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Effects of Different Levels of Copper Sulfate on Growth and Reproductive Performances in Guppy (P. *reticulate*)

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Abstract

Adult Guppies (Poecilia reticulate) were exposed to copper sulfate ($CuSO_4$ $5H_2O$) to evaluate the effects on growth, survival and reproduction performances. Total 480 individuals (mean age of 2.5-3 months) were employed in 5 experimental groups containing 16 fish per group and exposed to 4 sub-lethal levels of copper (0 as control, 0. 004, 0. 013, 0. 019 and 0. 026 mg $CuSO_4.l^{-1}$) for a period of 56 days. Control group had relative advantage than experimental ones at both growth and reproduction performances. As the copper concentration increased, Relative fecundity, gonadosomatic index, surviving rate, offspring production and feed conversion ratio decreased but specific growth rate increased significantly ($P<0.05$). Focused energy consumption in liver for Cu detoxification process and lack of energy for other physiological demands confirm low SGR and high FCR values in this study. It's obvious that copper has its toxic effects for guppy, even at lower concentrations than LC50 value (0. 46 mg $Cu.l^{-1}$)

Keywords: Guppy; Copper sulfate; Growth performance; Reproductive performance

Introduction

Copper (Cu) like other trace elements [zinc (Zn), iron (Fe), manganese (Mn), etc. serves important functions in living cells and is essential for fish [1]. Copper sulfate ($CuSO_4$) is the sulfated form of copper which routinely used as an algicide in commercial and recreational fish ponds. It is generally recognized that copper can be highly toxic to teleosts [2,3]. In aquaculture, copper is being used as eternal parasites, bacterial and fungal disease preventer and also weeds cleaning in sulfated form [4]. The ideal concentration of copper sulfate for weed termination has been suggested to be as much as $1mg.l^{-1}$ in which has lower poisonous effect on fish but highly affects the invertebrates [5]. According to the records of United States Environmental Protection Agency (EPA), copper sulfate is an ordinary compound which is being used broadly in aquaculture. Moreover, the Food and Drug Administration (FDA) has barred the medicinal use of copper in aquaculture.

It should be considered that copper could be accumulated in water body and gradually increases the concentration will raise to lethal concentration for fishes [4]. Toxicity of copper to aquatic species depends on factors such as organism sensitivity, concentration of copper and its bioavailability [6], total hardness, pH [7], organic particles or various other inorganic cations and anions [8]. So it is notable to avoid using copper in waters with lower alkalinity than 50 mg $CaCo_3.l^{-1}$. The range of copper sulfate is in aquaculture as much as 0.025-2 $mg.l^{-1}$ according to alkalinity and total hardness [9] but the usual effective use in aquaculture is reported as much as 0.01 of total alkalinity [10].

Guppy (P. *reticulata*) (or rainbow fish) is one of the most widely distributed tropical fish in the world. It is a member of the Poeciliidae family and, like all other members of the family, is live-bearing. Northeast South America is the native habitat for guppies but now, they could be found all around the world. High adaption ability makes them live in many different environmental and ecological conditions. Guppies exhibit sexual dimorphism and omnivorous feed habit (algal remains, diatoms, invertebrates, plant fragments, mineral particles, aquatic insect larva, etc.). Females produce offspring from first 10 weeks to 34 months of age, but first reproduction occur in 10-20 weeks of age. They are used as a model organism in the field of ecology, evolution, and behavioral studies [11]. Due to copper sulfate therapeutic trait which made it an ordinary drug for use in ornamental fish's hatcheries and personal aquariums despite its toxic effects and shortage of focused study on reproductive performance in guppy (P. *reticulate*), this study carried out.

Methods and Materials

This study performed at the aquatic laboratory of Shahid Fazli Bar Abadi located in Gorgan university of Agricultural Science and natural Resources, Golestan, Iran. Experimental fishes were bought from a local hatchery (Shast Kolah road, Gorgan province, Iran). Upon arrival, 480 individuals of 2.5-3 months aged guppies were acclimated to laboratory conditions for 2 weeks in a 1000 L round fiberglass tank measuring 1 m in diameter and fed a commercial diet (0.5-0.8 mm in size, Pars Kilka Corp, Babolsar, Iran) as much as 3% of body weight twice a day with two equal meals at 0800 and 1700 h. The experiment was conducted in a completely randomized design with six replications per treatment for 56 days. At the beginning of the experiment, 16 fish (N: 12 female and 4 male; mean length: 3.59 ± 0.11 cm; mean weight: 0.36 ± 0.01 g) were stocked in each aquarium (50×35×30 cm) designed to contain 35 L tap water (pH: 7.4 ± 0.12; salinity; 0.35 ± 0.12 ppt; DO: 7.36 ± 0.98 $mg.l^{-1}$; total hardness: 270-300 $mg.l^{-1}CaCO_3$) and equipped with airstones to maintain dissolved oxygen levels as much as possible. The water temperature was kept 28 ± 1°C by electrical heaters. Water quality variables were checked daily. Handmade plastic Happas were

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put in aquariums to act as shelters for new born offsprings from predation by parents.

