Biol Trace Elem Res (2017) 177:196–208
DOI 10.1007/s12011-016-0855-4

Dietary Supplementation of Magnesium Oxide (MgO) Nanoparticles for Better Survival and Growth of the Freshwater Prawn *Macrobrachium rosenbergii* Post-larvae

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Received: 23 June 2016 / Accepted: 20 September 2016 / Published online: 5 October 2016

Abstract This study was performed to assess the growth-promoting potential of dietary magnesium oxide nanoparticles (MgO NPs) in *Macrobrachium rosenbergii* post-larvae (PL). MgO NPs were supplemented at 0, 100, 200, 300, 400 and 500 mg kg^{-1} with the basal diet (containing 0.95 g Mg kg^{-1}); the concentrations of Mg in MgO NP-supplemented diets were increased correspondingly (1.07, 1.15, 1.24, 1.37 and 1.46 g Mg kg^{-1} respectively). MgO NP-supplemented diets were fed to *M. rosenbergii* PL (initial weight 0.11 ± 0.04 g) for a period of 90 days. In the carcasses of experimental prawns, the content of Mg was found to be elevated significantly with respect to the individual diet (102.14, 183.29, 205.46, 221.03, 237.10 and 254.36 μg Mg g^{-1} respectively) when compared with that of the control. The contents of Cu, Zn, Fe, Ca, Na and K levels were also found to be elevated in the carcasses of experimental prawns. Significant ($P < 0.05$) improvements were observed in nutritional indices [survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER)], activities of digestive enzymes (protease, amylase and lipase), concentrations of basic biochemical constituents (total protein, amino acid, carbohydrate, lipid, profiles of amino acids and fatty acids) and population of haemocytes [total and differential (hyalinocytes, semigranulocytes and granulocytes)] in all the test PL. Maximum performance was recorded in 500 mg kg^{-1} MgO NP-supplemented-feed-fed PL. There were no significant elevations recorded in activities of antioxidant enzymes [superoxide dismutase (SOD) and catalase (CAT)], lipid peroxidation (LPO) and metabolic enzymes [glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)] recorded in any of the MgO NP-supplemented-feed-fed PL when compared with the control. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed increases in the staining intensity of polypeptide bands resolved in 500 mg kg^{-1} MgO NP-supplemented-feed-fed PL when compared with the control. Based on the gradual improvement in attaining survival, growth, FCR, biochemical constituents and haemocyte population, this study recommends MgO NP supplementation of 500 mg kg^{-1} for sustainable maintenance of *M. rosenbergii* PL. As the studied highest concentration of MgO NPs showed the best performance, it is necessary to study with beyond 500 mg kg^{-1} of MgO NPs to optimize the actual concentration.

Keywords *Macrobrachium rosenbergii* · MgO NPs · Growth · FCR · Total protein · Haemocyte population · SOD · Catalase · Lipid peroxidation · GOT · GPT

Introduction

Aquaculture is one of the fastest-growing and expanding industries in the world [1]. The giant freshwater prawn *Macrobrachium rosenbergii* is one of the most important aquafarming species in the South and Southeast Asian countries as well as in the northern Oceanic and western Pacific islands [2]. In India, aquaculture of *M. rosenbergii* provides a nutritious delicacy with high-quality protein for human consumption and considerably contributes to employment opportunity and income generation [3].

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Micronutrients play a very important role in maintaining the good health and production of prawns [4]. Nanoforms of minerals, such as Cu, Zn, Fe, Mn, Ag and Se, are new tools to improve the quality and sustainability of aquaculture production. These mineral forms have been reported to improve the survival, growth performance, muscle compositions, activities of digestive enzymes, carcass minerals and non-specific immune responses of the prawn *M. rosenbergii*; shrimps *Fenneropenaeus indicus* and *Litopenaeus vannamei*, and fishes, *Labeo rohita* and crucian carp *Carassius auratus gibelio* [5–13].

As an essential mineral, magnesium is the second most abundant cation within the cell, and it is a cofactor for numerous enzymatic and metabolic pathways of protein, carbohydrate and lipid [14, 15]. It plays an important role in antioxidation and enhances immunity [16]. It has been reported that the dietary supplementation of Mg reduced oxidative stress and free radical generation, and improved the meat quality of the Pacific white shrimp, *L. vannamei* [17, 18] and fish, *Ctenopharyngodon idella* [19]. Dietary Mg deficiency adversely affects growth and survival of fish and shrimp [14, 20]. Magnesium from the environment is insufficient to satisfy the metabolic demand. Therefore, it has to be supplemented through diet.

In nanoscience, MgO is a functional semiconductor; it has a role in toxic waste remediation [21] and optoelectronics [22]. In the medical field, MgO has many applications including in drug delivery [23], cell signaling and imaging [24], catalysis [25] especially as successful potent antimicrobial and antioxidant agents fighting against the most provocative antibiotic-resistant dreadful diseases, relief of heartburn and sore stomach, bone regeneration and tumor treatment [26–29]. MgO NPs have considerable potential as food additives and an antibacterial agent [25, 30]. However, there is a dearth of information pertaining to MgO NPs on the basic physiology and biochemistry of crustaceans. Hence, the present study was conducted to understand the importance of MgO NPs on survival and growth of *M. rosenbergii* post-larvae (PL). In this study, at the end of the feeding trial, the following parameters were analysed: the carcass contents of trace elements and mineral salts; nutritional indices [weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER)]; activities of digestive enzymes (protease, amylase and lipase); contents of basic biochemical constituents (total protein, amino acids, carbohydrate and lipid); profiles of proteins, amino acids and fatty acids; populations of total and differential haemocytes; activities of antioxidant enzymes [superoxide dismutase (SOD) and catalase (CAT)]; status of lipid peroxidation (LPO); and activities of metabolic enzymes [glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)].

Material and Methods

Test Animal

The post-larvae (PL-10) of the freshwater prawn, *M. rosenbergii*, were procured from ADAK Hatchery, Odayam, Varkala (8.733° N, 76.717° E), Thiruvananthapuram, Kerala, India. They were transported to the laboratory in polythene bags filled with oxygenated water. The prawns were acclimatized to the ambient laboratory condition with groundwater in cement tanks (6 × 3 × 3 ft) for 2 weeks. The groundwater satisfied the required physicochemical parameters (temperature 26 ± 1.0 °C; pH 7.10 ± 0.28; total dissolved solids 0.92 ± 0.05 g L^{-1}; dissolved oxygen 7.00 ± 0.29 mg L^{-1}; BOD 35.00 ± 1.42 mg L^{-1}; COD 127.00 ± 4.00 mg L^{-1}; ammonia 0.024 ± 0.005 mg L^{-1}). During acclimatization, the prawns were fed ad libitum with boiled egg albumin, live *Artemia* nauplii and commercially available scampi feed alternatively. In order to maintain a good and healthy aquarium water quality, more than 75 % of tank water was routinely changed every day. In order to supply sufficient oxygen to the prawns and maintain an environment devoid of accumulated metabolic wastes, adequate aeration was provided. The unfed feeds, faeces, moult and dead prawns, if any, were removed by siphoning without disturbing the prawns.

Preparation of Diet

As per *Pearson's square method*, the basal diets were prepared with 40 % of the protein value by using locally available feed ingredients (g kg^{-1}), fish meal (330) and soybean meal (330) as protein sources, wheat bran (100) as the carbohydrate source, tapioca flour (100) and egg albumin (100) as binding agents, and cod liver oil (20) as the lipid source. Vitamins and minerals free of MgO (20) were also added in the form of Zincovit® tablets (Apex Laboratories Private Limited, Chennai, India). Each tablet contains energy 3 kcal; total carbohydrate 0.2 g; carbohydrate (as sugar) 0.2 g; protein and fat 0 g each; vitamins: vitamin C 40 mg, vitamin B_3 18 mg, vitamin E 10 mg, vitamin B_5 3 mg, vitamin B_2 1.6 mg, vitamin B_1 1.4 mg, vitamin B_6 1 mg, vitamin A 600 μg, folic acid 200 μg, biotin 150 μg, vitamin B_{12} 1 μg and vitamin D_3 200 IU; minerals: zinc 10 mg, magnesium 3 mg, manganese 250 μg, iodine 100 μg, copper 30 μg, selenium 30 μg and chromium 25 μg; and natural extract, grape seed extract 50 mg.

The required proportion of each ingredient was taken in powder form, steam cooked and cooled at room temperature (28 °C). Then egg albumin, cod liver oil, vitamins and minerals free of MgO were added one by one. MgO (or magnesia) is a white hygroscopic solid mineral that occurs naturally as pericalis and is a source of magnesium. It consists of a lattice of Mg^{2+} ions and O^{2-} ions held together by ionic bonding.

