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The effect of copper nanoparticles supplementation on freshwater prawn *Macrobrachium rosenbergii* post larvae

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ABSTRACT

The present study was performed to assess the effects of dietary supplementation of copper nanoparticles (Cu-NPs) on growth, biochemical constituents, digestive enzyme activities, antioxidant, metabolic enzyme levels, and non specific immune response of the freshwater prawn, *Macrobrachium rosenbergii* post larvae (PL). The Cu-NPs (200 nm) were synthesized by facile and environmental friendly hydrothermal method. Cu-NPs were supplemented at 0, 10, 20, 40, 60, and 80 mg kg^{-1} with the basal diets. These Cu-NPs supplemented diets were fed to *M. rosenbergii* PL for 90 days. Results showed significant ($P < 0.05$) improvements were observed in survival, growth, digestive enzyme activities, concentrations of biochemical constituents and total and differential haemocytes count of prawns fed with 20 mg Cu-NPs kg^{-1} supplemented feed. Prawns fed with 40–80 mg Cu-NPs kg^{-1} supplemented feed showed negative performance. Activity of antioxidants and metabolic enzymes in the muscle and hepatopancreas of prawns showed no significant alterations ($P > 0.05$) prawns fed with up to 20 mg Cu-NPs kg^{-1} supplemented feeds. Whereas, prawns fed with 40–80 mg Cu-NPs kg^{-1} supplemented feed showed significant ($P < 0.05$) elevations in antioxidant and metabolic enzymes activities. Hence, 40–80 mg Cu-NPs kg^{-1} diets may have toxic effect to *M. rosenbergii*. Hence, present study suggests that 20 mg Cu-NPs kg^{-1} can be supplemented for regulating better survival, growth and immune response of *M. rosenbergii* PL.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* is one of the edible species among crustaceans. In world freshwater prawn culture, *M. rosenbergii* holding top place due to its better environment tolerance, faster growth, larger in size, delicious meat quality and high market value. China, Thailand, Bangladesh, Vietanm, Taiwan and India are the major producers of *M. rosenbergii*. As per the report from MPEDA, the production of *M. rosenbergii* is 3332 MT during 2012–2013 in India [1].

Copper (Cu) is an essential micronutrient for humans and animals. It is an essential component of numerous oxidation reduction enzymes systems, such as cytochrome c oxidase, uricase, tyrosinase, superoxide dismutase, amine oxidase, lysyl oxidase, and ceruloplasmin. Cu intimately involved with iron metabolism and necessary for the formation of the pigment melanin, and it also needed for normal connective tissue metabolism and has functions within the central nervous system [2–4]. Cu has essential roles in biological, physiological and immune response of aquatic animals. The quantitative Cu requirement has been reported in few fish and crustaceans [5–9]. The optimum level of dietary Cu promoted better survival, growth, specific and non-specific immune response of crustaceans, such as *Eriocheir sinensis*, *M. rosenbergii*, and *Penaeus monodon* [6,9–12]. However, elevated Cu level may be toxic to fish and crustaceans due to association of heavy metals with formation of reactive oxygen species (ROS), that led to enhance cellular oxidative stress [13]. Higher concentrations of Cu may suppress the survival, growth and immunity has also been reported in fish and crustaceans [6,8,9].

Nanoscience and technology is an extensive and interdisciplinary area of research and development activity that has been growing explosively worldwide in recent years. The fish and shellfish industries can be revolutionized by nanotechnology with new tools to rapid disease detection and enhance the ability of cultivable species to uptake nutrients, hormones, drugs, and vaccines [14]. Nanoparticles (NPs) such as Se-NPs, Al-NPs, Fe-NPs, FeO-NPs, Zn-NPs, and ZnO-NPs play a vital role in aquaculture operations. The dietary supplementation of nanoparticles produced better survival,

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growth, antioxidant levels and immunity in aquatic organisms. However, the excess of nanoparticles in diets may be toxic to aquatic organisms [15,16].

In the present study was designed to synthesize and characterize the copper nanoparticles (Cu-NPs), and assess the effects of dietary Cu-NPs on survival, growth performance, activities of digestive enzyme (protease, amylase and lipase), concentrations of biochemical constituents (crude protein, total amino acids, carbohydrate and lipid), populations of haemocytes (total and differential haemocytes count), level of antioxidants (superoxide dismutase, catalase, and lipid peroxidation), metabolic enzymes (glutamate–oxaloacetate transaminase and glutamate–pyruvate transaminase) of *M. rosenbergii* post larvae (PL).

2. Materials and methods

2.1. Synthesis and characterization of Cu-NPs

In a typical procedure for the synthesis of Cu-NPs, 5 mmol L^{-1} of $CuCl_2{\cdot}2H_2O$ (99% purity, Sigma–Aldrich) and 5 mmol L^{-1} sodium dodecyl benzenesulfonate (SDBS) were dissolved in 30 mL of distilled water with vigorous stirring for 30 min. Consequently, 5 mL hydrazine hydrates ($N_2H_4{\cdot}H_2O$) was added drop wise to the above solution. The mixture was transferred into a 45 mL of Teflon lined stainless steel autoclave and maintained at 180 °C for 12 h in hot air oven and cooled at room temperature, the brown color precipitate was washed several times with double distilled water and absolute ethanol and separated by centrifugation. The obtained samples were collected and dried with vacuum oven at 80 °C for overnight. The formation of Cu-NPs in this work can be described on the basis of the following reactions:

$$Cu^{2+} + 2DBS^- \rightarrow Cu(DBS)_2 \quad (1)$$

$$Cu(DBS)_2 + N_2H_4 + 4OH^- \rightarrow 2Cu + N_2 + 4DBS^- + 4H_2O \quad (2)$$

The structural studies of the prepared samples were measured through X-ray diffraction (XRD) pattern by using a Bruker D8 Discover diffractometer with Cu Kα radiation (λ = 0.15406 nm) in the 2θ range from 10° C to 90 °C. Morphological and elemental compositions analyses were performed by field emission scanning electron microscope (FESEM) (JEOL JSM7000F).

