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Effects of dietary copper on the growth physiology and biochemistry of the freshwater prawn *Macrobrachium rosenbergii* post larvae

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ABSTRACT

The present study was performed to assess the growth promoting potency of dietary Cu in the post larvae (PL) of commercially important freshwater prawn, Macrobrachium rosenbergii. 99.9% pure Cu metal was supplemented at 0, 10, 20, 40, 60 and 80 mg kg^{-1} with the basal diet formulated with 41.78%, 29.37% and 7.69% of protein, carbohydrate and lipid ratio respectively. Cu supplemented diets were fed to M. rosenbergii PL (initial weight, 0.18 ± 0.02) for a period of 90 days. Significant ($P < 0.05$) improvement was seen in survival, growth, activities of digestive enzymes (protease, amylase and lipase), concentrations of biochemical constituents (total nitrogen, crude protein, amino acid, carbohydrate and lipid) and mineral salts (Cu, Zn, Fe, Ca, Mg, Na and K) at 10-40 mg kg^{-1} Cu supplemented feeds fed PL, whereas, 60 and 80 mg Cu kg^{-1} supplementation showed negative performance. There were no significant alterations in activities of enzymatic antioxidants (superoxide dismutase and catalse), metabolic enzymes (glutamic oxaloacetate transaminase and glutamic pyruvate transaminase) and lipid peroxidation up to 40 mg kg^{-1} Cu supplementations, whereas, 60 and 80 mg of Cu kg^{-1} supplementations showed significant ($P < 0.05$) alterations in these parameters. Hence, the dietary Cu showed toxic effects on M. rosenbergii PL beyond 40 mg kg^{-1}. Thus, this study suggests that Cu up to 40 mg kg^{-1} can be supplemented for regulation of survival and growth of M. rosenbergii PL sustainably.

Keywords: *Prawn, Copper, survival, Growth.*

INTRODUCTION

Increasing demand and rising prices for seafood are raising the profile of some species of *Macrobrachium* as an important aquaculture commodity in the world. The farming of the giant freshwater prawn, *Macrobrachium rosenbergii* popularly known as 'scampi' is an emerging industry in the world aquaculture. *M. rosenbergii* holding top place due to its better environment tolerance, faster growth, large size, delicious meat quality and high market value. As per a recent report of FAO[1], India has produced 3,332 MT of *M. rosenbergii* during 2012-2014.

Copper (Cu) is an essential trace nutrient for humans and animals. It is involved in iron metabolism, melanin formation, connective tissue metabolism and functions of central nervous system[2]. It has essential roles in biological, physiological, immune response of aquatic animals. The deficiency of Cu leads to increases the physiological demand in fish and crustaceans[3-5]. The dietary supplementation of Cu can promote the survival, growth, specific and non-specific immunity of fish and crustaceans[4-7]. However, excessive Cu level can produces toxic effects to these organisms due to association with formations of reactive oxygen species (ROS), which led to produce cellular oxidative stress followed by reduced survival, growth and immunity[3,6-8].

Study regarding the optimum mineral requirement is fragmentary in *M. rosenbergii* and no report is available with dietary supplementation of Cu. Hence, the present study was designed to optimize the dietary Cu and assess its effects on the survival, growth, activities of digestive enzymes (protease, amylase and lipase), antioxidant enzymes [superoxide dismutase (SOD) and catalase (CAT)], metabolic enzymes [glutamate–oxaloacetate transaminase (GOT) and glutamate–pyruvate transaminase (GPT)], status of lipid peroxidation (LPO), concentrations of biochemical constituents (crude protein, total amino acids, carbohydrate and lipid) in *M. rosenbergii* PL.

MATERIALS AND METHODS

Collection and acclimatization of experimental prawns

Post larvae of *M. rosenbergii* (PL-5) were procured from Aqua Hatchery, Koovathur, Kanchipuram district, Tamil Nadu, India. They were safely transported to the laboratory in plastic bags with well-oxygenated hatchery water. They were acclimatized to ambient laboratory conditions for two weeks in large cement tank (1000 L) with ground water (Temperature, 26 ± 2.0 °C; pH, 7.12 ± 0.31; total dissolved solids, 0.95 ± 0.07 g L^{-1}; dissolved oxygen, 7.41 ± 0.27 mg L^{-1}; BOD, 26.00 ± 1.83 mg L^{-1}; COD, 136.0 ± 74.00 mg L^{-1}; ammonia, 0.019 ± 0.004 mg L^{-1}). During acclimatization ¾ of the tank water was renewed daily, and prawns were fed with boiled egg albumin and newly hatched *Artemia* nauplii alternatively two times per day. Then the PL was transferred to plastic aquaria and maintained for a week before the commencement of feeding trial. They were fed with *Artemia* nauplii and the control feed (prepared without addition of Cu) alternatively twice a day, and the entire aquarium water was renewed daily. In both conditions, faeces, moult and unfed feed were removed and the aquarium water was adequately aerated.