MgO NP was procured from a local chemical supplier [<50 nm particle size, Sigma-Aldrich, USA, Product No: 549649; characterized by XRD, Raman FTIR and Brunauer–Emmett–Teller (BET) surface area measurement analysis] and supplemented with the basal diet at 0, 100, 200, 300, 400 and 500 mg kg^{-1}. The concentration of MgO NPs was chosen based on its requirement for crustaceans [18]. Diet with '0' % supplementation of MgO NPs served as the control. Separate dough for each formulation was prepared and quizzed with a 3.00-mm-sized die by using a hand pelletizer. The pellets were dried in a thermostatic oven at 40 °C until they reached constant weight. The dried feeds were stored in airtight plastic jars at −20 °C and consequently fed to *M. rosenbergii* PL during the feeding trial.

The proximate composition of organic matter of the basal diet prepared was determined by adopting the AOAC [31] methodology. It was calculated as 40.37 % crude protein, 5.79 % crude fat, 2.70 % crude fibre, 8.84 % total ash, 7.51 % moisture and 35.42 % carbohydrate (total nitrogen-free extract) with an energy value of 15.19 (kJ g^{-1}). In order to maintain palatability and ensure freshness throughout the feeding trial, the diets were freshly prepared once in every 30 days. The water stability of the feeds formulated was checked by the immersion and drying method, and the leaching percentage after 8 h of immersion was found to be between 20 and 22 %.

Feeding Trial

Six groups of PL-30-staged prawns (1.47 ± 0.39 cm and 0.11 ± 0.04 g) were taken in a triplicate experimental set-up. One group served as the control and was fed with '0' mg kg^{-1} MgO NP-supplemented diet. The remaining five groups were fed with 100, 200, 300, 400 and 500 mg kg^{-1} MgO NP-supplemented diets respectively. Each group consisted of 40 PL in an aquarium maintained with 40 L of groundwater. The water medium was renewed every 24 h by siphoning without severe disturbance to the PL and aerated adequately. The PL were fed with the above prepared diets at 10 % of body weight twice a day (8.00 a.m. and 8.00 p.m.) consecutively for 90 days continuously. During the feeding trial, the unfed feed, faeces and moults, if any, were collected on a daily basis while renewing the aquarium water. A similar experimental set-up was maintained then and there to study different parameters.

Contents of Trace Elements and Mineral Salts of Diets and Prawn Carcasses

The dietary and whole-body mineral contents, such as Fe, Cu and Zn and (in metal forms) and Ca, Mg, Na and K (in salt forms), were analyzed by adopting triple-acid digestion (H_2SO_4: HNO_3: $HClO_4$ at 9:3:1 ratio) of the dry sample using an atomic absorption spectrophotometer (Perkin-Elmer-2380) under an air acetylene flame [31].

Survival and Nutritional Indices

The survival rate [SR (no. of live prawns/no. of prawns introduced × 100)] and nutritional index parameters, such as feed intake [feed eaten (g)/total number of days], length gain [final length (cm) − initial length (cm)], weight gain [final weight (g) − initial weight (g)], specific growth rate [*log* final weight (g) − *log* initial weight (g)/total number of days × 100], feed conversion ratio [total feed intake (g)/total weight gain (g)] and protein efficiency ratio [total weight gain (g)/total protein intake (g)] were calculated by adopting the formulae of Tekinay and Davies [32].

Activities of Digestive Enzymes

The activities of digestive enzymes, such as protease, amylase and lipase, were assayed. The digestive tract of the prawns was carefully dissected out, homogenized in ice-cold distilled water and centrifuged at 9329*g* under 4 °C for 20 min. The supernatant was used as a crude enzyme source. Total protease activity was determined by casein-hydrolysis method of Furne et al. [33]; one unit of enzyme activity represents the amount of enzyme required to liberate 1 μg of tyrosine per minute under assay conditions. Amylase activity was determined by the starch-hydrolysis method put forth by Bernfeld [34]; the specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg g^{-1} h^{-1}). Lipase activity was determined by the method of Furne et al. [33]; one unit of lipase activity was defined as the amount of free fatty acid released from triacylglycerol per unit time, estimated by the amount of NaOH required to maintain pH constant and represented as mille equivalents of alkali consumed.

Concentrations of Biochemical Constituents

The contents of basic biochemical constituents, such as total protein, total amino acid, total carbohydrate and total lipid, in the muscle of the prawns were determined. Total protein was estimated by the method of Lowry et al. [35]. Total amino acid was estimated by the method of Moore and Stein [36]. Total carbohydrate was estimated by the method of Roe [37]. Total lipid was extracted with a chloroform–methanol mixture following the method of Folch et al. [38] and estimated by the method of Barnes and Blackstock [39].

Profiles of Proteins

SDS-PAGE analysis was done on the samples of the control and 500 mg kg^{-1} MgO NP-supplemented-feed-fed prawns.

The muscle tissue sample was first defrosted in phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4, pH 7.4), homogenized under ice-cold condition and centrifuged at 1500 rpm under 4 °C for 5 min. The soluble protein content in supernatant was determined [35]. SDS-PAGE was performed on vertical slab gel with 4 % stacking and 10 % separating gels [40]. Protein markers consisting of six different molecular weights (Medox-Biotech Pvt. Ltd., India), such as β-galactosidase (116 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), soybean trypsin inhibitor (20 kDa) and lysozyme (14 kDa), were also ran simultaneously. The polypeptide banding patterns between the control and test prawns were compared by using the information on apparent molecular masses of bands and their intensities.

Profiles of Amino Acids

The profiles of amino acids were analysed on the samples of the control and 500 mg kg^{-1} MgO NP-supplemented-feed-fed prawns by using the high-performance thin-layer chromatographic (HPTLC) method of Hess and Sherma [41]. The prawns were dried (80 °C for 3 h), digested with 6 M aqueous HCl and dried under vacuum. Samples (5 μL of distilled H_2O dissolved) were loaded on a TLC plate pre-coated with Silica gel-60F254 (8 mm thick; 20 cm × 15 cm), processed by using a CAMAG-LINOMAT 5 instrument and developed under butane–ammonia–pyridine–water (3.9:1:3.4:2.6) as the mobile phase. The gel was sprayed with ninhydrin reagent prepared in propan-2-ol and dried. The developed gel was documented under a CAMAG-11REPROSTAR 3 at 254- and 366-nm UV lights. Finally, the gel was scanned at 500 nm using the CAMAG-TLC SCANNER 3. TLC for four groups of standard amino acids: lysine, asparagine, glutamine, glutamic acid and methionine (group I); proline, serine, cystine, tyrosine and tryptophan (group II); histidine, arginine, aspartic acid, threonine and leucine (group III); and glycine, alanine, valine, isoleucine and phenyl alanine (group IV) were also performed simultaneously. The peak area of the sample was compared with that of standard amino acids and quantified.

Profiles of Fatty Acids

The profiles of fatty acids were analyzed on the samples of the control and 500 mg kg^{-1} MgO NP-supplemented-feed-fed prawns by using the gas chromatographic (GC) method of Nichols et al. [42]. Fatty acid samples were obtained from lipid by saponification using NaOH dissolved in a methanol–H_2O mixture (hydrolysis with alkali). They were methylated into fatty acid methyl ester using a methanol–HCl mixture. The fatty acid methyl ester was separated using a hexane–anhydrous diethyl ether mixture. For the organic phase, aqueous NaOH was used as base wash and the upper organic layer was separated. Two microlitres of sample was injected and analysed using Chemito 8610 Gas Chromatography, with a BPX70 capillary column and a flame ionization detector. Nitrogen was used as carrier gas. Standard fatty acids were analyzed simultaneously. Based on the retention time of the standard fatty acids, each fatty acid in the unknown sample was identified. The peak areas of the standard and the unknown were compared and quantified.

Populations of Total and Differential Haemocytes

Haemolymph (100 μL) was withdrawn from the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on a 1-mL syringe. Each syringe was pre-filled with 200 μL of anticoagulant (10 mM Tris-HCl, 250 mM sucrose, 100 mM sodium citrate, pH 7.6). More anticoagulants were added to make the volume up to 1 mL in the anticoagulated haemolymph. Further, a volume of 200 μL anticoagulated haemolymph was fixed with an equal volume of formalin (10 %) for 30 min. The fixed haemolymph was used to enumerate the total and differential haemocyte numbers (total haemocyte count (THC) and differential haemocyte count (DHC) respectively).

For THC, 100 μL of fixed haemolymph was diluted at a 1:2 ratio (*v*/*v*) with 20-mM ice-cold phosphate-buffered saline (PBS), pH 7.2. The diluted haemolymph was stained with 20 μL of Rose Bengal strain (1.2 % Rose Bengal in 50 % ethanol) and incubated at room temperature for 20 min. THC was determined by a haemocytometer (Neubauer improved, Germany) under a light microscope at RP 1000X (Labomed, CXR2). THC (106 cells mL^{-1}) = counted cells × depth of chamber × dilution factor/number of 1^{-} ^{mm} square.

For DHC, fixed haemolymph was stained with Rose Bengal solution (10 %) for 10 min and smeared on a slide. The numbers of differential haemocytes, such as hyalinocytes, semigranulocytes and granulocytes, were characterized according to Tsing et al. [43], and 350–400 cells from each smear were counted under a trinocular inverted microscope (model number INVERSO 3000) RP 1000X.

