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Growth and antioxidant activity of juvenile oriental river prawn *Macrobrachium nipponense*, fed diets containing different copper levels under nitrite exposure

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The present study investigated the effect of dietary copper on alleviation the nitrite stress on the juvenile oriental river prawn, *Macrobrachium nipponense*. Under two levels of nitrite (low nitrite: 0.01 mg/L; high nitrite: 1.90 mg/L), four semi-purified diets with graded levels of copper (0, 30, 60 and 180 mg/kg diet) as $CuSO_4 \cdot 5H_2O$ were fed to juvenile prawn (initial weight 0.103 ± 0.003 g) for 8 weeks. Growth and antioxidant ability of prawns with low nitrite were higher than those of prawns with high nitrite. Prawns fed with Cu of 30 and 60 mg/kg diet had significantly higher weigh gain, specific growth rate and antioxidant enzymes (T-AOC, Cu-Zn SOD and GPx) activities than those prawns fed diets with copper deficiency and excess regardless of nitrite level ($P < 0.05$). MDA was the highest in prawns fed diets supplemented with 180 mg/kg and the lowest in 30 mg/kg group ($P < 0.05$) regardless of nitrite level. The present study indicated that nitrite stress could inhibit growth and antioxidant ability in prawn, but supplemented Cu of 30-60 mg/kg diet could alleviate these adverse effects of nitrite.

Keywords: *Macrobrachium nipponense*, copper, nitrite, growth, antioxidant activity

INTRODUCTION

Nitrite has become one of the common stresses in aquatic animal with the aggravation of water pollution and intensive cultivation development. Nitrite is an intermediate product of bacterial nitrification and denitrification, and can adversely affect the physiological function in organism especially aquatic animal (Mevel and Chamroux, 1981; Jensen, 2003; Knudsen and Jensen, 1997). For crustacean species, water nitrite exposure typically results in the delay of larval development (Mallasen and Valenti, 2006), reduction of survival, weight gain and reproduction (Hayd *et al*., 2014), damage of gill histological structure (Romano and Zeng, 2009) and moulting frequency leading to precocity of crustacean species (Koo *et al*., 2005). But so far, information on release of nitrite from the aspect of nutritional manipulation for aquatic animals is still limited, though researchers have found that dietary manipulation can be a practical way to alleviate the damage of stress on organism (Teles, 2012).

Copper (Cu) is the fundamental trace elements to normal physiological function of animals, and its importance is well documented (O'Dell, 1976). Physiologically, Cu is a co-factor for some enzymes such as cytochrome c oxidase, tyrosinase, superoxide dismutase (SOD) (Lall, 2002; Watanabe *et al*., 1997). The optimal dietary Cu requirement in crustacean has been reported to be in the range of 15-50 mg/kg (Sun *et al*., 2011; Lee and Shiau, 2002; Davis *et al*., 1993), much higher than that in fishes of 2-6 mg/kg (Gatlin and Wilson, 1986; Tan *et al*., 2011; Shiau and Ning, 2003). Studies have also shown that moderate dietary Cu increase animal oxidative activity and antimicrobial infection ability (Hill, 1980; Lee and Shiau, 2002; Wang *et al*., 2009). Xian *et al*. (2013) found that feeding with Cu of 25-55 mg Cu/kg could effectively reduce the oxidative damage for hemocytes caused by nitrite stress, but excessive feeding with Cu of 110 mg/kg would exacerbate the damage on hemocytes. But till now, it is not clear whether dietary supplement moderate Cu can relieve the nitrite stress damage on prawn and the positive physiological effect of Cu was suppressed by nitrite exposure.

Therefore, the purpose of the present study was to investigate the effect of dietary copper levels on growth and antioxidant activities in oriental river prawn which is a traditional and popular aquaculture species in P.R. China, Japan and other South-East Asian countries under nitrite stress. Results will provide basis information to alleviate nitrite stress by dietary manipulating for aquatic animals.

