tilapia  
554 (*Oreochromis niloticus*). *The JSFS 85th Anniversary-Commemorative International Symposium*  
555 “*Fisheries Science for Future Generations*”, pp. 1–2.
- 556 Talpur, A. D.; Ikhwanuddin, M., 2012: Dietary effects of garlic (*Allium sativum*) on haemato-  
557 immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi*  
558 infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture.*, **364–365**, 6–12.
- 559 Tamadoni Jahromi, S.; Pourmozaffar, S.; Rameshi, H.; Gozari, M.; Nahavandi, R., 2020:  
560 Evaluation of hemolymph biochemical properties, clearance rate, bacterial microbiota and  
561 expression of HSP genes of gulf pearl oyster *Pinctada radiata* in response to salinity changes.  
562 *Fisheries Science.*, **86**, 1055–1065.
- 563 Tamadoni Jahromi, S.; Pourmozaffar, S.; Jahanbakhshi, A.; Rameshi, H.; Gozari, M.; Khodadadi,  
564 M.; Sohrabipour, J.; Behzadi, S.; Bazrkar, N.; Nahavandi, R.; Zahedi, M. R.; Moezzi, M., 2021:  
565 Effect of different levels of dietary *Sargassum cristaefolium* on growth performance,  
566 hematological parameters, histological structure of hepatopancreas and intestinal microbiota of  
567 *Litopenaeus vannamei*. *Aquaculture.*, **533**, 1–17.
- 568 Van Hai, N., 2015: The use of medicinal plants as immunostimulants in aquaculture: A review.  
569 *Aquaculture.*, **446**, 88–96.
- 570 Wan, J.; Ge, X.; Liu, B.; Xie, J.; Cui, S.; Zhou, M.; Xia, S.; Chen, R., 2014: Effect of dietary  
571 vitamin C on non-specific immunity and mRNA expression of three heat shock proteins (HSPs)  
572 in juvenile *Megalobrama amblycephala* under pH stress. *Aquaculture.*, **434**, 325–333.
- 573 Yearbook, F. A. O., 2020: *Fishery and Aquaculture Statistics 2018*; FAO: Rome, Italy, 2020.

## Garlic extract on physio-biochemical responses of *M. rosenbergii*

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

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

580 Zaefarian, A.; Yeganeh, S.; Adhami, B., 2017: Dietary effects of garlic powder (*Allium sativum*)  
581 on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo*  
582 *caspicus*) and resistance against *Yersinia ruckeri* infection. *Aquaculture International.*, **25**, 1987–  
583 1996.

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

588 Zeng, S. L.; Long, W. Q.; Tian, L. X.; Xie, S. W.; Chen, Y. J.; Yang, H. J.; Liang, G. Y.; Liu, Y.  
589 J., 2016: Effects of dietary aflatoxin B<sub>1</sub> on growth performance, body composition, haematological  
590 parameters and histopathology of juvenile Pacific white shrimp (*Litopenaeus vannamei*).  
591 *Aquaculture Nutrition.*, **22**, 1152–1159.

592 Zhou, X. Q.; Zhao, C. R.; Lin, Y., 2007: Compare the effect of diet supplementation with uncoated  
593 or coated lysine on juvenile Jian Carp (*Cyprinus carpio* Var. Jian). *Aquaculture Nutrition.*, **13**,  
594 457–461.

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

Ingredients	Treatments			
	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 <sup>a</sup>	10	10	10	10
Proximate analysis				

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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

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638 <sup>a</sup>Vitamins and mineral mixes: Vitamin A (10,000,000 IU), Vitamin D3 (3,000,000 IU), Vitamin E  
639 (10,000 IU), Vitamin B1 (400 mg), Vitamin B2 (1,200 mg), Vitamin B6 (1,200 mg), coated  
640 Vitamin C (25,000 mg), Folic acid (600 mg), Niacin (6,000 mg), Calcium pantothenate (10,000  
641 mg), Biotin (20,000 mcg), Choline Chloride (10,000 mg), Iron (12,000 mg), Copper (1,200 mg),  
642 Iodine (400 mg), Manganese (5,000 mg), Zinc (6,000 mg), Cobalt (20 mg), Selenium (20 mg)

643  
644 **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
	TTCAGTGCATCCACGTCCTC	21		
HSP70	TGACAAGGGTCGCCTCAGTA	20	158	EU884290.2
	CATTATCTTGTTGCGATCCTC	21		
β-actin	TCCGTAAGGACCTGTATGCC	20	96	AY651918.2
	TCGGGAGGTGCGATGATTTT	20		

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648 **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 <sup>c</sup>	12.93 ± 0.31 <sup>b</sup>	13.15 ± 0.26 <sup>b</sup>	16.43 ± 0.41 <sup>a</sup>
Weight gain(g)	8.39 ± 0.11 <sup>c</sup>	11.83 ± 0.31 <sup>b</sup>	12.02 ± 0.47 <sup>b</sup>	15.31 ± 0.22 <sup>a</sup>
SGR <sup>1</sup>	4.73 ± 0.33 <sup>c</sup>	5.78 ± 0.32 <sup>b</sup>	5.82 ± 0.27 <sup>b</sup>	6.93 ± 0.31 <sup>a</sup>
FCR <sup>2</sup>	2.12 ± 0.01 <sup>c</sup>	1.76 ± 0.02 <sup>b</sup>	1.73 ± 0.01 <sup>b</sup>	1.42 ± 0.03 <sup>a</sup>

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

653 <sup>1</sup>Specific growth rate  
654 <sup>2</sup>Feed conversion ratio

655  
656 **Table 4.** Muscle biochemical constituent of shrimp fed with experimental diets.