Feed formulation

The experimental diets were prepared with the following locally available feed ingredients (g kg^{-1}), fish meal **(**400), soybean meal (200), wheat bran (180), tapioca flour (150), egg albumin (30), and Cod liver oil (20). Of which fish meal and soybean meal were served as protein sources, wheat flour and tapioca flour were served as carbohydrate sources, Cod liver oil was used as lipid source, tapioca flour and egg albumin were served as binding agents. 10 g kg^{-1} of vitamin B-complex with vitamin C tablets (each tablet containing thiamine mononitrate IP, 10 mg; riboflavin IP, 10 mg; pyridoxine hydrochloride IP, 3 mg; vitamin B_{12} (as tablets 1:100) IP, 15 mcg; niacinamide IP, 100 mg; calcium pantothenate IP, 50 mg; folic acid IP, 1.5 mg; biotin USP, 100 mcg; ascorbic acid IP, 150 mg) and 10 g kg^{-1} of Cu free mineral mix (each gram containing $ZnSO_4$, 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) were also added.

The basal diet prepared by these ingredients containing proximate composition in the following ratio: crude protein, 41.78; crude lipid, 7.69; ash, 11.46; moisture, 8.56; total nitrogen free extract, 29.37 (energy value, 14.76 k.J g^{-1}).

Cu metal (99.9% pure) was purchased from Sigma Aldrich. The concentration range of dietary Cu was designed based on its requirements in crustaceans[3] and it was supplemented with the basal diet at 0, 10, 20, 40, 60 and 80 mg kg^{-1} and the analyzed Cu concentration in these diets was 0.82, 10.82, 20.76, 40.80, 60.83 and 80.72 mg kg^{-1} respectively (Table 1). The other minerals such as Zn, Fe, Ca, Mg, Na and K in the experimental diets were also provided in Table 1. Further, 3.0 ± 0.97 mm sized pellets were prepared as essentially described previously by Muralisankar *et al*.,[9]. The prepared feed was kept in airtight plastic containers at -20 ºC and consequently used during the feeding trial.

Feeding trial

Six groups of prawns (~PL 30; 1.42 ± 0.31 cm and 0.18 ± 0.03 g of initial length and weight respectively) were assigned in triplicate experimental set up. A group was served as control and fed with '0' mg Cu kg^{-1} supplemented diet. The remaining five groups were fed with 10, 20, 40, 60 and 80 mg Cu kg^{-1} supplemented diets respectively. Each group consisted of 40 PLs in an aquarium maintained with 40 L of ground water.

The water medium was renewed every 24 hr by siphoning method without severe disturbance to the PLs and aerated adequately. The PLs were fed with above prepared feeds at 10% of body weight twice a day (6.00 a.m. and 6.00 p.m.) for 90 days. During feeding trial, the unfed feed, feces and moults if any were collected on a daily basis while renewing aquarium water.

Survival and nutritional indices

At the end of the feeding trial, the survival rate (No. of live prawns/ no. of prawns introduced × 100) and nutritional indices 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 rate (feed intake (g)/ weight gain (g), and protein efficiency ratio (weight gain (g)/ protein intake (g) were all calculated by adopting the formulae of Tekinay and Davies[10].

Assays of digestive enzymes

Activities of digestive enzymes, such as protease, amylase and lipase were assayed on initial and final days of feeding trial. For this section, three prawns per group were sacrificed (3 × 6 = 18 PLs × 3 (triplicate) = 54 PLs), followed by dissected out the digestive tract including hepatopancreas of prawn, homogenized in ice-cold distilled water and centrifuged at 9329 g under 4 ºC 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*.,[11], 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 starch-hydrolysis method put forth by Bernfeld[12], the specific activity of amylase was calculated as milligrams of maltose liberated per gram of protein per hour (mg/g/h). Lipase activity was determined by method of Furne *et al.*,[11], 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.