In this study, five levels - one as blank and four as experimental groups - of copper sulfate penta-hydrate ($CuSO_4$ $5H_2O$, Merck, Germany) concentrations including 0 (as control), 0.004, 0.013, 0.019 and 0.026 mg $CuSO_4.l^{-1}$. Prior to start the trial, the mean 96 h LC50 in guppies determined as much as 0.046 mg $Cu.l^{-1}$ then, four concentrations were selected randomly. According to each level of copper sulfate, selected concentration prepared [12] into four reserved tanks to renew the replaced water volume. All experimental groups have been monitored daily by atomic absorption [13] to ensure the determined concentrations achieved [14].

Acute median lethal concentration (LC50) and their 95% confidence limits for all tests obtained by Finney's method were calculated with the formula of Mohapatra and Rengarajan. LC50 values of 24, 48, 72 and 96h were determined using Finney's method of probit analysis" and with SPSS computer statistical software. The mean lethal concentration LC50 for an exposure period of 24, 48, 72 and 96 hr was designated by trial and error. Amounts of LC1, LC10, LC30, LC50, LC70, LC90, LC99 were calculated by probit tables, mortality and probit regression.

At the end of the trial, percentage of body Specific Growth Rate (SGR) [ln(final weight)-ln(initial weight)/experimental period)]×100, Feed Conversion Ratio (FCR) [dry feed consumed (g)/gain in wet weight (g) [15], relative fecundity (RF) [absolute fecundity/body weight] [16], Gonadosomatic Index (GSI) [ovary weight/body weight]×100 [17], Surviving Rate (SR) [final fish number/initial fish number]×100 [18] and Offspring Production (OP) [new born offspring/number of adult females] calculated.

All data were reported as mean ± standard deviation. Statistics were performed by using one-way analysis of variance (ANOVA) followed by Duncan multiple comparisons test if significant differences were found. A Kolmogrov–Smirnov test was used to assess normality of distribution and abnormal data were log transformed. Significance was set at $P<0.05$ level.

Results and Discussion

Liver, brain, heart, kidney, and muscle are the main storing places for copper; in tissues and blood cells, it bounds to proteins, including many enzymes. It exerts a wide range of physiological effects on vital), and other hematopoietic tissues in which threats the present fishes in area [19]. Copper sulfate toxicity differs among fish species [20]; For instance, the amounts of 8, 8.97 and 16 mg $Cu.l^{-1}$ reported to have negative effects on tilapia (*Oreochromis niloticus* × *O-aureus*), grupper (*Epinephelus malabaricus*) and channel catfish (*Ictalurus punctatus*) respectively [21,22].

In 8 weeks, toxic effects of copper sulfate exposure were recorded (Table 1). As seen, the best growth performance resulted in control group (0 mg $CuSO_4.l^{-1}$) and growth diminished as copper sulfate increased.

Despite the same initial weight, age and feed consumption, rising amounts of copper had negative effects on weight gain, specific growth rate and feed conversion ratio (Figure 1). There are hypothesis for reduction in growth performance including:

1) Intestine tissue interruption [23]: Heavy metals have their special recipients in which act as a stimulator to them. Intestine can be considered as a target tissue for metals. Copper which enter into the intestine can damage the tissue and by means of that, reduces the nutrition intake from intestine. These researchers found out that, the existence of copper sulfate around catfishes reduced their growth performance due to interruption in intestine tissue. Furthermore, copper sulfate decreases the Zn [24] and Se [25] intake from intestine.

2) High energy consumption for copper detoxification in liver [26] reported that, in freshwater prawn (*Macrobrachium rosenbergii*) copper usually transported by the haemolymph to other organs primarily hepatopancreas for storage and detoxification. Ignoring the physiological differences, at the same polluted condition (Here by copper sulfate) guppies transfer copper to liver for detoxification and for fulfilling the detoxification process, body needs extra energy which is provided by more food consumption. Lack of energy for other metabolic processes result in low growth performance.

3) Chronic stress [27-29]: Chronic stress reduced the growth too [30]. The increase in blood glucose and cortisol concentrations is known as a general secondary response to stress of fish to toxic effects [31]. Griffin et al. [32] reported that, copper sulfate raised blood cortisol level (as stressor factor) in channel catfish (I. *punctatus*). These researches showed that the initial signs of stress (cortisol level) decreased consequently as copper sulfate diminished in water. At present study, a combination of three mentioned hypothesis is reliable reason for low growth performance. These results were in accordance with Berntssen et al. [33] on Atlantic salmon (*Salmo salar*), [34] on channel catfish (I. *Punctatus*). These researchers noted that increase in copper sulfate lead to low growth in fish.