Activities of Antioxidant Enzymes and Status of Lipid Peroxidation

Tissues of hepatopancreas and muscle (100 mg each) were homogenized (10 % *w*/*v*) in ice-cold 50 mM Tris buffer (pH 7.4) and centrifuged at 9329*g* for 20 min at 4 °C, and the supernatant was used to assay the activities of superoxide dismutase (SOD) and catalase (CAT).

SOD activity was measured by using pyrogallol (10 mM) autoxidation in Tris buffer (50 mM, pH 7.0) by adopting the method of Marklund and Marklund [44]. The reaction was

initiated by the addition of NADH. The mixture was incubated at 30 °C for 90 s and arrested by the addition of glacial acetic acid. The reaction mixture was then shaken with *n*-butanol, and the intensity of the chromogen in the butanol layer was measured at 560 nm using a spectrophotometer. The specific activity of the enzyme was expressed in units/milligram protein.

CAT activity was measured by using H_2O_2 as the substrate in phosphate buffer by adopting the method of Sinha [45]. The reaction was initiated by the addition of phosphate buffer (0.01 M, pH 7.1) and H_2O_2 (0.2 M). After 60 s, the reaction was stopped by the addition of dichromate acetic acid reagent. The absorbance of the chromophore was read at 620 nm. The activity of CAT was expressed as micromolars of hydrogen peroxide consumed/minute/milligram protein.

Lipid peroxidation (LPO) in tissue homogenate was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS) and malondialdehyde (MDA) by adopting the method of Ohkawa et al. [46]. The absorbance of the supernatant was measured at 535 nm against the reagent blank and expressed as nanomolars of malondialdehyde (MDA)/milligram protein. The concentration of soluble proteins was determined by the method of Lowry et al. [35].

Activities of Metabolic Enzymes

The metabolic enzymes such as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were analyzed by the method of Reitman and Frankel [47] using a kit (Medsource Ozone Biomedicals Pvt. Ltd., Haryana, India). The hepatopancreas and muscle tissues (each 100 mg) were homogenized in 0.25 M sucrose and centrifuged at 3300*g* for 20 min in a high-speed cooling centrifuge at 4 °C. The supernatant was used as the enzyme source.

For GOT analysis, the substrate, buffered L-aspartic acid (500 μL; pH 7.4), was added to a 100-μL sample and incubated at 37 °C for 1 h. With this, 500 μL of 2, 4-dinitrophenyl hydrazine was added and allowed to stand at room temperature for 20 min followed by addition of 3 mL of freshly prepared sodium hydroxide solution (4 N). The colour development was read at 505 nm using a spectrophotometer within 15 min. Sodium pyruvate (160 U L^{-1}) was used as a calibrator. The activity of GOT was expressed as units per litre.

For GPT analysis, the substrate, buffered L-alanine, 2-oxoglutarate (500 μL; pH 7.4), was added to a 100-μL sample and incubated at 37 °C for 20 min. With this, 500 μL of 2, 4-dinitrophenyl hydrazine was added and allowed to stand at room temperature for 30 min followed by the addition of 3 mL of freshly prepared sodium hydroxide solution (4 N). The colour development was read at 505 nm using a spectrophotometer within 15 min. Sodium pyruvate (170 U L^{-1}) was used as a calibrator. The activity of GPT was expressed as units per litre.

Statistical Analysis

The data were expressed as mean ± SD and analysed by one-way analysis of variance (ANOVA) using SPSS (version-20), followed by Duncan's multiple range test (DMRT) to compare the significant differences among treatments at $P < 0.05$. The data obtained for profiles of amino acids and fatty acids were analyzed by Student *t* test. The actual requirement of dietary MgO NPs for optimal growth of *M. rosenbergii* PL was calculated by the polynomial regression method based on the weight gain [48].

Results

Contents of Trace Elements and Mineral Salts in Diets and Carcasses of Prawns

In the control diet, the contents of trace elements (mg kg^{-1}), Fe, Cu and Zn, and mineral salts (g kg^{-1}), Ca, Mg, Na and K, presented were 5.45, 2.10 and 4.52, and 0.40, 0.95, 0.32 and 2.19 respectively. In the experimental diets supplemented with 100–500 mg kg^{-1} of MgO NPs, the level of Mg alone was elevated gradually based on the supplementation of MgO NPs, whereas Fe, Cu, Zn, Ca, Na and K levels were not fluctuated much, because any forms of these trace elements and minerals were not supplemented (Table 1).

At the end of the feeding trial, the content of Mg was gradually increased significantly ($P < 0.05$) in the carcasses of prawns fed with 100–500 mg kg^{-1} MgO NP-supplemented feeds when compared with the control, based on the original contents present in different experimental diets (Table 2). The contents of trace elements (Fe, Cu and Zn) and other dietary minerals, such as Fe, Cu, Zn, Ca, Na and K, also showed elevation in the carcasses of prawns (Table 2).

Survival, Nutritional Indices, Activities of Digestive Enzymes and Contents of Basic Biochemical Constituents

The survival rate (SR), nutritional indices [length gain (LG) and weight gains (WG), feed intake (FI), specific growth rate (SGR) and protein efficiency ratio (PER)], activities of digestive enzymes (protease, amylase and lipase) and concentrations of basic biochemical constituents (total protein, amino acids, carbohydrate, lipid and ash) were found to be gradually and progressively increased in PL fed with 100–500 mg kg^{-1} MgO NP ($P < 0.05$)-supplemented diets when compared with those of the control (Table 3). In contrast, the food conversion ratio (FCR)

Table 1 Concentrations of trace elements and mineral salts in MgO NP-supplemented diets

Parameters		Concentrations of MgO NPs (mg kg^{-1})						*F* value
		Control	Experimental					
			100	200	300	400	500	
Trace elements (mg kg^{-1})	Fe	5.45 ± 0.67a	5.47 ± 0.64a	5.50 ± 0.62a	5.51 ± 0.65a	5.55 ± 0.71a	5.52 ± 0.63a	0.009
	Cu	2.10 ± 0.15a	2.12 ± 0.17a	2.15 ± 0.14a	2.11 ± 0.13a	2.14 ± 0.15a	2.18 ± 0.12a	0.125
	Zn	4.52 ± 0.80a	4.57 ± 0.74a	4.60 ± 0.65a	4.58 ± 0.59a	4.61 ± 0.72a	4.55 ± 0.64a	0.007
Mineral salts (g kg^{-1})	Ca	0.40 ± 0.03a	0.42 ± 0.04a	0.41 ± 0.02a	0.43 ± 0.03a	0.44 ± 0.02a	0.43 ± 0.03a	0.765
	Mg	0.95 ± 0.02f	1.07 ± 0.05e	1.15 ± 0.04d	1.24 ± 0.03c	1.37 ± 0.05b	1.46 ± 0.04a	68.109
	Na	0.32 ± 0.02a	0.34 ± 0.03a	0.33 ± 0.04a	0.35 ± 0.02a	0.34 ± 0.03a	0.35 ± 0.04a	0.424
	K	2.19 ± 0.10a	2.21 ± 0.07a	2.23 ± 0.08a	2.24 ± 0.11a	2.22 ± 0.08a	2.24 ± 0.07a	0.152

Each value is the mean ± SD; $n = 3$; mean values within the same row sharing different letters are statistically significant at $P < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT). Mean values within the same row sharing the same letters are not statistically significant at $P > 0.05$

Fe iron, *Cu* copper, *Zn* zinc, *Ca* calcium, *Mg* magnesium, *Na*, sodium, *K* potassium

and tissue moisture content were gradually decreased from 100 to 500 mg kg^{-1} MgO NP-supplemented diets fed PL when compared with those of the control ($P < 0.05$). The polynomial (cubic order) regression analysis is defined as the maximum concentration of any dietary mineral required for attaining maximum growth and beyond which growth would be depressed [19, 48]. In the present study, this analysis was approximating the relationship between maximum weight gain and essential nutrient (MgO NPs) intake, which revealed that no breakpoint appeared at any of the dietary concentrations of MgO NPs; therefore, the curve was straight and actually at the highest concentration of MgO NPs (500 mg kg^{-1}) was the maximum growth (weight gain) of the test prawn recorded (Fig. 1). Since WG was found to be increased in all the dietary concentrations of MgO NPs, it is essential to study with >500 mg kg^{-1} MgO NPs to see the optimum concentration of MgO NPs as far as *M. rosenbergii* PL is concerned.