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MATERIALS AND METHODS

Diet preparation

The basal diet was formulated using casein and fish meal as protein sources, fish oil and soybean oil as lipid sources. Copper sulfate (Analytical Reagent, Shanghai Chemical Co., Shanghai, China) was added to a basal diet at 0, 30, 60, and 180 mg/kg dry diet with corresponding decreases in the amount of cellulose. Process of diet preparation was similar to that described by Li et al. (2010). Copper concentrations in the diets were analyzed by flame atomic absorption photometry (AOAC, 1990) to be 1.9, 33.9, 62.7 and 179.1 mg/kg, respectively. Contents of crude protein, crude lipid and ash in feed were analyzed to be 40.3%, 7.78% and 7.39%, respectively. The dry pellets were sealed in plastic bags and stored at -20°C until use. The compositions of the experimental diet are given in Table 1.

Experimental procedure

All juvenile prawns were obtained from a farm in huzhou (Zhejiang, China). Prior to the experiment, prawns were acclimatized to the laboratory conditions for 2 weeks. At the beginning of the experiment, healthy prawns were randomly selected with an average initial weight of 0.103±0.003 g. Prawns were fed on four experimental diets and subjected to two levels of nitrite (low level: 0.01 mg/L, high level: 1.90

Table 1 Ingredients and approximate compositions of experimental diets (%)

Ingredient	Percentage dry weight
Casein[a]	30
Fish meal[b]	20
Corn starch	26
fish oil[c]	4
Soybean oil[d]	2
Vitamin mix[e]	2
Cu-free mineral mix[f]	3
Attractant[g]	3
Cholesterol[h]	0.5
Choline chloride[h]	0.5
lecithin[h]	0.5
cellulose[h]	6.5
sodium carboxymethylcellulose[h]	2
Proximate composition	
Crude protein	40.3
Crude lipid	7.78
Crude ash	7.39

[a]: Sigma-Aldrich Co., Shanghai, China

[b]: Tecnologica De Alimentos U.S.A.

[c]: Xiamen Xinsha Pharmaceutical Co. Ltd, Xiamen, China.

[d]: National Golden Dragon Fish Co. Ltd, Shanghai, China.

[e]: Vitamin mixture(mg/100g mixture): vitamin A 420000IU; vitamin C 6000mg; α-tocopherol acetate 2000mg; vitamin D3 120000 IU; vitamin K 1000mg; vitamin B1 1000mg; vitamin B2 1000mg; vitamin B6 1600mg; vitamin B12 2mg;niacin 5000mg; folic acid 400mg; inositol 6000mg; biotin 10mg; calcium pantothenic 3500mg;Hangzhou Minsheng Bio-Tech Co., Ltd., China.

[f]: Composition of mineral mixture(g kg^{-1} diet): KCl 0.84, $MgSO_4.7H_2O$ 3, NaH_2PO_4 6.45, KH_2PO_4 3, $Ca(H_2PO_4)_2.H_2O$ 7.95, $CaCO_3$ 3.15, $C_6H_{10}CaO_6.5H_2O$ 4.95, $FeC_6H_5O_7.5H_2O$ 0.36, $ZnSO_4.7H_2O$0.1428, $MnSO_4.H_2O$ 0.0321, $AlCl_3.6H_2O$ 0.0045, $CoCl_2.6H_2O$ 0.042, KI 0.0069.

[g]: alanine 0.6%, glycine 0.6%, glutamic acid 0.6%, betaine 1.2%.

[h]: China National Medicine Corporation Co., Ltd, Beijing, China.

mg/L) leading to in all of 8 experimental groups that were carried out in forty 300 L tanks with 50 prawns per tank (five replicates per group). Prawns were fed to apparent satiation by twice a day (0800 and 1700h). One third of the tank water was exchanged daily to maintain water quality. The temperature of water ranged from 26°C to 30°C, dissolved oxygen was maintained above 6.5 mg/L during the feeding trial. Prawns were exposed to a natural photoperiod and fed to for 8 weeks. The Cu concentration in rearing water was also monitored regularly and ranged from 1.2 to 1.6 μg/L in tanks during the trial.