Activities of enzymatic antioxidants and lipid peroxidation

100 mg of muscle, and hepatopancreas tissue was homogenized (10% w/v) in ice-cold 50 mM Tris buffer (pH 7.4), centrifuged at 9329 g for 20 min at 4 ºC and the supernatant was used to assay the activities superoxide dismutase (SOD) and catalase (CAT). SOD activity was measured by pyrogallol (10 mM) autoxidation in Tris buffer (50 mM, pH 7.0) by adopting the method of Marklund and Marklund[13]. 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 spectrophotometer. The specific activity of the enzyme was expressed in unit/mg protein. CAT activity was measured by using H_2O_2 as the substrate in phosphate buffer adopting the method of Sinha[14]. The reaction was initiated by the addition of phosphate buffer (0.01 M, pH 7.1), 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 µM of hydrogen peroxide consumed/ minute/ mg protein. Lipid peroxidation (LPO) in tissue homogenate was measured by estimating the formation of thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA) by adopting the method of Ohkawa *et al*.,[15]. The absorbance of the supernatant was measured at 535 nm against the reagent blank. TBARS was expressed as nM of malondialdehyde (MDA)/ mg protein. Concentration of soluble proteins was determined by the method of Lowry *et al*.,[16].

Activities of metabolic enzymes

100 mg of muscle, and hepatopancreas tissue was homogenized in 0.25 M sucrose and centrifuged at 3381g for 20 min in a high speed cooling centrifuge at 4 °C. The supernatant was used as the enzyme source. The metabolic enzymes, such as glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) were assayed according to the method prescribed by Reitman and Frankel[17] using a med source kit (Medsource Ozone Biomedicals Pvt. Ltd. Haryana, India). The colour development was read at 505 nm using spectrophotometer within 15 min. Sodium pyruvate (GOT, 160; GPT, 170 U/L) was used as a calibrator. The activity of GOT and GPT was expressed as unit/L.

Estimations of biochemical constituents and carcass mineral contents

Analysis of total nitrogen, crude protein, moisture and ash contents were performed according to standard AOAC[18] methods. Total nitrogen and crude protein (N*6.25) were analyzed after single acid digestion using Kjeldhal apparatus (Model: Kelplus DISTYL-BS manufactured by Pelican Equipments Pvt. Ltd. Chennai, India). Concentration of total amino acid was analyzed by adopting the standard procedures of Moore and Stein[19]. Total carbohydrate was estimated by the method of Roe[20]. The total lipid was extracted by the method of Folch *et al.*,[21] and estimated by the method of Barnes and Blackstock[22].

The dietary and whole body mineral contents, such as Cu^+, Zn^+ and Fe^+ (in metal forms), Ca^+, Mg^+, Na^+ and K^+ (in salt forms) were analyzed using the atomic absorption spectrophotometer (AAS of Perkin-Elmer; Model 2380) under air acetylene flame by adopting AOAC[18] method of triple acid digestion (H_2SO_4: HNO_3: $HClO_4$ at 9:3:1 ratio).

Statistical analysis

The data were expressed as mean ± S.D, and analyzed 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 $P < 0.05$.

RESULTS

Survival, growth and activities of digestive enzymes

The survival rate (SR), length (LG) and weight gains (WG), and other nutritional indices parameters, such as feed intake (FI), specific growth rate (SGR) and protein efficiency ratio (PER) were found to be increased in PL fed with 10-40 mg Cu kg^{-1} supplemented diets with a maximum better performance at 40 mg Cu kg^{-1} ($P < 0.05$) when compared with the control (Table 2). Whereas, this trend was turned towards reverse in other concentrations (60 and 80 mg kg^{-1}) of Cu. In contrast, the feed conversion ratio (FCR) was just opposite in 10-40 mg Cu kg^{-1} supplemented diets fed PL with a very lowest rate at 40 mg kg^{-1} of Cu when compared with the control ($P < 0.05$). Actually, this trend was turned towards increasing side in 60 and 80 mg Cu kg^{-1} supplemented diets fed PL (Table 2). Activities of digestive enzymes, such as protease, amylase and lipase showed increasing trend in PL fed with 10-40 mg Cu kg^{-1} supplemented diets with a significant elevation ($P < 0.05$) at 40 mg Cu kg^{-1} when compared with the control (Table 2). While, in other concentrations of Cu supplemented feeds fed PL this trend was started to decrease, particularly at 80 mg Cu kg^{-1} the activities of digestive enzymes were lower than that of control ($P < 0.05$).