Profiles of Proteins

Polypeptide bands of molecular weight between 116 and 13 kDa were resolved in the muscle tissue of the test prawns (Fig. 2). Generally, there were 16 Coomassie blue-stained protein bands (116, 99, 68, 66, 60, 50, 47, 45, 43, 41, 26, 20, 18, 15, 14 and 13 kDa) calculated against the standard markers of 116, 66, 45, 29, 20 and 14 kDa, which represent β-galactosidase, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme respectively. SDS-PAGE revealed that the polypeptide bands of 116, 99, 68, 66, 47, 45, 43, 41, 26, 20, 18, 15 and 14 kDa were found to

Table 2 Concentrations of trace elements and mineral salts in the carcasses of *M. rosenbergii* PL fed with MgO NP-supplemented diets

Parameters		Concentrations of MgO NPs (mg kg^{-1})						*F* value
		Control	Experimental					
			100	200	300	400	500	
Trace elements (μg g^{-1})	Fe	42.08 ± 1.35f	50.65 ± 2.11e	57.51 ± 2.82d	66.94 ± 2.29c	81.07 ± 2.40b	85.36 ± 2.05a	178.21
	Cu	35.05 ± 2.13f	71.33 ± 1.68e	80.97 ± 2.05d	86.51 ± 3.22c	93.28 ± 2.85b	105.72 ± 2.49a	294.82
	Zn	37.51 ± 1.58f	84.75 ± 2.29e	92.34 ± 2.43d	99.63 ± 3.56c	106.15 ± 2.93b	113.47 ± 3.14a	296.46
Mineral salts (μg g^{-1})	Ca	73.73 ± 3.17e	105.12 ± 2.45d	121.69 ± 3.17c	135.58 ± 2.73b	142.91 ± 3.02a	147.85 ± 2.83a	276.11
	Mg	102.14 ± 3.72f	183.29 ± 3.09e	205.46 ± 3.42d	221.03 ± 4.29c	237.10 ± 3.55b	254.36 ± 3.67a	663.24
	Na	118.69 ± 2.78d	167.94 ± 3.51c	172.82 ± 3.18c	179.57 ± 3.73b	184.63 ± 3.49b	192.58 ± 2.32a	201.97
	K	145.27 ± 2.55e	182.58 ± 3.37d	186.54 ± 3.25cd	191.86 ± 3.59c	198.41 ± 4.07b	204.61 ± 3.58a	111.93

Each value is the mean ± SD; $n = 3$; mean values within the same row sharing different letters are statistically significant at $P < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT)

Fe iron, *Cu* copper, *Zn* zinc, *Ca* calcium, *Mg* magnesium, *Na* sodium, *K* potassium

Table 3 Survival rate, nutritional indices, concentrations of biochemical constituents and activities of digestive enzymes in *M. rosenbergii* PL fed with MgO NP-supplemented diets (initial length and weight 1.47 ± 0.39 cm and 0.11 ± 0.04 g respectively)

Parameters		Concentrations of MgO NPs (mg kg^{-1})						*F* value
		Control	Experimental					
			100	200	300	400	500	
Survival rate (%)		80.03 ± 3.81b	82.50 ± 2.50b	84.17 ± 2.89b	85.83 ± 3.82b	86.67 ± 2.89b	92.50 ± 2.50a	5.12
Nutritional indices	Length (cm)	4.83 ± 0.77f	5.12 ± 0.38e	5.44 ± 0.40d	5.72 ± 0.52c	5.93 ± 0.63b	6.33 ± 0.95a	128.06
	LG (cm)	3.36 ± 0.03f	3.65 ± 0.04e	3.97 ± 0.07d	4.25 ± 0.12c	4.46 ± 0.10b	4.86 ± 0.08a	136.28
	Weight (g)	0.85 ± 0.08f	1.26 ± 0.04e	1.51 ± 0.06d	1.85 ± 0.05c	1.95 ± 0.08b	2.20 ± 0.06a	5624.17
	WG (g)	0.74 ± 0.02f	1.15 ± 0.02e	1.40 ± 0.01d	1.74 ± 0.02c	1.84 ± 0.03b	2.09 ± 0.01a	2131.08
	FI (g day^{-1})	0.44 ± 0.01e	0.46 ± 0.02d	0.51 ± 0.02c	0.57 ± 0.01b	0.59 ± 0.01b	0.64 ± 0.01a	108.47
	DM (NM day^{-1})	2.67 ± 0.09e	2.74 ± 0.05e	2.87 ± 0.08d	3.03 ± 0.07c	3.18 ± 0.06b	3.46 ± 0.04a	54.04
	SGR (%)	0.99 ± 0.06d	1.17 ± 0.06c	124 ± 0.06bc	1.33 ± 0.06ab	1.36 ± 0.06a	1.41 ± 0.06a	21.18
	FCR (g)	1.34 ± 0.06a	0.90 ± 0.03b	0.82 ± 0.03c	0.74 ± 0.01d	0.72 ± 0.02d	0.69 ± 0.01d	190.12
	PER (g)	1.85 ± 0.08e	2.75 ± 0.08d	3.01 ± 0.11c	3.34 ± 0.05b	3.47 ± 0.09ab	3.60 ± 0.06a	187.79
Digestive enzymes (U mg^{-1} protein)	Protease	1.15 ± 0.12e	1.32 ± 0.15de	1.50 ± 0.14cd	1.68 ± 0.16bc	1.93 ± 0.13ab	2.12 ± 0.17a	18.99
	Amylase	0.68 ± 0.05d	0.77 ± 0.12cd	0.84 ± 0.10cd	0.97 ± 0.12bc	1.11 ± 0.15b	1.36 ± 0.11a	14.76
	Lipase[a]	0.23 ± 0.04e	0.30 ± 0.03d	0.37 ± 0.02c	0.45 ± 0.04b	0.50 ± 0.03ab	0.56 ± 0.05a	35.49
Biochemical constituents (mg g^{-1} wet wt.)	Protein	168.63 ± 4.13f	184.02 ± 4.56e	192.14 ± 3.63d	207.31 ± 2.80c	238.60 ± 4.30b	252.56 ± 2.73a	224.58
	Amino acid	101.39 ± 3.64e	108.05 ± 3.82d	112.67 ± 2.16d	125.01 ± 3.22c	144.47 ± 2.97b	157.22 ± 2.21a	153.51
	Carbohydrate	38.31 ± 2.37d	40.13 ± 1.56cd	43.58 ± 2.24c	49.32 ± 2.34b	52.97 ± 3.30ab	56.46 ± 2.00a	28.49
	Lipid	18.53 ± 1.09d	20.46 ± 1.18cd	22.61 ± 2.15c	25.64 ± 1.10b	27.04 ± 1.22ab	28.51 ± 1.74a	21.26
	Moisture (%)	74.87 ± 2.30a	73.43 ± 1.46ab	70.47 ± 1.05b	66.37 ± 2.58c	64.70 ± 2.40c	63.23 ± 1.32c	18.16
	Ash (%)	13.33 ± 0.51e	14.13 ± 0.32d	15.63 ± 0.29c	16.97 ± 0.21b	17.53 ± 0.31b	18.40 ± 0.36a	99.33

Each value is the mean ± SD; $n = 3$; mean values within the same row sharing different letters are statistically significant at $P < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT)

LG length gain, *WG* weight gain, *FI* feeding intake, *DM* daily moult, *SGR* specific growth rate, *FCR* feed conversion ratio, *PER* protein efficiency ratio

[a] $\times 10^2$ U mg^{-1} protein

be stained in higher intensity in the test sample, i.e. 500 mg kg^{-1} MgO NP-supplemented-feed-fed prawns when compared with the control.

Profiles of Amino Acids

In the present study, there were 18 amino acids detected in the muscle of prawns PL, of which ten were essential amino acids (EAA), arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, and eight were non-essential amino acids (NEAA), alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline and tyrosine (Table 4). Generally, the contents of all the EAA and NEAA were found to be significantly higher ($P < 0.05$) in the test prawns PL when compared with those of the control.

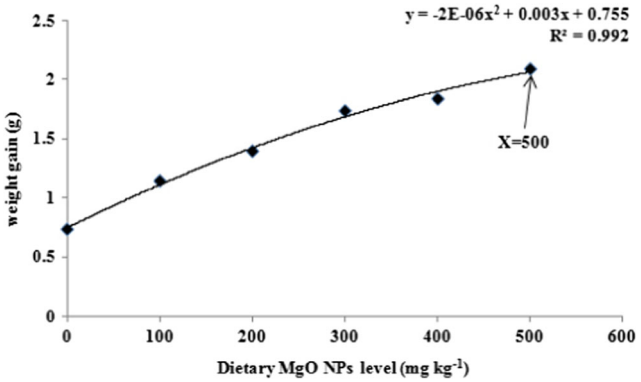

Fig. 1 Relationship between weight gain (g) and dietary MgO NP levels for *M. rosenbergii*. Each *point* represents the mean of triplicates per treatment. The optimal requirement is beyond 500 mg kg^{-1} MgO NPs in the diet as no breakpoint appeared in this polynomial regression analysis

Profiles of Fatty Acids

In the present study, 11 fatty acids were detected in the muscle of prawns PL, of which five were saturated fatty acids (SFA), lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid; two were monounsaturated fatty acids (MUFA), palmitoleic acid and oleic acid; and four were polyunsaturated fatty acids (PUFA), linoleic acid (n-6), arachidonic acid (n-6), EPA (n-3) and DHA (n-3) (Table 5). The contents of all the SFA, MUFA and PUFA were found to be significantly higher ($P < 0.05$) in the test prawn PL when compared with those of the control. In addition, SFA and MUFA were found to be predominant than PUFA in both control and test prawn PL.

