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PHYSIOLOGICAL ASPECTS IN NILE TILAPIA UNDER EFFECT OF COPPER SULFATE TOXICITY

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Abstract

In this study, Nile tilapia, *Oreochromis niloticus* (L.), (2 to 3g) was distributed into the four groups, control and three treated groups with copper sulfate at concentrations 5, 10 and 15 ml mol/L, according to design of experiment to four groups each group three replicate (control group) was contain 10 fish /100 L. and three treated groups with copper sulfate at concentrations 5, 10 and 15 ml mol/L for three months respectively. At the end of the experimental trial, blood samples were taken to determine the different physiological aspects. The growth parameters were affected by copper sulfate at concentrations 5, 10 and 15 ml mol/L. The high growth performance (WG. SGR and RGR) of tilapia, subject at control group and group exposed to copper sulfate at concentration 5 ml mol/L, respectively than the groups exposed to copper sulfate at concentration 10 and 15 ml mol/L, respectively. The best feed conversion ratio was obtained with control plus exposed to copper sulfate at concentration 5 ml mol/L, respectively. Also The highest values of protein efficiency ratio and protein productive were obtained with control plus exposed to copper sulfate at concentration 5 ml mol/L, respectively. The physiological aspects including erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct) were significantly affected by exposed to copper sulfate at concentrations than control one. The overall results presented here indicate that the best growth performance of Nile tilapia was obtained at control fish.

Key words: *Oreochromis niloticus*; Growth parameters; Copper sulfate

Introduction

Nile tilapia are the source of protein for many people throughout the world and fish consumption has increased in importance among health-conscious people because it provides healthy and low cholesterol, sources of protein and other nutrients (Burger and Gechfeld, 2005; Agusa *et al.*, 2005). Aquaculture is the most developed sector in terms of the supply of high- quality food products that meet human nutritional requirements (Ottinger *et al.*, 2016). Pervasive population growth has led to increased demand for fish as food, which has affected wild fishery stocks and altered coastal environments, causing water pollution and habitat deterioration (Yoo and Bai, 2014) The expansion in aquaculture practices accompanied by intensification of aquaculture caused pollution that is directly linked to the elevated levels of fish stressors, both environmental and physiological. Tilapias are the world's second most important fish species for aquaculture after the carp and this is due to their high growth rates, being prolific breeders, completing their life cycle in captivity, tolerance to environmental stress and high market demand (El-Sayed, 2006.). The effect of population is usually seen to be either density dependent or density independent. They suggested that stocking density that negatively affects fish growth is density dependent. Stocking density is an important parameter in fish culture as the health, growth and survival of fish depend upon this factor. Stocking density is one of the most important factors in aquaculture because it directly influences Growth, survival behavior, health, feeding and production of fish under farmed conditions Rahman and Rahman , 2003) Water pollution is one of the most common issues encountered around the world in aquaculture by nitrogenous compounds on. The major hazardous nitrogen metabolic product in aquaculture is ammonia, constituting about 70% of nitrogenous waste excretion in fish ((Shokr, E. A. M., 2019). Due to those conceivable impacts on fish health and survival, ammonia accumulation is of specific concern in aquaculture (Evans *et al.*, 2006). Ammonia is the end product of the protein catabolism by living organisms, Total Ammonia Nitrogen (TAN) is a total result of NH3 (non-ionized) and NH4+ (ionized). Only the NH3 is considered the TAN's most toxic form as it excreted freely through fish gills (Silva *et al*., 2013). Besides affecting land usability that subsequently impacting on profitability of an aquaculture venture, stocking density is believed to affect growth performance and survival of fish species stocked. (Ugando *et al*., 2014). Aquaculture is the main source of fish production in Egypt and it contributes about 61% of the total production (GAFRD, 2006). One of the main targets of the Egyptian government is to compensate the deficiency in meat production by increasing high quality fish production. Nile tilapia is by far the most important farmed tilapia species in the world. Tilapia is the most familiar and popular fishes in Egypt, as well as, in the Middle East and warm climate countries. Fish production should be increased in Egypt to meet the demand of the increasing population. Several problems face fish production in Egypt. Among these problems are the mortality of fish due to low water quality because of pollution with heavy metal. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems (Censi *et al.,* 2006 and Shokr, E. A. M.., 2020). Fish cannot escape from the detrimental effects of these pollutants. The Cu is obligatory and monitoring of others is suggested. Heavy metals are regarded as serious pollutants in the aquatic environment because of their environmental persistence and tendency to concentrate in aquatic organisms (Shokr, 2015 and 2020). High concentrations of heavy metals in water, sediments, and organisms may result in serious ecological consequences. Most heavy metals released into the environment enter the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rain (Shokr, 2015 and 2020). Therefore, aquatic animals are often exposed to elevated levelsof heavy metals (Kalay and Canlı, 2000; and Farkas *et al.,* 2001). Heavy metals may be directly absorbed by organisms but are also transferred from lower to higher trophic levels of the food chain. The high accumulation of heavy metals in these components can result in serious ecological changes. The studies carried out on various fishes have shown that these metals alter the physiological activities and biochemical parameters both in tissues and blood (Basa and Rani, 2003). The toxic effects of heavy metals have been reviewed, including their bioaccumulation by several workers (Rani , 2000; Waqar, 2006 and Shokr, 2020). Copper compounds are commonly used to treat some of fish diseases in addition to its role for maintaining the normal biological activity of many enzymes. Copper was found as a trace metal in the natural water at a very low concentration. High levels of copper in the water are toxic to fish. Lead, the most toxic metal, is detectable in practically all phases of the inert environment and all biological systems, because it is toxic to most living things at high exposure levels (Shokr, 2020). Lead is non- essential element and it is a bone-seeking element, it is processed along with calcium because of its chemical resemblance to calcium. However, tissues other than bone are considered to be storage sites for lead in fish (Shokr, 2015).