Activities of enzymatic antioxidants and metabolic enzymes, and 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 muscle and hepatopancreas of PLs fed with 10-40 mg Cu kg^{-1}supplemented diets (Table 3). However, significant elevations ($P < 0.05$) were recorded in SOD, CAT, GOT and GPT activities, and LPO in PLs fed with 60 and 80 mg Cu kg^{-1} supplemented feeds when compared with control (Table 3).

Concentrations of total nitrogen and biochemical constituents

The levels of total nitrogen, concentrations of crude protein, amino acids, carbohydrate, lipid and ash were significantly increased in PLs fed with 10-40 mg Cu kg^{-1} supplemented diets with a maximum elevation in 40 mg Cu kg^{-1} ($P < 0.05$) when compared with the control. This trend was turned towards decreasing side in 60 and 80 mg Cu kg^{-1} supplementations except ash content. There was no significant difference was found in the cases of ash and moisture contents in 10-80 mg Cu kg^{-1} supplemented feeds fed PL when compared with control (Table 4).

Content of minerals

Concentration of Cu was gradually increased significantly ($P < 0.05$) in 10-80 mg kg^{-1} of Cu supplemented feeds fed PLs with a maximum increase in 80 mg kg^{-1} when compared with the control (Table 5). The contents of other dietary minerals, such as Zn, Fe, Ca, Mg, Na and K were also found to be significantly increased in the carcass of PLs up to 40 mg kg^{-1} of Cu supplementation ($P < 0.05$) when compared with control. This trend was turned towards opposite side in the cases of 60 and 80 mg kg^{-1} of Cu supplementation with a very steep decrease of Na and K (Table 5).

Table 1. Concentrations of minerals in Cu supplemented diets

Minerals	Cu supplementation (mg kg^{-1})					
	0	10	20	40	60	80
Cu	0.82 ± 0.50^{f}	10.82 ± 0.41^{e}	20.76 ± 0.37^{d}	40.80 ± 0.83^{c}	60.83 ± 0.12^{b}	80.72 ± 0.92^{a}
Zn	1.21 ± 0.012^{a}	1.18 ± 0.012^{a}	1.2 ± 0.011^{a}	1.23 ± 0.011^{a}	1.17 ± 0.019^{a}	1.21 ± 0.012^{a}
Fe	2.13 ± 0.017^{a}	2.12 ± 0.022^{a}	2.08 ± 0.02^{a}	2.12 ± 0.015^{a}	2.13 ± 0.018^{a}	2.11 ± 0.022^{a}
Ca	1.76 ± 0.014^{a}	1.73 ± 0.016^{a}	1.57 ± 0.015^{a}	1.66 ± 0.014^{a}	1.61 ± 0.015^{a}	1.71 ± 0.016^{a}
Mg	2.08 ± 0.021^{a}	2.07 ± 0.02^{a}	2.12 ± 0.023^{a}	2.06 ± 0.013^{a}	2.03 ± 0.02^{a}	2.1 ± 0.0210^{a}
Na	1.46 ± 0.015^{a}	1.42 ± 0.011^{a}	1.39 ± 0.012^{a}	1.50 ± 0.016^{a}	1.47 ± 0.018^{a}	1.51 ± 0.019^{a}
K	1.26 ± 0.012^{a}	1.30 ± 0.014^{a}	1.27 ± 0.015^{a}	1.24 ± 0.013^{a}	1.20 ± 0.018^{a}	1.29 ± 0.011^{a}

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts 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 alphabetical superscripts are not statistically significant at $P > 0.05$.