Sample collection

At the end of the feeding trial, the prawns were not fed 24h before sampling. Hepatopancreas were dissected and stored at -80°C. The concentration of maleic dialdehyde (MDA) and activities of total antioxidant competence (T-AOC), copper-zinc superoxide dismutase (Cu-Zn SOD) and glutathione peroxidase (GPx) of hepatopancreas were determined.

Analysis and measurement

At the end of the feeding trial, the prawns were not fed 24h before being weighted. All prawns were counted and weighed. Survival rate = 100 × (final prawn number) / (initial prawn number), weight gain (WG) = 100 × (final body weight - initial body weight)/initial body weight and specific growth rate (SGR) = 100 × [In(final weight) - In(initial weight)]/(days) were calculated.

Crude protein, crude lipid and ash in diets were measured following standard methods (AOAC, 1990). Crude protein was analyzed with Kjeldahl method, crude lipid was measured by the ether extraction method using the Soxhlet system (2055 Soxhlet Avanti; Foss Tecator, Hoganas, Sweden) and ash content was determined by a muffle furnace at 550°C overnight.

MDA was measured by thiobarbituric acid (TBA) method (Buege and Aust, 1978). T-AOC was determined based on the Fe^{3+} reduction process (Tang *et al.*, 2005).Cu-Zn SOD activity was analyzed according to the method of Xanthine oxidase (Elstner and Heupel, 1976). Reduced GSH in the enzymatic reaction was analyzed to determine GPx activity (Sedlak and Linday, 1968).

Statistical analysis

All data are expressed as mean ± S.D. A two-way analysis of variance (ANOVA) in the general linear models (GLM) procedure of SPSS 16.0 with three levels of dietary Cu and two levels of nitrite as the main effects was used to determine significant difference between groups. The significant variance was set at $P < 0.05$. If a significant interaction was detected between the main effects, then the differences between treatment means were compared by Turkey multiple range tests.

RESULTS

As shown in Table 2, survival rate of prawns was not affected by the dietary copper levels and water nitrite levels, although the survival rate were relatively lower in the groups of high nitrite ($P > 0.05$). High nitrite decreased weight gain (WG) and specific growth rate (SGR) of prawns compared to low nitrite in all diets ($P < 0.05$). Prawns in the 30 and 60 mg/kg groups exhibited significantly higher weight gain and SGR than prawns in the 0 and 180 mg Cu/kg groups regardless of nitrite levels ($p < 0.05$).

High nitrite resulted in a decrease of hepatopancreas copper-zinc superoxide dismutase (Cu-Zn SOD), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) activities of prawns compared to low nitrite in all diets ($P < 0.05$, Table 3). Cu-Zn SOD, GSH-Px and T-AOC activities in the prawns supplemented with Cu of 30 and 60 mg /kg were significantly higher than those in the prawns supplemented Cu of 0 and 180 mg/kg regardless of nitrite levels (P <0.05). Hepatopancreas malondialdehyde

Table 2 Effect of dietary copper level on growth performances of juvenile *Macrobrachium nipponense* under nitrite exposure (mean ± SD, n=5)

Nitrite level (mg/L)	Cu level (mg/kg)	WG (%)	SGR (%/d)	Survival (%)
0.01	0	693.98±28.03^{Bb}	3.70±0.06^{Bb}	79.97±5.65
	30	729.71±36.54^{Cb}	3.78±0.08^{Cb}	84.09±4.02
	60	725.24±43.65^{Cb}	3.77±0.09^{Cb}	83.28±4.96
	180	658.45±29.92^{Ab}	3.62±0.07^{Ab}	80.46±3.47
1.90	0	632.62±34.40^{Ba}	3.55±0.08^{Ba}	78.55±3.71
	30	698.45±16.44^{Ca}	3.71±0.04^{Ca}	82.78±3.13
	60	706.99±28.48^{Ca}	3.73±0.06^{Ca}	82.53±4.77
	180	609.13±28.57^{Aa}	3.50±0.07^{Aa}	78.04±4.83
ANOVA	Cu	0.000	0.000	0.064
P value	Nitrite	0.000	0.000	0.297
	Cu × Nitrite	0.447	0.357	0.980

$^{A, B, C, D}$ For variable with a significant effect of dietary Cu ($P < 0.05$).