Therefore, the main objectives of the present study was to study the effect of heavy metals on the growth performance and feed utilization of Nile tilapia (*Oreochromis niloticus*). Also, to investigate the effect of heavy metals on the carcass chemical composition of fish body and on the residues in different parts of fish body.

Materials and Methods

This study was carried out Central Laboratory for Aquaculture Research, Abbassa , Abo-Hammed, Sharkia, Egypt. 2-3 g Nile tilapia were acclimated in laboratory conditions for 2 weeks before the begging of the experimental work. Fish were distributed in twelve glass aquaria of about 100-liter capacity each and stocked at a rate of 10 fish/aquarium. The glass aquaria were supplied with dechlorinated tap water and continuous aeration was adapted by using an air pump and air stones. Average water temperature was maintained at 27± 2 C. The aquaria were divided into 4 groups with three replicates per group. The 1st group (T1) was kept as control have10 fish/ aquarium and three treated groups with copper sulfate at concentrations 5, 10 and 15 ml mol/L respectively. These groups are illustrated in Tables (1). Fish of the experimental groups were fed on a pelleted fish diet containing 32 % CP. (Table 2) and the diet was fed at a rate of 3 % of live body weight twice daily for 90 days. Semi–dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water.

Measurements of fish growth:

At the end of the experimental period, the following growth and feed utilization indices were calculated: weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR), feed efficiency (FE) and protein efficiency ratio (PER) using the following formulae: according to Jauncey and Rose (1982).

WG = Final average weight (g) − initial average weight (g); SGR (% d – 1) = 100 × (ln Wt − ln W0)/t

Where Wt and W0 represent final and initial body weights of fish, respectively, and t represents the duration of the feeding trial;

FCR = Dry weight of feed (g) / wet weight gain by fish (g); PER = weight gain by fish (g) / protein intake (g) ,RGR= weight gain/ initial weight , FER=weight gain / feed intake *100 and PPV= relative protein/ protein intake*100

Where protein intake (g) = Protein (%) in feed × total weight (g) of diet consumed /100

Table 1. Design of the experimental.