Table 2. Survival, nutritional indices and activities of digestive enzymes in *M. rosenbergii* fed with Cu supplemented diets

Parameters		Cu supplementation (mg kg^{-1})					
		0	10	20	40	60	80
SR (%)		75.83 ± 3.81^{bc}	78.33 ± 2.88^{abc}	81.66 ± 5.20^{ab}	85.00 ± 2.50^{a}	73.33 ± 3.81^{cd}	67.50 ± 2.50^{d}
Nutritional indices	Length (cm)	4.20 ± 0.96^{c}	5.40 ± 0.52^{ab}	5.64 ± 0.622^{ab}	6.08 ± 0.76^{a}	4.92 ± 0.43^{bc}	$4.86 \pm 0.41b^{c}$
	Weight (g)	0.60 ± 0.23^{d}	1.40 ± 0.39^{abc}	1.54 ± 0.44^{ab}	1.91 ± 0.65^{a}	1.03 ± 0.30^{bcd}	0.93 ± 0.16^{cd}
	LG (cm)	3.85 ± 0.12^{e}	5.05 ± 0.21^{bc}	5.29 ± 0.25^{ab}	5.73 ± 0.43^{a}	4.57 ± 0.27^{c}	4.51 ± 0.29^{d}
	WG (g)	0.42 ± 0.02^{f}	1.22 ± 0.03^{c}	1.36 ± 0.04^{b}	1.73 ± 0.07^{a}	0.85 ± 0.01^{d}	0.75 ± 0.03^{e}
	FI (g d^{-1})	0.39 ± 0.01^{d}	0.47 ± 0.02^{bc}	0.49 ± 0.01^{ab}	0.51 ± 0.02^{a}	0.45 ± 0.01^{c}	0.41 ± 0.01^{d}
	DM (moults d^{-1})	2.50 ± 0.08^{c}	2.53 ± 0.5^{c}	2.94 ± 0.11^{b}	3.17 ± 0.12^{a}	2.47 ± 0.15^{c}	2.33 ± 0.12^{c}
	SGR (% d^{-1})	0.57 ± 0.04^{d}	0.97 ± 0.07^{b}	1.02 ± 0.06^{ab}	1.13 ± 0.07^{a}	0.91 ± 0.06^{b}	0.78 ± 0.07^{c}
	FCR (g)	2.12 ± 0.14^{a}	0.87 ± 0.09^{c}	0.81 ± 0.08^{cd}	0.67 ± 0.05^{d}	1.19 ± 0.13^{b}	1.22 ± 0.10^{b}
	PER (g)	1.61 ± 0.09^{d}	4.20 ± 0.18^{b}	4.25 ± 0.14^{b}	4.80 ± 0.21^{a}	2.65 ± 0.30^{c}	2.58 ± 0.25^{c}
Digestive enzymes (U/mg protein)	Protease	1.01 ± 0.11^{cd}	1.11 ± 0.14^{cd}	1.43 ± 0.13^{b}	1.81 ± 0.11^{a}	1.20 ± 0.12^{c}	0.94 ± 0.15^{e}
	Amylase	0.62 ± 0.18^{d}	0.86 ± 0.12^{ab}	0.90 ± 0.23^{ab}	0.98 ± 0.21^{a}	0.65 ± 0.11^{cd}	0.59 ± 0.08^{de}
	Lipase $x10^{2}$	0.20 ± 0.06^{d}	0.25 ± 0.03^{cd}	0.31 ± 0.01^{b}	0.45 ± 0.03^{a}	0.26 ± 0.04^{bc}	0.18 ± 0.01^{de}

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

Initial length and weight were 1.42 ± 0.35 and 0.18 ± 0.02 respectively.

SR, Survival rate; LG, Length gain; WG, Weight gain; FI, Feeding intake; DM, Daily moult; SGR, Specific growth rate; FCR, Feed conversion ratio; PER, Protein efficiency ratio.

Initial protease, amylase and lipase activities were found to be 0.27 ± 0.06, 0.16 ± 0.04, 0.74 ± 0.05 respectively.

Table 3. Activities of SOD (µmol/min/mg protein), CAT (U/mg protein), LPO (nmol MDA/mg protein), GOT (Unit/L) and GPT (Unit/L) in the muscle and hepatopancreas of *M. rosenbergii* fed with Cu supplemented diets