$^{a, b}$ For variable with a significant effect for ambient nitrite ($P < 0.05$)

Table 3 Effect of dietary copper level on hepatopancreas antioxidant activities and lipid peroxidation of juvenile *Macrobrachium nipponense* under nitrite exposure (mean ± SD, n=3)

Nitrite level (mg/L)	Cu level (mg/kg)	MDA (nmol/mg prot)	Cu-Zn SOD (U/mg prot)	GSH-Px (U/mg prot)	T-AOC (U/mg prot)
0.01	0	7.28±0.57^{Ca}	11.88±1.50^{Ab}	104.63±5.10^{Ab}	20.96±1.83^{Ab}
	30	3.82±0.58^{Aa}	19.69±1.02^{Bb}	180.59±4.36^{Cb}	26.08±0.98^{Cb}
	60	5.88±0.30^{Ba}	21.06±0.76^{Cb}	188.76±8.49^{Db}	26.66±0.77^{Cb}
	180	10.49±1.23^{Da}	10.76±1.12^{Ab}	157.84±2.32^{Bb}	21.16±1.41^{Bb}
1.90	0	12.72±0.69^{Cb}	8.79±0.82^{Aa}	83.68±5.11^{Aa}	13.98±1.40^{Aa}
	30	5.28±0.50^{Ab}	17.70±1.37^{Ba}	162.81±7.06^{Ca}	22.46±0.64^{Ca}

Table 3. Continue

	60	8.21±0.97^{Bb}	20.14±0.22^{Ca}	173.28±4.33^{Da}	24.34±0.49^{Ca}
	180	15.81±0.51^{Db}	10.32±0.47^{Aa}	139.52±4.93^{Ba}	17.90±1.54^{Ba}
ANOVA	Cu	0.000	0.001	0.000	0.000
P value	Nitrite	0.000	0.000	0.000	0.000
	Cu × Nitrite	0.000	0.140	0.860	0.023

$^{A, B, C, D}$ For variable with a significant effect of dietary Cu ($P < 0.05$).

$^{a, b}$ For variable with a significant effect for ambient nitrite ($P < 0.05$).

(MDA) content in the prawns of high nitrite was significantly higher than low nitrite group in all diets ($P < 0.05$). MDA content in prawns supplemented with Cu of 30 mg/kg exhibited the lowest value, and successively increased in 60 mg/kg, 0 mg/ kg and 180 mg/kg groups regardless of nitrite levels ($P < 0.05$). Significant interactions were detected between dietary Cu and water nitrite levels on MDA content and T-AOC activity ($P < 0.05$).

DISCUSSION

In this study, high nitrite significantly reduced the growth performance of *M. nipponense*. Growth performance is commonly used as an indicator in chronic stress experiment for its sensitivity and credibility. (De Boeck *et al*., 1997). Literatures have showed that chronic nitrite stress could suppress the growth of *Gadus morhua* (Siikavuopio and Sæther; 2006), *M. amazonicum* (Hayd *et al*., 2014) and *Penaeus monodon* (Chen and Chen, 1992). It could be the reason that animal increased energy demand to maintain metabolism in response to environmental stresses (Iwama, 1998; Chen and Lai, 1992), and more nutrients were employed to energy expenditure, and resulted in the decrease of growth (Frances *et al*., 1998). Besides, nitrite stress can affect the efficiency of feed utilization and the intake of feed, leading to the reduction of growth (Koo *et al*., 2005). Survival rate of prawn was not significantly affected by nitrite exposure, which is in agreement with the findings in *P. monodon* (Chen and Chen, 1992), implying that prawns can tolerate higher nitrite concentration as 1.9 mg/L (Colt *et al*., 1981).