Treatment	copper sulfate	Stoking density
control	0 ml mol/L	10 fish X 3 aquaria
Group1	5 ml mol/L	10 fish X 3 aquaria
Group2	10 ml mol/L	10 fish X 3 aquaria
Group3	15 ml mol/L	10 fish X 3 aquaria

Table 2. Chemical analysis of commercial diets, used in the experiment (on dry matter basis).

Item	%
Dry matter (DM %)	93.12
Crud protein (CP %)	29.65
Ether extract (EE %)	6.23
Crud fiber (CF %)	6.67
Ash	12.12
NFE	45.33
GE Kcal / 100g	412.7
P / E ratio	71.84

Physiological Analyses:

Blood samples were taken from the caudal vein of no anaesthetized fish by sterile syringe containing EDTA as an anticoagulant. Erythrocyte count according to Dacie and Lewis (1984), hemoglobin content according to Van kampen (1961) and hematocrit value according to Britton (1963) were detected.

Plasma was obtained by centrifugation of the blood at 3000 rpm for 15 min and the non haemolyzed plasma was stored in a deep freezer at -20 ^{0}C till analysis. Treatment of nile tilapia that exposed to copper for 90 days by sodium bicarbonate at dose 10 ml mol NaHCO3.

Statistical analysis:

One way analysis of variance (ANOVA) was conducted to test the effect of copper sulfate on fry Nile tilapia during different stoking density. This analysis was done using the computer program SPSS and least Significant difference (LSD) post hoc were done to determine significant differences (Tamhane and Dunlop, 2000).

RESULTS

Growth performance and feed utilization:

The effect of copper sulfate at concentrations 5, 10 and 15 ml mol/L respectively in water at fish to reduction growth (Table 3). Results of growth performance are summarized in Table (3) after 90 days of raised fish treatment with copper sulfate at concentrations 5, 10 and 15 ml mol/L had a low significantly in final weight, weight gain, relative growth rate and specific growth rate than fish at control one. There were lower significantly in all parameter of growth performance of fish exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L. Moreover, WG, ADG and SGR improved significantly with control one.

The results in Table (4) shows that feed conversation rate (FCR) was decreased significantly in *O. niloticus* exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively when compared to the control group and significantly different ($P < 0.05$) from other treatments. However, in same Table (4) indicated that protein efficiency ratio (PER), feed efficiency ratio (FER) and protein production value (PPV) decreased in fish treated with exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L

when compared to the control. FCR decreased with exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L. FER and PER are used to assess protein utilization and turnover.

Table 3. Growth performance of Nile tilapia (*O.niloticus*) exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L

Growth Parameters	Control	5 ml mol / L copper	10 ml mol / L copper	15 ml mol /L copper
Initial weight (g)	3.123± 0.009[a]	3.12± 0.26[a]	3.09± 0.018[a]	3.11± 0.009[a]
Final weight (g)	19.07±0.236[a]	19.01±0.222[a]	17.72± 0.104[b]	14.34±0.024[c]
Weight gain (g)	15.95± 0.244[a]	15.89± 0.142[a]	14.63± 0.104[b]	11.23± 0.025[c]
RGR	5.11± 0.092[a]	5.09± 0.08[a]	4.72± 0.04[b]	3.62± 0.015[c]
SGR (%/day)	3.23± 0.027[a]	3.23± 0.229[a]	3.12± 0.013[b]	2.73± 0.006[c]
Survival rate (%)	100.00± 0.00[a]	100.00± 0.00[a]	93.00± 3.33[a]	83.33± 3.33[b]

The same letter in the same column is not significantly different at $P<0.05$

Table 4. Feed intake, feed conversion ratio (FCR) , feed efficiency ratio (FER), protein efficiency ratio and protein production value (PPV) in Nile tilapia exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L

Items	**Treatment (**copper sulfate**)**			
	0 ml mol/L	5 ml mol/L	10 ml mol/L	15 ml mol/L
Feed Intake	37.73± 2.31[a]	34.19± 1.162[a]	37.19± 0.329[a]	35.72± 1.652[a]
FC	1.748± 0.055[a]	1.607± 0.017[a]	1.737± 0.050[a]	1.787± 0.800[a]
FER	8.312± 0.358[d]	22.07± 0.478[c]	28.871± 0.731[b]	29.39± 2.265[a]
PER	3.73± 2.31[a]	3.19± 1.162[a]	3.19± 0.329[a]	3.72± 1.652[a]
PPV	1.48± 0.055[a]	1.07± 0.017[a]	1.3± 0.050[a]	1.1± 0.800[a]