Parameters		Initial	Cu supplementation (mg kg^{-1})					
			0	10	20	40	60	80
Muscle	SOD	4.81 ± 1.01	8.38 ± 1.52 ^{b}	8.44 ± 1.30 ^{b}	8.56 ± 1.41 ^{b}	8.71 ± 1.21 ^{b}	13.56 ± 1.34 ^{a}	15.50 ± 1.22 ^{a}
	CAT	11.20 ± 1.01	21.28 ± 2.00 ^{b}	21.35 ± 1.24 ^{b}	21.67 ± 1.45 ^{b}	21.85 ± 1.90 ^{b}	26.97 ± 1.29 ^{a}	29.89 ± 2.23
	LPO	0.13 ± 0.01	0.62 ± 0.02 ^{c}	0.64 ± 0.02 ^{c}	0.65 ± 0.03 ^{c}	0.65 ± 0.02 ^{c}	2.19 ± 0.01^{b}	3.30 ± 0.02^{a}
	GOT	6.32 ± 0.67	8.51 ± 0.50 ^{b}	8.53 ± 1.01 ^{b}	8.52 ± 1.19 ^{b}	8.52 ± 1.26 ^{b}	12.41 ± 2.34 ^{a}	15.20 ± 2.47 ^{a}
	GPT	7.95 ± 1.01	9.87 ± 0.22 ^{c}	9.86 ± 0.26 ^{c}	9.86 ± 0.14 ^{c}	9.85 ± 0.26 ^{c}	12.44 ± 1.38^{b}	18.29 ± 1.72^{a}
Hepatopancreas	SOD	5.81 ± 1.01	15.65 ± 2.34 ^{b}	15.66 ± 2.00 ^{b}	15.70 ± 2.32 ^{b}	15.72 ± 2.65 ^{b}	21.10 ± 2.47 ^{a}	24.83 ± 2.38 ^{a}
	CAT	14.20 ± 1.01	26.89 ± 3.00 ^{b}	26.92 ± 2.45 ^{b}	26.93 ± 2.51 ^{b}	26.95 ± 2.87 ^{b}	33.95 ± 2.43 ^{a}	37.22 ± 2.86 ^{a}
	LPO	0.14 ± 0.010	1.82 ± 0.04 ^{c}	1.84 ± 0.06 ^{c}	1.85 ± 0.05 ^{c}	1.87 ± 0.08 ^{c}	3.03 ± 0.12^{b}	5.76 ± 0.28^{a}
	GOT	8.32 ± 0.67	13.86 ± 1.24 ^{c}	13.87 ± 1.24 ^{c}	13.87 ± 1.15 ^{c}	13.75 ± 1.95 ^{c}	17.02 ± 1.76^{b}	21.68 ± 1.84^{a}
	GPT	9.15 ± 1.01	14.33 ± 1.41 ^{b}	14.34 ± 1.07 ^{b}	14.34 ± 1.13 ^{b}	14.35 ± 1.55 ^{b}	23.48 ± 3.03 ^{a}	26.25 ± 2.40 ^{a}

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts are 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; GOT, glutamate–oxaloacetate transaminase; GPT, glutamate–pyruvate transaminase.

Table 4. Proximate composition of *M. rosenbergii* fed with Cu supplemented diets

Parameters (mg/g wet wt.)	Initial	Cu supplementation (mg kg^{-1})					
		0	10	20	40	60	80
Total nitrogen (%)	4.10 ± 0.18	8.16 ± 0.29^{c}	8.65 ± 0.31^{bc}	9.24 ± 0.42^{b}	9.87 ± 0.32^{a}	9.13 ± 0.23^{b}	8.67 ± 0.37^{bc}
Crude protein (%)	25.66 ± 1.16	51.04 ± 1.82^{c}	54.10 ± 1.97^{bc}	57.75 ± 2.62^{b}	61.68 ± 2.00^{a}	57.07 ± 1.45^{b}	54.19 ± 2.35^{bc}
Total amino acid	25.33 ± 2.30	96.06 ± 3.57^{d}	99.40 ± 2.42^{cd}	106.00 ± 4.00^{b}	117.46 ± 2.83^{a}	102.66 ± 3.05^{bc}	88.00 ± 3.46^{e}
Total Carbohydrate	18.07 ± 1.07	33.34 ± 1.25^{d}	36.73 ± 1.80^{cd}	41.66 ± 2.68^{b}	50.82 ± 1.66^{a}	41.43 ± 2.39^{b}	39.67 ± 3.07^{bc}
Total lipid	10.68 ± 0.74	18.71 ± 2.05^{c}	19.57 ± 1.06^{c}	21.96 ± 3.53^{bc}	27.03 ± 1.36^{a}	24.700 ± 2.32^{ab}	20.08 ± 1.29^{c}
Ash (%)	10.40 ± 0.69	14.16 ± 1.76^{b}	16.36 ± 1.19^{a}	17.23 ± 1.07^{a}	18.46 ± 1.10^{a}	16.76 ± 1.36^{ab}	15.90 ± 1.57^{ab}
Moisture (%)	78.33 ± 1.52	76.00 ± 3.60^{a}	74.00 ± 4.35^{a}	72.33 ± 6.50^{a}	70.66 ± 1.52^{a}	73.30 ± 1.47^{a}	75.00 ± 2.00^{a}