Prawns fed diets with Cu of 30 and 60 mg/kg gained better growth than 0 and 180 mg /kg groups in this study, showing that the requirement of dietary copper for *M. nipponense* would be around 30-60 mg/kg, but more studies should be conducted to determine the precise values. Similar result was found in Chinese mitten crab, crabs fed with Cu of 20.78-40.34 mg/kg gain better growth, dietary Cu deficit and excess decreased growth (Sun *et al.,* 2013).

Previous study showed that short-term nitrite stress increased oxygen free radicals in prawn (Wang *et al*., 2004), whereas free radicals can attack biomolecules, destroy protein and result to lipid peroxidation (Hermes-Lima, 2004). Malondialdehyde (MDA) is a final product of lipid peroxidation. It is commonly used as an indicator of endogenous oxidative damage. Normally, to reduce oxidative stress and maintain the poise state of free radicals, organisms have developed various antioxidant defenses, i.e. specialized antioxidant enzymes SOD to eliminate the negative effect of free radicals (Fattman *et al*., 2003; Nozik-Grayck *et al*., 2005). In the present study, high nitrite significantly increased MDA content of prawn. Similar result was seen in *Litopenaeus vannamei* (Liao *et al*., 2012). This illustrated that nitrite induce the unbalance of free radicals in prawn and resulted in oxidative stress. So the Cu-Zn SOD, GSH-Px and T-AOC activities in prawns of high nitrite group were lower than that prawns of low nitrite group. Wang *et al.* (2004) found that acute exposure of 4-13.3 mg/L nitrite also significantly decreased SOD, CAT and GSH-Px activities in prawn, on the contrary, acute exposure of 2 mg/L nitrite increased SOD activity. It is the reason that short-term nitrite exposure induced a phenomenon of stress-intoxication effect to SOD activity (Stebbing, 1982). Thus improve the ability of organism to cope with stress. The stress-intoxication effect phenomenon was diminished and enzyme activity gradually decreased with the time elapsing (Parihar *et al*., 1997; Hong *et al*., 2011). So the results of our present study and Wang *et al.* (2004) don't conflict with each other. Nitrite stress reduced the ability of scavenging free radical in prawn and induced oxidative stress.

Researchers have found that optimal dietary Cu significantly enhanced organism antioxidant ability, whereas Cu deficit or excess induced oxidative stress, decreased antioxidant ability (Wang *et al*., 2009; Shao *et al*., 2012). Similarly, in present study, Cu-Zn SOD, GSH-Px and T-AOC activities were significantly affected by dietary Cu level regardless of nitrite levels, supplement with Cu of 30-60 mg/kg significantly enhanced antioxidant ability of prawn. So our study showed that the positive effect of Cu to physiological function in prawn wan not suppressed by nitrite of 1.9 mg/L, fed with Cu of 30-60 mg/ kg effectively release the nitrite stress on prawn.

Overall, *M. nipponense* growth and antioxidant activity were affected by the dietary Cu concentrations and water nitrite levels. High nitrite resulted to a decrease in growth and antioxidant ability of prawn. Diets supplemented with Cu of 30-60 mg /kg could effectively reduce oxidative damage of nitrite stress on prawn, but excess dietary Cu would intensify this damage on prawn.

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REFERENCE

AOAC (1990). Official methods of analyses of association of analytical chemist (15th ed.). Washington, DC: AOAC.

Buege JA, Aust SD (1978). Microsomal lipid peroxidation. Methods in Enzymology 52: 302-310.

Chen JC, Chen SF (1992). Effects of nitrite on growth and molting of *Penaeus monodon* juveniles. Comparative Biochemistry and Physiology-Part C: Comparative & Pharmacology 101(3): 453-458.

Chen JC, Lai SH (1992). Oxygen consumption and ammonia-N excretion of *Penaeus japonicas* adolescents exposed to ambient ammonia. Comparative Biochemistry Physiology-Part C: Toxicology and Pharmacology 102: 129-133.