2.2. Collection and acclimatization prawns

M. rosenbergii post larvae (PL) were procured from Rosen fisheries, Thrissur, Kerala, India. They were safely transported to the laboratory in plastic bags with well-oxygenated hatchery water. PL were acclimatized to ambient laboratory conditions for three weeks in large cement tank (1000 L) with ground water (pH, 7.10 ± 0.30; temperature, 28.17 ± 1.47 °C; total dissolved solids, 0.93 ± 0.07 g L^{-1}; dissolved oxygen, 7.40 ± 0.26 mg L^{-1}; BOD, 10.40 ± 1.15 mg L^{-1}; COD, 66.0 ± 4.00 mg L^{-1}; ammonia, 0.019 ± 0.004 mg L^{-1}; salinity, 6.8 ± 0.15 ppt; suspended copper, 0.018 mg L^{-1}) with adequate aeration. During which they were fed with boiled egg albumin (for protein source), *Artemia nauplii* (for essential fatty acids source) and control feed prepared with basal ingredients (for adopting the PL to artificial diet) alternatively three times per day, and 80% of aquarium water was changed daily at 6 a.m.

2.3. Formulation of feeds

The experimental feeds were prepared with locally available feed ingredients and proximate composition of basal diets was analyzed (Table 1). Fishmeal and soybean meal were used as protein sources; wheat flour and tapioca flour were used as carbohydrate sources; cod liver oil was used as lipid source; tapioca flour and egg albumin were served as binding agents; vitamin B complex with vitamin C and Cu free mineral mix was also added. The concentrations of dietary Cu-NPs were designed according to dietary requirements Cu in crustaceans [6] and it was supplemented with the basal diets at different concentrations like 0, 10, 20, 40, 60, and 80 mg kg^{-1}. However, the content of Cu concentrations were 10.82, 20.88, 30.95, 51.10, 71.13, and 91.22 mg Cu kg^{-1} for 0, 10, 20, 40, 60 and 80 mg kg^{-1} supplemented diets respectively (Table 2). The 3.0 ± 0.57 mm sized pellets were prepared as previously described [15]. The prepared feeds were stored individually in airtight plastic containers at −20 °C until used for the feeding trials. Proximate composition such as protein, carbohydrate, fiber, lipid, ash, moisture and energy were analyzed according to standard procedures of AOAC [17] (Table 1).

Table 1
Ingredients and composition of experimental diets.

Ingredients	(g kg^{-1})
Fish meal	400
Soybean meal	200
Wheat bran	180
Tapioca flour	150
Egg albumin	30
Cod liver oil	20
Vitamin mix[a]	10
Cu free mineral mix[b]	10
Analyzed proximate composition (%) on dry basis	
Protein	41.78
Carbohydrate	29.37
Fiber	5.10
Lipid	6.29
Ash	10.46
Moisture	7.0
Energy (kJ g^{-1})	14.76

[a] Thiamine mononitrate IP 10 mg; riboflavin IP 10 mg; pyridoxine hydrochloride IP 3 mg; vitamin B_{12} (as tablets 1:100) 1 P 15 mcg niacinamide 1 P 100 mg; calcium pantothenate IP 50 mg; folic acid IP 1.5 mg; biotin USP 100 mcg; ascorbic acid IP 150 m.

[b] Copper free mineral mix contains, $ZnSO_4$, 6 mg; $CaCO_3$, 164 mg; NaH_2PO_4, 148 mg; KH_2PO_4, 337.6 mg; $CaCl_2$, 66.64 mg; $MgSO_4$, 80 mg; KCl, 22.40 mg; $AlCl_3$, 0.96 mg; $MnSO_4$, 11.45 mg; $FeSO_4$, 90 mg; $CoCl_2$, 1.41 mg; KI 1.81, mg; cellulose, 69.74 mg.

2.4. Experimental procedure

In the present study, six groups of prawn PL (age PL 27 days) ranged from 0.9 to 1.8 cm length and 0.15 to 0.22 g weight were assigned in triplicate for 90 days. One group was served as control and diet fed with without Cu-NPs supplementation. The remaining five groups were fed with different concentration of (10, 20, 40, 60 and 80 mg kg^{-1} respectively) Cu-NPs supplemented diets. Each group consisted with 40 prawns in an aquarium maintained with 40 L of ground water (PL were acclimatized for three days before the 0 day of the experiment for adapting the new conditions). The water was renewed every 24 h without severe disturbance to the prawns and aerated adequately. The experimental feeds were fed to these prawns at 10% of body weight twice per day (6.00 a.m. and 6.00 p.m.). The unfed feed, feces and moult were removed on daily basis while renewing 80% of aquarium water at early morning.

2.5. Survival, growth and nutritional index analysis

After 90 days feeding trial, the survival rate (SR), length gain (LG), weight gain (WG), feed intake (FI), daily moult (DM), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were determined [18].

SR (%) = no. of live prawns/no. of prawns introduced × 100