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

Table 5. Concentrations of minerals in the whole body of *M. rosenbergii* fed with Cu supplemented diets

Minerals (µg g^{-1})	Cu supplementation (mg kg^{-1})					
	0	10	20	40	60	80
Cu	55.90 ± 2.66^{f}	69.55 ± 2.78^{e}	75.50 ± 2.59^{d}	95.40 ± 2.75^{c}	102.6 ± 4.03^{b}	144.15 ± 4.01^{a}
Zn	50.40 ± 2.95^{e}	56.50 ± 2.65^{d}	56.60 ± 3.01^{d}	98.35 ± 3.92^{a}	82.85 ± 3.92^{c}	70.80 ± 2.45^{b}
Fe	36.95 ± 2.45^{e}	57.65 ± 2.54^{c}	92.55 ± 4.02^{b}	107.75 ± 4.03^{a}	49.90 ± 2.11^{d}	47.35 ± 1.46^{d}
Ca	25.35 ± 1.44^{f}	49.20 ± 2.53^{e}	87.25 ± 3.65^{d}	246.4 ± 5.75^{a}	138.8 ± 3.25^{b}	106.2 ± 3.23^{c}
Mg	104.65 ± 3.21^{d}	126.05 ± 4.63^{c}	122.50 ± 5.76^{c}	150.20 ± 4.33^{a}	140.80 ± 4.37^{b}	113.55 ± 2.55^{e}
Na	129.52 ± 3.42^{c}	132.12 ± 3.54^{c}	144.89 ± 3.49^{b}	174.82 ± 2.78^{a}	114.83 ± 3.45^{d}	110.65 ± 2.62^{d}
K	149.78 ± 3.61^{c}	151.05 ± 2.11^{c}	164.65 ± 3.31^{b}	186.28 ± 2.91^{a}	142.28 ± 3.21^{d}	136.19 ± 3.16^{e}

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts 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 alphabetical superscripts are not statistically significant at $P > 0.05$.

DISCUSSION

Cu plays an essential role to regulate physiological, immunological and metabolic process in aquatic animals. The dietary supplementations of Cu promote the survival and growth of fishes and crustaceans[4,7,23]. In the present study, the improved survival, and growth parameters indicate the fact that 40 mg Cu kg^{-1} has the potency to influence. It has been reported that Cu has produced better survival, improved feed intake, total body weight and specific growth rate in Pacific white shrimp, *Litopenaeus vannamei*, the tiger shrimp, *Penaeus monodon*, the European sturgeon, *Huso huso*, the grass carp, *Ctenopharyngodon idella*, the Pacific abalone, *Haliotis discus hannai*, the Malabar grouper, *Epinephelus malabaricus*, the rainbow trout, *Salmo gairdneri*, the largemouth bass, *Micropterus salmoide*, the Atlantic salmon, *Salmo salar* and the Wuchang bream, *Megalobrama amblycephala*[3,6,8,23-25]. The higher value of feed conversion recorded in 60 and 80 mg Cu kg^{-1} supplementation shows their negative influence on feed utilization, survival and growth. This may be due to over dosage. Cu content beyond the optimum level have been reported to decrease the survival, feed intake, growth, feeding efficiency, specific growth rate and protein efficiency ratio in crustaceans, *Penaeus vannamei, Fenneropenaeus indicus* and *P. monodon*[3,5,24] and in fishes, *H. huso, S. salar, E. malabaricus, M. amblyephala* and the abalone, *H. discus hannai*[6,8,23,25,26].