Copper sulfate also had negative effects on reproduction performance (Table 1). In sensitive species of teleosts, copper adversely affects reproduction and survival from 0.01-0.02 mg $Cu.l^{-1}$. At present study, surviving rate, gonadosomatic index, relative fecundity and finally offspring production decreased by increase in copper sulfate levels ($P<0.05$) and best performance of mentioned factors resulted in control group (with no copper sulfate). Although relative fecundity between first two treatments (0 and 0.004 $CuSO_4.l^{-1}$) were not analytically significant; but it would be due to rare concentration of experimental treatment (0.004 $CuSO_4.l^{-1}$). Copper may affect reproductive success of fish through disruption of hatch coordination with food availability or through adverse effects on larval fishes. Chronic exposure of representative species of teleosts to low concentrations (0.005 to 0.04 $mg.l^{-1}$) of copper in water containing low concentrations of organic material adversely affects survival and spawning. Dethloff et al. reported that cortisol is released to the blood via stimulation of the Hypathalamo-Pituitary-Adrenal (HPI) axis by heavy metal exposure. Cortisol has depressive effects on a number of immune responses in fish, including phagocytosis and lymphocyte mitogenesis [35]. Despite the valuable role of HPI hormones in reproductive processes of fishes, cortisol hormone which made in response to stress resistance in fish would interrupt HPI hormones. It will be probably the reason for lowering slope gonadosomatic index in response of rise in copper sulfate concentrations.

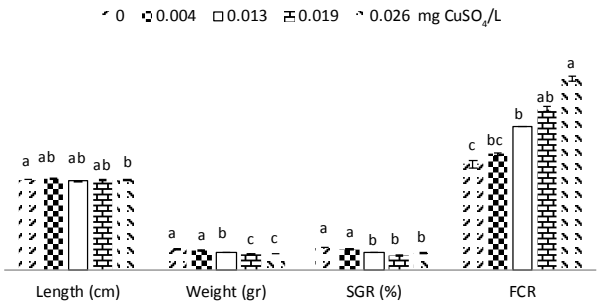
Pulsford et al. [36] showed that, cortisol restricts the metabolic activities of macrophage and lymphocyte cells which especially spread in kidney and spleen; on one side, negative effects of copper on intestine inhibited absorption of nutrients like electrolytes and fatty acid intake [37]; on the other side, inducing hypertrophy in gill cells [38], blocking calcium transport in gills through interference with chloride cells and ionic and gas exchanges [39] make fishes weak to stand with situation. That's why low survival rate appears in high doses of copper sulfate.

Parameters *	Copper sulfate concentrations (mg $CuSO_4$. l^{-1})				
	0 (Control)	0.004	0.013	0.019	0.026
Initial length (cm)	3.61 ± 0.034^{a}	3.58 ± 0.026^{a}	3.57 ± 0.014^{a}	3.6 ± 0.031^{a}	3.59 ± 0.04^{a}
Final length (cm)	3.76 ± 0.02^{a}	3.71 ± 0.037^{ab}	3.65 ± 0.017^{ab}	3.7 ± 0.026^{ab}	3.6 ± 0.01^{b}
Initial weight (gr)	0.36 ± 0.005^{a}	0.36 ± 0.017^{a}	0.37 ± 0.011^{a}	0.37 ± 0.023^{a}	0.36 ± 0.011^{a}
Final weight (gr)	0.82 ± 0.023^{a}	0.78 ± 0.023^{a}	0.72 ± 0.011^{b}	0.63 ± 0.005^{c}	0.65 ± 0.003^{c}
Specific growth rate	0.91 ± 0.013^{a}	0.86 ± 0.02^{a}	0.71 ± 0.015^{b}	0.69 ± 0.041^{b}	0.59 ± 0.018^{b}
Feed conversion ratio	4.36 ± 0.16^{c}	4.76 ± 0.065^{bc}	5.88 ± 0.00^{b}	6.62 ± 0.31^{ab}	7.88 ± 0.89^{a}
Relative fecundity	56.82 ± 1.82^{a}	51.42 ± 3.52^{a}	32.45 ± 1.63^{b}	34.89 ± 1.51^{b}	25.57 ± 1.33^{b}
Gonadosomatic index	6.1 ± 0.115^{a}	5.61 ± 0.034^{b}	5.32 ± 0.005^{c}	5.29 ± 0.023^{c}	5.31 ± 0.011^{c}
Surviving rate	88.88 ± 2.77^{a}	55.55 ± 7.34^{b}	52.77 ± 7.34^{b}	30.55 ± 10.01^{c}	27.77 ± 2.77^{c}
Offspring production	3.88 ± 0.22^{a}	3.33 ± 0.17^{b}	1.94 ± 0.07^{c}	1.83 ± 0.096^{c}	1.55 ± 0.07^{c}

exposure to several concentrations of Cooper

*Mean ± SD values with different superscript letters within a raw for a parameter are significantly different (P<0.05).

Table 1: The mean results of growth and reproduction performances of guppy (*P. reticulate*) in response of



*Different superscript letters within a raw for a parameter are significantly different (P<0.05).

Figure 1: The mean results of growth performance of guppy (*P. reticulate*) in response of exposure to several concentrations of Cooper during 56 days of trial

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