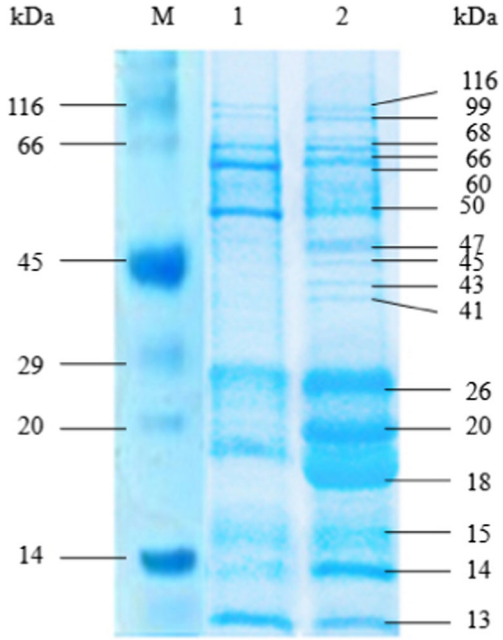

Fig. 2 SDS-PAGE pattern of muscle protein of *M. rosenbergii* PL fed with MgO NP-supplemented diet. *M* marker, *lane 1* control-diet-fed prawn, *lane 2* MgO NP (500 mg kg^{-1})-supplemented-diet-fed prawn

Haemocyte Populations

The haemocyte populations (total and differential, hyalinocytes, semigranulocytes and granulocytes) were found to be increased in *M. rosenbergii* PL fed with 500 mg kg^{-1} MgO NP ($P < 0.05$)-supplemented diet when compared with those of the control (Table 6).

Activities of Antioxidant and Metabolic Enzymes and Status of Lipid Peroxidation

In this study, no significant alterations were observed in activities of antioxidant enzymes (SOD and CAT) and metabolic enzymes (GOT and GPT) and lipid peroxidation (LPO) in the hepatopancreas and muscle of *M. rosenbergii* PL fed with 100–500 mg kg^{-1} MgO NP-supplemented diets when compared with control (Table 7).

Discussion

The concentration of Mg stored in body can be used to help determine the Mg requirement in crustaceans [17]. In this study, the level of Mg was found to be increased in test prawns based on MgO NP incorporation in diets. Mg is readily absorbed through the integument, antennal glands, gills and gastro-intestinal tracts of crustaceans [49–51]. Presumably, excess Mg might cost more energy in osmotic and ionic regulation and interact with the utilization of other nutrients. In the study, the concentration of MgO NPs (500 mg kg^{-1}) up-regulated the absorption of other minerals, such as Fe, Cu, Zn, Ca, Na and K. Therefore, 500 mg kg^{-1} MgO NPs may be harmless to *M. rosenbergii* PL. Moreover, no supplementation of any other mineral (of any form) was done in diet formulation. Therefore, the carcass mineral content may only depend on dietary concentration. The absorption is continuous; in all five levels, 100–500 mg kg^{-1} of MgO NPs showed positive responses/regulation on overall growth and survival of *M. rosenbergii* PL. In fact, a dietary concentration of >500 mg kg^{-1} MgO NPs may be required for optimal growth (to attain maximum weight gain) of test prawn PL, because all the five test concentrations showed positive response. This result can be compared with optimized levels of bulk Mg (300–574 mg kg^{-1}), which were reported to produce better survival and growth of *M. rosenbergii* larvae [52, 53]. Moreover, the optimized dose of Mg for the juveniles of *L. vannamei* was in the range of 3–3.2 g kg^{-1} [17, 18, 20, 54]. Furthermore, the optimized dose of Mg for fishes, *Ictalurus punctatus* fingerlings, *C. idella* juveniles and

Table 4 Profiles of amino acids (g/100 g dry wt.) in *M. rosenbergii* PL fed with MgO NP-supplemented diets

Amino acids		Concentrations of MgO NPs (mg kg^{-1})		*t* value
		Control	Experimental (500 mg kg^{-1})	
EAA	Arginine	2.90 ± 0.12	3.07 ± 0.18	−4.90
	Histidine	7.54 ± 0.45	8.72 ± 0.52	−29.19
	Isoleucine	2.23 ± 0.11	2.93 ± 0.17	−20.20
	Leucine	3.31 ± 0.17	3.49 ± 0.11	−5.19
	Lysine	2.57 ± 0.15	2.88 ± 0.19	−13.42
	Methionine	2.42 ± 0.09	3.19 ± 0.15	−22.22
	Phenylalanine	2.88 ± 0.14	3.10 ± 0.21	−5.44
	Threonine	3.58 ± 0.17	4.32 ± 0.24	−19.54
	Tryptophan	6.34 ± 0.44	6.92 ± 0.47	−33.48
	Valine	3.72 ± 0.21	4.37 ± 0.31	−11.258
NEAA	Alanine	2.71 ± 0.15	3.46 ± 0.24	−14.43
	Aspartic acid	8.62 ± 0.73	9.21 ± 0.69	−25.54
	Cystine	2.30 ± 0.12	2.90 ± 0.16	−25.98
	Glutamic acid	4.80 ± 0.20	5.67 ± 0.23	−50.22
	Glutamine	2.83 ± 0.16	3.43 ± 0.18	−51.96
	Glycine	2.03 ± 0.11	2.56 ± 0.20	−10.20
	Proline	5.04 ± 0.31	5.28 ± 0.22	−4.61
	Tyrosine	2.70 ± 0.15	3.15 ± 0.29	−5.56
ΣAA		68.47 ± 3.98	78.65 ± 4.76	−22.60
ΣEAA		37.44 ± 2.05	42.99 ± 2.55	−19.22
ΣNEAA		31.03 ± 1.93	35.66 ± 2.21	−28.64

Each value is the mean ± SD; $n = 3$; values are significant ($P < 0.05$) by paired-sample *t* test

EAA essential amino acids, *NEAA* non-essential amino acids

Table 5 Profiles of fatty acids (%) in *M. rosenbergii* PL fed with MgO NP-supplemented diets

Fatty acids		Concentrations of MgO NPs (mg kg^{-1})		*t* value
		Control	Experimental (500 mg kg^{-1})	
SFA	C12:0 (lauric acid)	0.05 ± 0.01	0.21 ± 0.05	−6.92
	C14:0 (myristic acid)	0.12 ± 0.03	0.46 ± 0.07	−14.72
	C16:0 (palmitic acid)	1.17 ± 0.10	7.74 ± 0.15	−227.59
	C18:0 (stearic acid)	0.44 ± 0.07	8.84 ± 0.12	−290.98
	C20:0 (arachidic acid)	0.20 ± 0.04	1.12 ± 0.09	−31.87
MUFA	C16:1 (palmitoleic acid)	0.11 ± 0.02	0.69 ± 0.04	−50.22
	C18:1 (olecic acid)	2.81 ± 0.09	10.86 ± 0.17	−174.28
PUFA	C18:2 n-6 (linoleic acid)	0.17 ± 0.03	0.25 ± 0.04	−13.85
	C20:4 n-6 (arachidonic acid)	0.04 ± 0.01	0.15 ± 0.02	−19.05
	C20:5 n-3 (EPA)	0.10 ± 0.02	0.24 ± 0.04	−12.12
	C22:6 n-3 (DHA)	0.07 ± 0.01	0.21 ± 0.02	−24.24
ΣFA		5.28 ± 0.53	30.77 ± 0.81	−157.70
ΣSFA		1.98 ± 0.35	18.37 ± 0.48	−218.37
ΣMUFA		2.92 ± 0.11	11.55 ± 021	−149.50
ΣPUFA		0.38 ± 0.07	0.85 ± 0.12	−16.28
n-3		0.17 ± 0.03	0.45 ± 0.06	−16.16
n-6		0.21 ± 0.04	0.40 ± 0.06	−16.45
n-3/n-6		0.81 ± 0.01	1.12 ± 0.02	−73.68

Each value is the mean ± SD; $n = 3$; values are significant ($P < 0.05$) by paired-sample *t* test

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Lateolabrax japonicus juveniles, was in the range of 0.5–1.0 g kg^{-1} [19, 55, 56]. This study suggested that MgO NP supplementation significantly increased the absorption and bioavailability of Mg. Similarly, the significant elevation in the whole body of Cu, Zn, Fe, P, Ca, Mg, NaCl and K has been reported in *M. rosenbergii*, *L. vannamei* and *Penaeus indicus* when fed graded levels of these minerals in supplemented diets [8–11, 20, 53, 57]. Significant increases in Mg content have also been reported in fishes, *Oncorhynchus mykiss*, *C. idella* and *L. japonicas*, fed with dietary Mg-supplemented diets [19, 56, 58].