Colt J, Ludwig R, Tchobanoglous G, Cech JJ (1981). The effect of nitrite on the short-term growth and survival of channel catfish, *Ictalurus punctatus*. Aquaculture 24: 111-122.

Davis DA, Lawrence AL, Gatlin III D (1993). Dietary copper requirement of *Penaeus vannamei*. Nippon Suisan Gakkaishi 59: 117-122.

De Boeck G, Vlaeminck A, Blust R (1997). Effects of sub-lethal copper exposure on copper accumulation, food consumption, growth, energy stores, and nucleic acid content in common carp. Archives of Environmental Contamination and Toxicology 33: 415-422.

Elstner EF, Heupel A (1976). Inhibition of nitrite formation from hydroxyl-ammonium chloride: a simple assay for superoxide dismutase. Analytical Biochemistry 70: 616-620.

Fattman CL, Schaefer LM, Oury TD (2003) Extracellular superoxide dismutase in biology and medicine. Free Radical Biology & Medicine 35: 236-256.

Frances J, Allan GL, Nowak BF (1998). The effects of nitrite on the short-term growth of silver perch (*Bidyanus bidyanus*). Aquaculture 163: 63-72.

Gatlin III DM Wilson RP (1986). Dietary copper requirement of fingerling channel catfish. Aquaculture 54: 277-285.

Hayd LA, Lemos D, Valenti WC (2014). Effects of ambient nitrite on amazon river prawn, *Macrobrachium amazonicum*, larvae. Journal of the World Aquaculture Society 45(1): 55-64.

Hermes-Lima M (2004). Oxygen in Biology and Biochemistry: Role of Free Radicals. In Kenneth B. S. (Eds), Functional Metabolism: Regulation and Adaptation, John Wiley and Sons, Inc. 319-368.

Hill CH (1980). Influence of time of exposure to high levels of minerals on the susceptibility of chicks to *Salmonella gallinarum*. Journal of Nutrition 110: 433-436.

Hong ML, Chen LQ, Sun XJ, Li EC, Gu SZ, Yu N (2011). Effects of acute nitrite exposure on immunity indicators and HSP70 expression in Chinese mitten-hand crab (*Eriocheir sinensis*). Chinese Journal of Applied and Environmental Biology 17(5): 688-693.

Iwama G (1998). Stress in fish. Annuals of the New York Academy of Sciences 851: 304-310.

Jensen FB (2003). Nitrite disrupts multiple physiological functions in aquatic animals. Comparative Biochemistry and Physiology-Part A: Physiology 135: 9-24.

Knudsen PK, Jensen FB (1997). Recovery from nitrite-induced methaemoglobinaemia and potassium balance disturbances in carp. Fish Physiology and Biochemistry 16: 1-10.

Koo JG, Kim SG, Jee JH, Kim JM, Bai SC, Kang JC (2005). Effects of ammonia and nitrite on survival, growth and moulting in juvenile tiger crab, *Orithyia sinica* (Linnaeus). Aquaculture Research 36: 79-85.

Lall SP (2002). The minerals. In: Halver J.E., Hardy R.W., editors. Fish Nutrition. New York: Academic Press 259-308.

Lee MH, Shiau SY (2002). Dietary copper requirement of juvenile grass shrimp, *Penaeus monodon*, and effects on non-specific immune responses. Fish & Shellfish Immunology 13: 259–270.

Li EC, Yu N, Chen LQ, Zeng C, Liu LH, Qin JG (2010). Dietary Vitamin B_6 Requirement of the Pacific White Shrimp, *Litopenaeus vannamei*, at low salinity. Journal of World Aquaculture Society 41: 756-763.

Liao SA, Li Q, Wang AL, Xian JA, Chen XD, Gou NN, Zhang SP, Wang L, Xu XR (2012). Effect of nitrite on immunity of the white shrimp *Litopenaeus vannamei* at low temperture and low salinity. Ecotoxicology 21: 1603-1608.