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## Garlic extract on physio-biochemical responses of *M. rosenbergii*

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 <sup>c</sup>	15.6 ± 0.11 <sup>b</sup>	16.1 ± 0.34 <sup>b</sup>	18.5 ± 0.42 <sup>a</sup>
Fat	9.82 ± 0.45 <sup>a</sup>	7.62 ± 0.52 <sup>b</sup>	7.41 ± 0.22 <sup>b</sup>	5.22 ± 0.17 <sup>c</sup>
Ash	3.82 ± 0.04 <sup>c</sup>	4.46 ± 0.01 <sup>b</sup>	4.58 ± 0.06 <sup>b</sup>	5.24 ± 0.05 <sup>a</sup>
Moisture	65.86 ± 0.18	65.82 ± 0.10	65.62 ± 0.15	65.59 ± 0.12

658

659 **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 <sup>c</sup>	33.5 ± 1.31 <sup>b</sup>	38.7 ± 1.62 <sup>a</sup>	39.2 ± 1.39 <sup>a</sup>
SOD (U/mg protein)	87.5 ± 1.42 <sup>c</sup>	98.2 ± 1.83 <sup>b</sup>	109.4 ± 1.59 <sup>a</sup>	110.3 ± 1.42 <sup>a</sup>
GSH-Px (U/mg protein)	25.3 ± 1.15 <sup>c</sup>	30.3 ± 1.25 <sup>b</sup>	35.1 ± 1.19 <sup>a</sup>	36.8 ± 1.61 <sup>a</sup>

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

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664 **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 <sup>c</sup>	113.3 ± 1.41 <sup>b</sup>	115.8 ± 1.67 <sup>b</sup>	133.2 ± 1.59 <sup>a</sup>
AST (U/ml)	60.1 ± 1.42 <sup>c</sup>	71.7 ± 1.10 <sup>b</sup>	73.6 ± 1.32 <sup>b</sup>	83.7 ± 1.70 <sup>a</sup>
ALP (U/ml)	192.2 ± 2.56 <sup>c</sup>	231.8 ± 3.17 <sup>b</sup>	233.5 ± 2.72 <sup>b</sup>	265.3 ± 3.27 <sup>a</sup>
Total protein (g/l)	23.3 ± 1.15 <sup>c</sup>	29.7 ± 1.28 <sup>b</sup>	38.8 ± 1.34 <sup>a</sup>	39.7 ± 1.41 <sup>a</sup>
Albumin (g/l)	3.3 ± 0.12 <sup>c</sup>	4.6 ± 0.22 <sup>b</sup>	5.7 ± 0.17 <sup>a</sup>	5.9 ± 0.27 <sup>a</sup>

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

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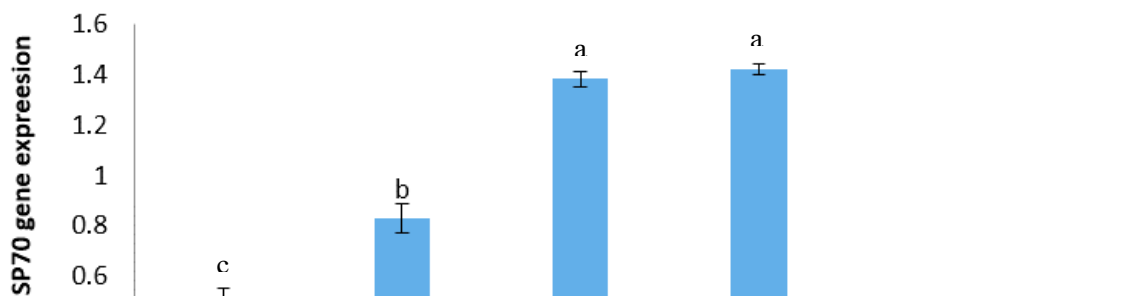
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## Garlic extract on physio-biochemical responses of *M. rosenbergii*

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

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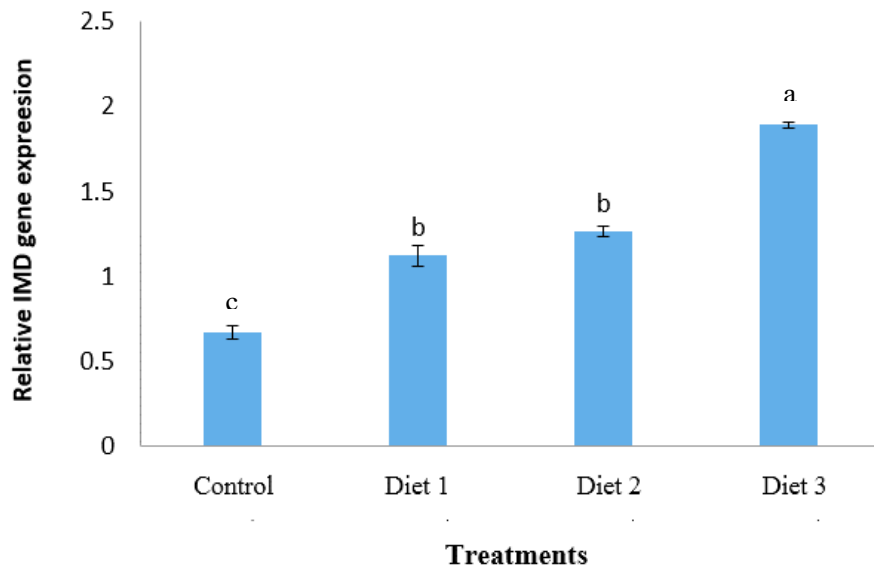
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692 **Fig 2.** The relative gene expression of IMD in hepatopancreas of *M. rosenbergii* fed experimental  
693 diets. Data are expressed as the mean SD. Different lowercase letters indicate statistically  
694 significant differences between groups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