In the present study, 500 mg kg^{-1} MgO NPs has maximum influence on the survival and growth of *M. rosenbergii* PL. Similar increases in survival and growth performance have been reported in marine shrimps, *Marsupenaeus japonicus* [59] and *L. vannamei* [17, 18, 20, 60], fed with Mg-supplemented diets**.** In fishes (*L. japonicus* and *O. mykiss*) also similar improved performance in survival and growth due to Mg supplementation has been reported [56, 58].

Dietary supplementations of minerals have influenced the digestive enzyme activities in fishes and prawns [60, 61]. In the present study, the increased activities of protease, amylase and lipase in MgO NP-supplemented-feed-fed prawns indicate its importance. The enhanced activities of digestive enzymes recorded in the test prawns led to enhanced food consumption and food conversion, which in turn ultimately led to

Table 6 Populations of haemocytes (total and differential counts) in *M. rosenbergii* PL fed with MgO NP-supplemented diets

Parameters		Concentrations of MgO NPs (mg kg^{-1})						*F* value
		Control	Experimental					
			100	200	300	400	500	
Haemocytes (×10^6 mL^{-1})	Total Haemocytes	4.96 ± 0.22e	6.15 ± 0.17d	7.42 ± 0.25c	7.79 ± 0.31c	8.92 ± 0.44b	9.57 ± 0.49a	78.81
	Hyalinocytes	2.64 ± 0.37d	3.45 ± 0.28c	3.77 ± 0.32bc	4.01 ± 0.25b	4.67 ± 0.29a	4.82 ± 0.27a	22.04
	Semigranulocytes	1.23 ± 0.14e	1.54 ± 0.07d	1.86 ± 0.12c	2.05 ± 0.09b	2.18 ± 0.07b	2.37 ± 0.10a	52.17
	Granulocytes	0.92 ± 0.09e	1.23 ± 0.12d	1.42 ± 0.10cd	1.61 ± 0.07bc	1.79 ± 0.14ab	1.96 ± 0.11a	37.65

Each value is the mean ± SD; $n = 3$; mean values within the same row sharing different letters are statistically significant at $P < 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT)

Table 7 Activities of antioxidant and metabolic enzymes and lipid peroxidation in the hepatopancreas and muscle of *M. rosenbergii* PL fed with MgO NP-supplemented diets

Parameters		Concentrations of MgO NPs (mg kg^{-1})						*F* value
		Control	Experimental					
			100	200	300	400	500	
Hepatopancreas	SOD (μmol min^{-1} mg^{-1} protein)	15.84 ± 1.21a	15.91 ± 1.47a	15.98 ± 1.32a	16.12 ± 1.38a	16.15 ± 1.41a	16.18 ± 1.26a	0.03
	CAT (U mg^{-1} protein)	27.35 ± 2.08a	27.38 ± 1.95a	27.43 ± 2.08 a	27.39 ± 2.13a	27.41 ± 2.09a	27.42 ± 2.15a	0.001
	LPO (nmol MDA mg^{-1} protein)	1.87 ± 0.09a	1.89 ± 0.11a	1.90 ± 0.07a	1.95 ± 0.18a	1.98 ± 0.16a	2.02 ± 0.12a	0.65
	GOT (U L^{-1})	14.32 ± 1.14a	14.41 ± 1.79a	14.46 ± 1.36a	14.52 ± 1.52a	14.47 ± 1.69a	14.56 ± 1.27a	0.010
	GPT (U L^{-1})	14.74 ± 1.07a	14.79 ± 1.14a	14.76 ± 1.21a	14.80 ± 1.29a	14.78 ± 1.35a	14.81 ± 1.38a	0.001
Muscle	SOD (μmol min^{-1} mg^{-1} protein)	9.02 ± 1.37a	9.12 ± 1.25a	9.20 ± 1.57a	9.32 ± 1.34a	9.36 ± 1.41a	9.44 ± 1.22a	0.04
	CAT (U mg^{-1} protein)	22.96 ± 1.04a	23.04 ± 1.58a	23.16 ± 1.39a	23.45 ± 1.42a	23.49 ± 1.54a	23.42 ± 1.61a	0.08
	LPO (nmol MDA mg^{-1} protein)	0.71 ± 0.06a	0.73 ± 0.05a	0.72 ± 0.03a	0.74 ± 0.06a	0.76 ± 0.07a	0.77 ± 0.04a	0.56
	GOT (U L^{-1})	9.38 ± 1.18a	9.42 ± 1.12a	9.47 ± 1.28a	9.46 ± 1.39a	9.52 ± 1.35a	9.55 ± 1.18a	0.01
	GPT (U L^{-1})	10.56 ± 1.05a	10.60 ± 1.56a	10.59 ± 1.31a	10.62 ± 1.44a	10.61 ± 1.59a	10.64 ± 1.55a	0.001

Each value is the mean ± SD; $n = 3$; mean values within the same row sharing different letters are not statistically significant at $P > 0.05$ (one-way ANOVA and subsequent post hoc multiple comparison with DMRT)

SOD superoxide dismutase, *CAT* catalase, *LPO* lipid peroxidation, *MDA* malondialdehyde, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase

better survival and growth of *M. rosenbergii* PL. Similarly, dietary Cu, Zn, ZnO, Fe, Mn, Cd and Pb supplementations increased protease, amylase and lipase activities in *Cyprinus carpio*, *C. idella*, *Oreochromis niloticus*, *Oreochromis aureus* and *M. rosenbergii* [8–11, 61–64].

In the present study, the higher levels of total protein, amino acid, carbohydrate and lipid recorded in MgO NP-supplemented-feed-fed PL suggest its influence on the metabolism of such constituents. Similarly, Mg supplementations have shown synthesis and storage of protein and lipid in juvenile *L. japonicus* [56], *O. mykiss* [65] and *C. idella* [19, 66]. The bulk and nanoforms of other minerals, such as Zn, Cu, Fe, Mn and NaCl, have also been reported to improve basic biochemical constituents in *M. rosenbergii* and fishes (*L. rohita, Cirrhinus mrigala* and *C. carpio*) [8–11, 60].

Mg is involved in the fat body protein synthesis in soft tissues and energy metabolism [15]. The dietary supplementation of minerals, such as Cu, Zn, Fe, Ca, Mg, Na and K, has improved the synthesis of protein in aquatic animals, *M. rosenbergii* [8, 9], *L. vannamei* [17], *Macrobrachium olfersii* [51], *L. japonicus* [56] and *Huso huso* [67]. In this study, the recorded increase in the staining intensity of 116-, 99-, 68-, 66-, 47-, 45-, 43-, 41-, 26-, 20-, 18-, 15- and 14-kDa proteins in the muscle tissue of test PL suggests that MgO NPs have influenced the protein synthesis. It has been reported that optimum dietary concentration of Mg can improve the synthesis of protein in shrimps and fishes [19, 20, 65, 68].

Naturally, prawns are good sources of essential amino acids [69, 70]. In the present study, the significant elevation of essential amino acids, such as arginine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan and valine, suggest that dietary supplementation of MgO NPs has promoted the absorption of nutrients, which led to accumulation of essential amino acids in the test prawns. The elevation of non-essential amino acids, such as alanine, aspartic acid, cystine, glutamic acid, glutamine, glycine, proline and tyrosine, in the test prawns suggested that MgO NPs have some useful roles in synthesis of these amino acids. Moreover, generally, fish and prawns contain high concentrations of non-essential amino acids, alanine, glutamine, glycine and proline, which support osmoregulatory functions [71–73].

In this study, the good content of SFA (lauric, myristic, palmitic, stearic and arachidic acids), MUFA (oleic and palmitoleic acids) and PUFA (linoleic acid (n-6), arachidonic acid (n-6), EPA (n-3) and DHA (n-3)) recorded in the test prawns suggests that MgO NPs in some way help in maintaining the levels of these fatty acids, which support better survival and growth of *M. rosenbergii*. A similar opinion has also been derived in literature [74–76].

The haematological parameters are indicators of the health state of aquatic animals as well [8, 77]. In the present study, the increase in THC and DHC (hyalinocytes, semigranulocytes and granulocytes) recorded in the test prawns suggests that MgO NPs have influenced the

production of haemocytes in order to maintain good health. Similarly, optimum levels of dietary Mg, nano-Zn, Cu, Fe and Mn have also produced better values on THC and DHC in *M. rosenbergii* PL and *L. japonicus* [8–11, 56]. Moreover, Mg and Fe have been reported to improve TBC, RBC, Hb, Hct and the macrophage chemotaxis ratio in fishes [7, 19, 78].