Digestive enzymes play a vital role in nutritional physiology and directly or indirectly regulate the growth and moult cycle[26,27]. The increased activity of protease, amylase and lipase in 40 mg Cu kg^{-1} supplementation indicates that this level of dietary Cu has influenced the digestive enzymes activity in *M. rosenbergii* PL. Similarly, Cu supplemented feed fed fishes, the Nile tilapia, *Oreochromis niloticus*, the blue tilapia, *Oreochromis aureus* and *C. idella* showed significant improvement in digestive enzymes (protease, amylase, trypsin and chymotrypsin) secretion[25,28]. Kotorman *et al.*,[29] reported that dietary administration of Cu influenced on trypsin and lipase activities in the young carp, *Cyprinus carpio*. In the present study, the decreased levels of digestive enzymes in PLs fed with 60 and 80 mg Cu supplementation suggests that these levels of dietary Cu is toxic and inhibit the activity of digestive enzymes in *M. rosenbergii*. To support this, Tang *et al.*,[25] have reported that the increased dietary Cu led to significant reduction of digestive enzymes secretions in the fish *C. idella.*

Further, this was evident from the fluctuation of antioxidant enzymes (SOD and CAT) activities and produced cellular oxidative damage by reactive oxygen species (ROS), which was evident from the elevated LPO status under 60 and 80 mg Cu kg^{-1} supplementation. Dietary copper influences respiratory and antioxidant functions in crustaceans[30]. The toxic nature of dietary Cu at 60 and 80 mg kg^{-1} was further evident from the increased activities of metabolic enzymes (GOT and GPT) in the present study. This hepatotoxic effect of Cu in turn led to poor metabolic performance and thus, poor survival, growth, nutritional indices was resulted in *M. rosenbergii*. Since, 10-40 mg Cu kg^{-1} were not toxic to *M. rosenbergii*, there were no significant alterations recorded in the activities of SOD, CAT, GOT and GPT, and MDA production. Hence, 40 mg Cu kg^{-1} can be taken as an optimum dietary level as far as *M. rosenbergii* PL is concerned.

The optimum concentration of dietary Cu is vary from species to species and the fluctuations in SOD, CAT, MDA, GOT and GPT have been reported in *Sebastes schlegeli*[31], *E. malabaricus*[32]**,** *F. indicus*[5], *M. amblycephala*[6]**,** *C. idella*[25]**,** *H. huso*, and the marine crab, *Eriocheir sinensis*[6,33].

Body biochemical compositions are good indicator of the physiological condition of any cultivable species. The elevation recorded in biochemical constituents, such as total nitrogen, crude protein, amino acid, carbohydrate and lipid at 40 mg Cu kg^{-1} suggests that this level of Cu has influenced maximum on the physiology of *M. rosenbergii*, led absorption, synthesis and storage of protein, amino acids, carbohydrate and lipid. Mohseni *et al.*,[7] reported that the dietary Cu supplementation has promoted the storage of protein, lipid and ash contents in juvenile beluga, *H. huso*. The decrease in these biochemical constituents at 60 and 80 mg Cu kg^{-1} was associated with its toxicity due to its excessiveness. I has been reported that higher dietary Cu led to decrease in whole body and muscle protein, lipid and ash contents in *H. huso*[7], *H. discus hannai*[23] and the flathead catfish, *Pylodictis olivaris*[34].

In the present study, the contents of minerals (Zn, Fe, Ca, Mg, Na and K) except Cu were found to be similar in all experimental feeds. Whereas, the whole body mineral contents were gradually elevated in 10-40 mg Cu kg^{-1} supplemented feeds fed PLs and decreased in 60 and 80 mg Cu kg^{-1}. It clearly indicated the fact that supplementation of Cu positively regulates mineral absorption up to certain levels. Lorentzen and Maage[35] reported that fishmeal based Cu supplemented diets fed *S. salar* showed increase and decrease liver selenium content at optimum and beyond the optimum level of supplementations respectively. Similarly, elevation of whole body Cu content has been reported in *E. malabaricus* and *H. discus hannai* fed with Cu supplemented feeds[6,23]. The increase of minerals in the carcasses of prawns indicates the fact that dietary addition of Cu can promotes absorption of other minerals as well.

Inclusion of Cu (10-40 mg kg^{-1}) has significantly improved the survival, growth, activities of digestive enzymes, contents of biochemical constituents and minerals in *M. rosenbergii* PL. Whereas these levels of Cu did not produced any adverse effects on activities of antioxidant enzymes and metabolic enzymes, and lipid peroxidation. The enhanced activities of digestive enzymes in turn increased the food consumption and food conversion, which in turn ultimately led to better survival and growth of *M. rosenbergii* PL as these levels of Cu did not produce any adverse effect on the activities of antioxidant and metabolic enzymes, and lipid peroxidation status. Therefore, for sustainable maintenance of survival and growth of *M. rosenbergii* PL up to 40 mg kg^{-1} Cu can be supplemented in aqua feed formulations.

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