Mallasen M, Valenti WC (2006). Effect of nitrite on larval development of giant river prawn *Macrobrachium rosenbergii*. Aquaculture 261: 1292-1298.

Mevel G, Chamroux G (1981). A study on the nitrification in the presence of prawns (*Penaeus japonicus*) in marine enclosed systems. Aquaculture 23: 29-43.

Nozik-Grayck E, Suliman HB, Piantadosi CA (2005). Extracellular superoxide dismutase. The International Journal of Biochemistry and Cell Biology 37: 2466-2471.

O'Dell BL (1976). Biochemistry of copper. Symposium on trace elements. Medical Clinics of North America 60: 697-703.

Parihar MS, Javeri T, Hemnani T, Dubey AK Prakash P (1997). Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the freshwater catfish (*Heteropneustes fossills*) to short-term elevated temperature. Journal of Thermal Biology 22(2): 151-156.

Romano N, Zeng CS (2009). Sub-chronic exposure to nitrite, potassium and their combination on survival, growth, total haemocyte count and gill structure of juvenile blue swimmer crabs, *Portunus pelagicus*. Ecotoxicology and Environmental Safety 72: 1287-1295.

Sedlak J, Linday RH (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellmanx's reagent. Analytical Biochemistry 25: 192 -205.

Shao XP, Liu WB, Lu KL, Xu WN, Zhang WW, Wang Y, Zhu J (2012). Effects of tribasic copper chloride on growth, copper status, antioxidant activities, immune responses and intestinal microflora of blunt snout bream (*Megalobrama amblycephala*) fed practical diets. Aquaculture 338-341: 154-159.

Shiau SY, Ning YC (2003). Estimating of dietary copper requirements for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Animal Science 77: 287-292.

Siikavuopio SI, Sæther BS (2006). Effects of chronic nitrite exposure on growth in juvenile Atlantic cod, *Gadus morhua*. Aquaculture 255: 351-356.

Stebbing ARD (1982). Hormesis-the stimulation of growth by low levels of inhibitors. Science of Total Environment 22(1): 213-234.

Sun SM, Chen M, Chen LQ, Jiang HB, Li EC (2011). Optimal dietary copper, zinc requirements for juvenile Chinese mitten crab, *Eriocheir sinensis*. The Israeli Journal of Aquaculture 62(3): 580-587.

Sun SM, Qin JG, Yu N, Ge XP, Jiang HB, Chen LQ (2013). Effect of dietary copper on the growth performance, non-specific immunity and resistance to *Aeromonas hydrophila* of juvenile Chinese mitten crab, *Eriocheir sinensis*. Fish & Shellfish Immunology 34: 1195-1201.

Tan XY, Luo Z, Liu X, Xie CX (2011). Dietary copper requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture Nutrition 17: 170-176.

Tang R, Liu H, Wang T, Huang K (2005). Mechanisms of selenium inhibition of cell apoptosis induced by oxysterols in rat vascular smooth muscle cells. Archives of Biochemistry and Biophysics 441: 16-24.

Teles AO (2012). Nutrition and health of aquaculture fish. Journal of Fish Diseases 35: 83-108.

Wang WF, Mai KS, Zhang WB, Ai QH, Yao CF, Li HT, Liufu ZG (2009). Effects of dietary copper on survival, growth and immune response of juvenile abalone, *Haliotis discus hannai* Ino. Aquaculture 297: 122-127.

Wang WN, Wang AL, Zhang YJ, Li ZH, Wang JX, Sun RY (2004) Effects of nitrite on lethal and immune response of *Macrobrachium nipponense*. Aquaculture 232: 679-686.

Watanabe T, Kiron V, Satoh S (1997). Trace minerals in fish nutrition. Aquaculture 151: 185-207.

Xian JA, Miao YT, Ye CX, Ye JM, Guo H, Zhang SP, Wang AL (2013). Effects of feed copper content on haemocyte of *Penaeus monodon* under nitrite stress. Journal of Hydroecology 34(5): 71-75.