Aquatic animals have systems to fight in vivo oxidation and thus maintain health and prevent oxidation-induced lesions by physiological antioxidant [SOD, CAT, lysozyme (LSZ), thiobarbituric acid reactive substances (TBARS) and glutathione peroxidase (GPx)] protection involving a variety of chemical systems of endogenous and exogenous origins, both water and lipid soluble [19, 79]. Normal metabolism depends upon the ratio of free radical production and the activity of lipid peroxidation protection factors [8, 80]. Under normal conditions, the antioxidant defence system prevents the uncontrolled generation of reactive oxygen species through enzymes, such as SOD, CAT and LSZ [19]. In this study, since an unaltered LPO status was recorded, there were no requirements of activations of SOD and CAT in the test prawns fed with MgO NP-supplemented feed. In this study, even 500 mg kg^{-1} MgO NPs seemed to be not toxic to *M. rosenbergii* PL, which was evident from the unaltered activities of GOT and GPT in the hepatopancreas and muscle. Similar unaltered SOD, CAT, LPO, GOT and GPT systems have also been reported under optimum dietary concentrations of Mg and nano-Zn, Cu, Fe and Mn in *L. japonicus* and *M. rosenbergii* PL [8–11, 56].

Conclusion

In this study, even 500 mg kg^{-1} of MgO NPs did not produce a hepatotoxic effect, which was evident from the insignificant induction of GOT and GPT in the test prawns over the control. Therefore, there was a stable LPO system, which was further evident from the insignificant induction of SOD and CAT in the test prawns over the control. Therefore, 100–500 mg kg^{-1} of MgO NP supplementations produced better survival; growth; activities of protease, amylase and lipase; and concentrations of biochemical constituents, such as total protein, amino acids, carbohydrate and lipid including profiles of proteins, amino acids, fatty acids and the haemocyte population in *M. rosenbergii* PL over the control. This indicates the fact that the general health and non-specific immunity of the test prawns were improved in 100–500 mg kg^{-1} of MgO NP supplementations. Therefore, beyond 500 mg kg^{-1} of MgO NPs can also be considered as a safe concentration as far as *M. rosenbergii* PL is concerned. To reveal this, it is essential to study with >500 mg kg^{-1} MgO NPs to see the optimum concentration of MgO NPs as far as *M. rosenbergii* PL is concerned. However, this study recommends supplementation of 500 mg kg^{-1} of MgO NPs for sustainable maintenance of *M. rosenbergii* PL.

Acknowledgments Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Veterinary College and Research Institute, Namakkal, Tamil Nadu, India, is gratefully acknowledged for providing the outsourcing services.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

References

1. FAO (2010) The state of world fisheries and aquaculture. Rome
2. New MB (2005) Freshwater prawn farming: global status, recent research and a glance at the future. Aquac Res 36:210–230
3. FAO (2013) Fisheries and aquaculture. National aquaculture sector overview, India
4. Davis DA, Galtin DM III (1996) Dietary mineral requirements of fish and marine crustaceans. Rev Fish Sci 4:75–99
5. Zhou X, Wang Y, Gu Q, Li W (2009) Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*. Aquaculture 291:78–81
6. Rather MA, Sharma R, Aklakur M, Ahamad S, Kumar N, Khan M, Ramya VL (2011) Nanotechnology: a noval tool for aquaculture and fisheries development. Aprospectiv mini-review. Fish Aquac J 16:1–15
7. Behera T, Swain P, Rangacharulu PV, Samanta M (2014) Nano-Fe as feed additive improves the hematological and immunological parameters of fish, *Labeo rohita* H. Appl Nanosci 4:687–694
8. Muralisankar T, Bhavan PS, Radhakrishnan S, Seenivasan C, Manickam N, Srinivasan V (2014) Dietary supplementation of zinc nanoparticles and its influence on biology, physiology and immune responses of the freshwater prawn, *Macrobrachium rosenbergii*. Biol Trace Elem Res 160:56–66
9. Muralisankar T, Bhavan PS, Radhakrishnan S, Seenivasan C, Srinivasan V (2016) The effect of copper nanoparticles supplementation on freshwater prawn *Macrobrachium rosenbergii* post larvae. J Trace Elem Med Biol 34:39–49
10. Srinivasan V, Bhavan PS, Rajkumar G, Satgurunathan T, Muralisankar T (2016) Effects of dietary iron oxide nanoparticles on the growth performance, biochemical constituents and physiological stress responses of the giant freshwater prawn *Macrobrachium rosenbergii* post-larvae. Int J Fish Aquat Stud 4:170–182
11. Asaikkutti A, Bhavan PS, Vimala K, Karthik M, Cheruparambath P (2016) Dietary supplementation of green synthesized manganese-oxide nanoparticles and its effect on growth performance, muscle composition and digestive enzyme activities of the giant freshwater prawn *Macrobrachium rosenbergii*. J Trace Elem Med Biol 35:7–17
12. Vaseeharan B, Ramasamy P, Chen JC (2010) Antibacterial activity of silver nanoparticles (AgNps) synthesized by tea leaf extracts against pathogenic *Vibrio harveyi* and its protective efficacy on juvenile *Feneropenaeus indicus*. Lett Appl Microbiol 50:352–356
13. Sarkheil M, Sourinejad I, Mirbakhsh M, Kordestani D, Johari SA (2016) Application of silver nanoparticles immobilized on TEPA-

Den-SiO_2 as water filter media for bacterial disinfection in culture of penaeid shrimp larvae. Aquac Eng 74:17–29
14. Lall SP (2002) The minerals. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd edn. Academic Press Inc, San Diego, pp. 259–308
15. Vormann J (2003) Magnesium: nutrition and metabolism. Mol Asp Med 24:27–37
16. Tam M, Gomez S, Gonzalez-Gross M, Marcos A (2003) Possible roles of magnesium on the immune system. Eur J Clin Nutr 57: 1193–1197
17. Roy LA, Davis DA, Saoud IP, Henry RP (2007) Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. Aquaculture 262:461–469
18. Roy LA, Davis DA, Nguyen TN, Saoud IP (2009) Supplementation of chelated magnesium to diets of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low-salinity waters of West Alabama. J World Aquacult Soc 40:248–254
19. Wang FB, Luo L, Lin SM, Li Y, Chen S, Wang YG, Wen H, Hu CJ (2011) Dietary magnesium requirements of juvenile grass carp, *Ctenopharyngodon idella*. Aquac Nutr 7:691–700
20. Cheng KM, Hu CQ, Liu YN, Zheng SX, Qi XJ (2005) Dietary magnesium requirement and physiological responses of marine shrimp *Litopenaeus vannamei* reared in low salinity water. Aquac Nutr 11:385–393
21. Zhang K, An Y, Wang F, Lin L, Guo H (2011) Experimental investigation on water treatment by the combined nano MgO-nanofiltration technique. Water Sci Technol 63:2542–2546
22. Jackson JB, Westcott SL, Hirsch LR, West JL, Halas NJ (2003) Controlling the surface enhanced raman effect via the nanoshell geometry. Appl Phys Lett 82:257–259
23. Seferos DS, Giljohann DA (2007) Nano-flares-probes for transfection and mRNA detection in living cells. J Am Chem Soc 129: 15477–15479
24. Parak WJ, Pellegrino T, Plank C (2005) Labelling of cells with quantum dots. Nanotechnology 16:9–25
25. Tang ZX, Lv BF (2014) MgO nanoparticles as antibacterial agent: preparation and activity. Braz J Chem Eng 31:591–601
26. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002) Metal oxide nanoparticles as bactericidal agents. Langmuir 18: 6679–6686
27. Bertinetti L, Drouet C, Combes C, Rey C, Tampieri A, Coluccia S, Martra G (2009) Surface characteristics of nanocrystalline apatites: effect of MgO surface enrichment on morphology, surface hydration species, and cationic environments. Langmuir 25:5647–5654
28. Martinez-Boubeta C, Bacells L, Cristofol R, Sanfeliu C, Rodriguez E, Weissleder R, Lope-Piedrafita S, Simeonidis K, Angelakeris M, Sandiumenge F, Calleja A, Casas L, Monty C, Martinez B (2010) Self-assembled multifunctional Fe/MgO nanospheres for magnetic resonance imaging and hyperthermia. Nanomed Nanotech Biol Med 6:362–370
29. Di DR, He ZZ, Sun ZQ, Liu J (2012) A new nano-cryosurgical modality for tumor treatment using biodegradable MgO nanoparticles. Nanomed Nanotech Biol Med 8:1233–1241
30. Manuel-y-Keenoy B, Moorkens G, Vertommen J, Noe M, Ne've J, De Leeuw I (2000) Magnesium status and parameters of the oxidant-antioxidant balance in patients with chronic fatigue: effects of supplementation with magnesium. J Am Coll Nutr 19:374–382
31. AOAC (1995) Official methods of analysis of AOAC international, 16th edn. AOAC International Publishers, Arlington
32. Tekinay AA, Davies SJ (2001) Dietary carbohydrate level influencing feed intake, nutrient utilization and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. Turk J Vet Anim Sci 25:657–666
33. Furne M, Hidalgo MC, Lopez A, Garcia-Gallego M, Morales AE, Domezain A, Domezain J, Sanz A (2005) Digestive enzyme activities in Adriatic sturgeon *Acipenser naccarii* and rainbow trout *Oncorhynchus mykiss*. A comparative study. Aquaculture 250: 391–398
34. Bernfeld P (1955) Amylases. In: Colowick SP, Kaplan NO (eds) Methods in enzymology. Academic Press, New York, pp. 149–158
35. Lowry OH, Rosenbrough WJ, Fair AL, Randall RJ (1951) Protein measurement with the folinphenol reagent. J Biol Chem 193:265–275
36. Moore S, Stein WH (1948) Photometric ninhydrin method for use in the chromatography of amino acid. J Biol Chem 176:367–388
37. Roe JH (1955) The determination of sugar and blood and spinal fluid with anthrone reagent. J Biol Chem 212:335–343
38. Folch J, Lees M, Bloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 266:497–509
39. Barnes H, Blackstock J (1973) Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total' lipids. J Exp Mar Biol Ecol 12:103–118
40. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
41. Hess B, Sherma J (2004) Quantification of arginine in dietary supplement tablets and capsules by silica gel highperformance thin-layer chromatography with visible mode densitometry. Acta Chromatogr 14:60–69
42. Nichols DS, Nichols PD, McMeekin TA (1993) Polyunsaturated fatty acids in Antarctic bacteria. Antarct Sci 5:149–160
43. Tsing A, Arcier JM, Brehelin M (1989) Hemocytes of penaeid and palaemonid shrimps: morphology, cytochemistry and hemograms. J Invertebr Pathol 53:64–77
44. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474
45. Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47: 389–394
46. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351–358
47. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63
48. Lee MH, Shiau SY (2002) Dietary copper requirement of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune responses. Fish Shellfish Immun 13:259–270
49. Wilder MN, Ikuta K, Atmomarsono M, Hatta T, Komuro K (1998) Changes in osmotic and ionic concentrations in the hemolymph of *Macrobrachium rosenbergii* exposed to varying salinities and correlation to ionic and crystalline composition of the cuticle. Comp Biochem Physiol 119A:941–950
50. McNamara JC, Torres AH (1999) Ultracytochemical location of Na^+/K^+-ATPase activity and effect of high salinity acclimation in gill and renal epithelia of the freshwater shrimp *Macrobrachium olfersii* (Crustacea, Decapoda. J Exp Zool 284:617–628
51. Furriel RPM, McNamara JC, Leone FA (2000) Characterization of (Na^+, K^+)-ATPase in gill microsome of the freshwater shrimp *Macrobrachium olfersia*. Comp Biochem Physiol 126B:303–315
52. Tavabe KR, Rafiee G, Frinsko M, Daniels H (2013) Effects of different calcium and magnesium concentrations separately and in combination on *Macrobrachium rosenbergii* (de Man) larviculture. Aquaculture 412–13:160–166
53. Hangsapreuke K, Thamrongnawasawat T, Powtongsook S, Tabthipwon P, Lumubol P, Pratoomchat B (2008) Embryonic development, hatching, mineral consumption, and survival of *Macrobrachium rosenbergii* (de Man) reared in artificial seawater in closed recirculating water system at different levels of salinity. Maejo Int J Sci Technol 2:471–482
54. Davis DA, Saoud IP, Boyd CE, Rouse DB (2005) Effects of potassium, magnesium, and age on growth and survival of *Litopenaeus*