The same letter in the same row is not significantly different at $P<0.05$

In the present study, the results in Table (5) showed that protein content in fish body was significantly higher in fish exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L, while the protein content at control group decreased. Contrarily, total lipid content in fish body was decreased significantly in fish exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L, while increased at control ones. Ash content was higher significantly in fish control and compared to groups that exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L (Table 5).

Table 5. Chemical analysis of the experiment fish at the end of the experimental period (on dry matter basis) in fish exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L.

Items	**Treatment (**copper sulfate**)**			
	0 ml mol/L	5 ml mol/L	10 ml mol/L	15 ml mol/L
DM	23.5± 0.21 [bc]	25.03± 0.21 [a]	24.14± 0.11[b]	23.42± 0.1[6c]
CP	57.83± 0.07[b]	58.46± 0.15[a]	57.49±0.14[bc]	57.10± 0.12[d]
EE	21.56± 0.19[a]	20.24± 0.06[d]	21.36±0.09[ab]	21.07± 0.12[bc]
ASH	20.63± 0.26[c]	21.26±0.19[bc]	21.17±0.23[bc]	21.79± 0.22[b]

The same letter in the same row is not significantly different at $P<0.05$

Hematological parameters:

The results of erythrocyte count and hemoglobin content, hematocrit value obtained from fish exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L are given in (Table 6). This Table shows that the in fish exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L caused decreased significantly erythrocyte, Hb and Hct) in all blood parameter examined respectively as compared of the control one. In this study, the RBC and WBC counts, which are indicators of hematopoiesis, showed that yucca extract supplementation in water greatly enhanced the blood cell count along with the Hct percentage.

Table 6. Changes in erythrocyte (count x $10^6/mm^3$), hemoglobin content (g/100ml) and hematocrit value (%) in the blood of Nile tilapia (*O. niloticus*) exposed copper sulfate at concentrations 5, 10 and 15 ml mol/L

Items	**Treatment (**copper sulfate**)**			
	0 ml mol/L	5 ml mol/L	10 ml mol/L	15 ml mol/L
Erythrocyte count (RBCs)	2.07± 0.030 [b]	2.37± 0.044 [a]	2.27± 0.044 [b]	1.99± 0.020 [bc]
Hemoglobin (Hb)	7.93± 0.12[a]	7.83± 0.05[a]	7.04± 0.07[b]	6.98± 0.08 [b]
hematocrit value	26.32± 0.100[c]	27.00± 0.09[b]	27.79± 0.09[a]	27.85± 0.17 [a]

The same letter in the same row is not significantly different at P<0.05

The results of challenge test (Table 7) revealed that mortality rate was decreased in fish that exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L and treated with 10 ml mol NaCO3 at different doses respectively with copper sulfate. The mortality rate of control group was 0%. the addition of 10 ml mol NaCO3 at different doses respectively with copper sulfate in water showed the decreased mortality rate of *O. nilotiucus* at 5ml mol copper sulfate than 10 and 15 ml mol/L copper sulfate as shown in Table 7

Table 7. Challenge test of 10 ml mol NaCO3 and pattern of mortality among *O. niloticus*) exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L.