vannamei post-larvae reared in inland low salinity well waters in West Alabama. J World Aquacult Soc 36:403–406
55. Gatlin DM III, Robinson EH, Poe WE, Wilson RP (1982) Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. J Nutr 112:182–187
56. Zhang CX, Huang F, Li J, Wang L, Song K, Mai KS (2016) Interactive effects of dietary magnesium and vitamin E on growth performance, body composition, blood parameters and antioxidant status in Japanese seabass (*Lateolabrax japonicus*) fed oxidized oil. Aquac Nutr 22:708–722
57. Ahamad Ali S (1999) Calcium, phosphorus and magnesium requirements in the diet of shrimp *Penaeus indicus*. Asian Fish Sci 12:145–153
58. Shearer KD, Asgard T (1992) The effect of water-borne magnesium on the dietary magnesium requirement of the rainbow trout (*Oncorhynchus mykiss*. Fish Physiol Biochem 9:387–392
59. Kanazawa A, Teshima S, Sasaki M (1984) Requirements of the juvenile prawn for calcium, phosphorus, magnesium, potassium, copper, manganese, and iron. Mem Fac Fish Kagoshima Univ 33:63–71
60. Keshavanath P, Gangadhara B, Khadri S (2003) Growth enhancement of carp and prawn through dietary sodium chloride supplementation. Aquacult Asia 8:4–8
61. Li JS, Li JL, Wu TT (2007) The effects of copper, iron and zinc on digestive enzyme activity in the hybrid tilapia *Oreochromis niloticus* (L.) × *Oreochromis aureus*. (Steindachner. J Fish Biol 71:1788–1798
62. Kotorman M, Laszlo K, Nemcsok J, Simon LM (2000) Effects of Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} on activities of some digestive enzymes in carp (*Cyprinus carpio* L. J Environ Sci Health 35A:1517–1526
63. Tang QQ, Feng L, Jiang WD, Liu Y, Jiang J, Li SH, Kuang SY, Tang L, Zhou XQ (2013) Effects of dietary copper on growth, digestive, and brush border enzyme activities and antioxidant defense of hepatopancreas and intestine for young grass carp (*Ctenopharyngodon idella*. Biol Trace Elem Res 155:370–380
64. Hu CH, Xiao K, Jiao LF, Song J (2014) Effects of zinc oxide supported on zeolite on growth performance, intestinal barrier function and digestive enzyme activities of Nile tilapia. Aquac Nutr 20: 486–493
65. Ogino C, Takashima F, Chiou JY (1978) Requirement of rainbow trout for dietary magnesium. Bull Jpn Soc Sci Fish 44:1105–1108
66. Liang JJ, Tian LX, Liu YJ, Yang HJ, Liang GY (2012) Dietary magnesium requirement and effects on growth and tissue magnesium content of juvenile grass carp (*Ctenopharyngodon idella*. Aquac Nutr 18:56–64
67. Mohseni M, Pourkazemi M, Bai SC (2014) Effects of dietary inorganic copper on growth performance and immune response of juvenile beluga, *Huso huso*. Aquac Nutr 20:547–556
68. Shim KF, Ng SH (1988) Magnesium requirement of the guppy (*Poecilia reticulata* Peters. Aquaculture 73:131–141
69. Tidwell JH, Schulmeister G, Mahl C, Coyle (1997) Growth, survival, and biochemical composition of freshwater prawns *Macrobrachium rosenbergii* fed natural food organisms under controlled conditions. J World Aquacult Soc 28:123–132
70. Bhavan PS, Radhakrishnan S, Seenivasan C, Shanthi R, Poongadi R, Kannan S (2010) Proximate composition and profiles of amino acid and fatty acids in the muscle of adult males and females of commercially viable prawn species *Macrobrachium rosenbergii* collected from natural environments. Int J Biol 2:107–119
71. Cobb BF, Conte FS, Edwards MA (1975) Free amino acids and osmoregulation in penaeid shrimp. J Agric Food Chem 23:1172–1174
72. Fang LS, Tang CK, Lee DL, Chen IM (1992) Free amino acid composition in muscle and hemolymph of the prawn *Penaeus monodon* in different salinities. Nippon Suisan Gakkaishi 58: 1095–1102
73. Alam MD, Teshima SI, Yaniharto D, Sumule O, Ishikawa M, Koshio S (2005) Assessment of reference dietary amino acid pattern for juvenile red sea bream, *Pagrus major*. Aquac Int 13:369–379
74. Cavalli RO, Menschaert G, Lavens P, Sorgeloos P (2000) Maturation performance, offspring quality and lipid composition of *Macrobrachium rosenbergii* females fed increasing levels of dietary phospholipids. Aquac Int 8:41–58
75. Kamarudin MS, Roustaian P (2002) Growth and fatty acid composition of freshwater prawn, *Macrobrachium rosenbergii* larvae fed diets containing various ratios of cod liver oil–corn oil mixture. J Appl Ichthyol 18:148–153
76. Feliz GLA, Gatlin MD, Lawrence LA, Velazquez PM (2002) Effect of dietary phospholipid on essential fatty acid requirements and tissue lipid composition of *Litopenaeus vannamei* juveniles. Aquaculture 207:151–167
77. Kader MA, Koshio S, Ishikawa M, Yokoyama S, Bulbul M (2010) Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. Aquaculture 308:136–144
78. Shiau SY, LW S (2003) Ferric citrate is half as effective as sulfate in meeting the iron requirement of juvenile tilapia *Oreochromis niloticus* × *Oreochromis aureus*. J Nutr 133:483–488
79. pt?>Sun S, Ge X, Zhu J, Fu H, Jiang Z (2014) Effects of waterborne copper on the survival antioxidant status metallothionein-I mRNA expression and physiological responses of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Brachyura) larvae. Sci Mar 78:91–97
80. Mourente G, Diaz-Salvago E, Bell JG, Tocher DR (2002) Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidized oil: attenuation by dietary vitamin E. Aquaculture 214:343–361