Items	**Treatment (**copper sulfate**)**			
	0 ml mol/L	5 ml mol/L	10 ml mol/L	15 ml mol/L
No treated fish	30	30	30	30
Mortality No.	0	3	6	8
Treatment NaHCO3	10 ml mol NaHCO3	10 ml mol NaHCO3	10 ml mol NaHCO3	10 ml mol NaHCO3
No treated fish	30	27	24	22
Mortality No.	0	0	0	0

DISCUSSION

Water pollution is one of the most common issues encountered around the world in aquaculture by nitrogenous compounds. The major hazardous nitrogen metabolic product in aquaculture is ammonia, constituting about 70% of nitrogenous waste excretion in fish (Benli and KBksal, 2005). The effect of copper sulfate at concentrations 5, 10 and 15 ml mol/L on fish to reduction of growth compared with control group It is represented as significant decrease when compared to the control. Results of growth performance are raised in control fish than treatment with copper sulfate at concentrations 5, 10 and 15 ml mol/L. there are a high significantly in final weight, weight gain, relative growth rate and specific growth rate in control fish than fish that exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L. There were lower significantly in all parameter of growth performance of fish exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L. Moreover, WG, ADG and SGR improved significantly with control fish. Fish growth and feed utilization were significantly retarded herein with increasing the concentrations of copper. It has been demonstrated that rearing fish at high concentrations of copper may reduce their growth due to factors such as social interaction. and the deterioration of water quality, which can affect the feed utilization by fish (Ellis *et al*., 2002). These results are in agreement with that obtained by Zannatul (2014) who indicated that stocking density had a significant effect on growth and survival rates of monosex tilapia.

Fry held at the highest density exhibited lowest growth and survival rates also, Costa (2017) showed that the increase in stocking density caused a decrease in the final weight of fish, weight gain, daily weight gain, standard length and survival, as well as an increase in feed conversion. or the deterioration of water quality (Ellis *et al*., 2002) or decreased food consumption. Reductions in growth rate and food conversion efficiency of fish reared at high stocking density are attributed to an alteration in metabolism due to physiological stress (Lupatsch *et al*., 2010). The results showed that feed conversation rate (FCR) was decreased significantly in *O. niloticus* exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively when compared to the control group. However, results indicated that protein efficiency ratio (PER), feed efficiency ratio (FER) and protein production value (PPV) decreased in fish exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively when compared to the control group.. FCR decreased with increasing concentrations. FER and PER are used to assess protein utilization and turnover. These results are in agreement with that obtained by El-saidy and gaber (2004) showed that the best feed conversion ratio (FCR) was achieved with Y750 fed groups. The protein efficiency ratio (PER) and feed efficiency ratio (FER) of the Y750 and Y1000 fed groups were significantly ($P < 0.05$) higher than that of the control group. Also, Capeng *et al*. (2012) declines in serum concentrations of total T3 and free TH, as well as the reduction in food consumption coupled with the increase in FCR, caused by high stocking density may have contributed to the growth inhibition or due to increased of ammonia toxicity (Shokr, E. A. M., 2015 and 2020). In the present study , the results showed that protein content in fish body was significantly higher in control fish than exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively.Contrarily, total lipid content in fish body was decreased significantly in fish exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively when compared to the control group.. Ash content was higher significantly in control fish than exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively. These results are in agreement with that obtained by El-saidy and gaber (2004) indicated hat proximate composition of whole body moisture, protein, and lipid and ash contents was significantly influenced by adding Yucca with different density.

Hematological parameters:

The results of erythrocyte count and hemoglobin content, hematocrit value obtained from fish exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively caused decreased significantly when compared to the control group. In this study, the RBC and WBC counts, which are indicators of hematopoiesis, showed that exposed to copper sulfate at concentrations 5, 10 and 15 ml mol/L, respectively caused decreased significantly in the blood cell count along with the Hct percentage. Similar results were observed in previous studies by (Shokr, E. A. M., 2020 and Güroy *et al.* 2014) reported that the growth and hematological responses of striped catfish juveniles (*P. hypophthalmus*) and Nile tilapia were greatly inhibited by copper effect. The improved hematological and immune responses of D. labrax juveniles are consistent with the superior growth performance observed in the present study at control group. Many studies have shown that exposed to copper caused significantly decreased the growth proportion of channel catfish juveniles (*Ictalurus punctatus*) and *O. Niloticus* (Shokr, 2020 and Kelly and Kohler, 2003). In vertebrates including fish, blood is the most frequently examined tissue in efforts to establish their health status or physiological status. Accordingly, health status such as oxygen carrying capacity has been directly determined by reference to main hematological indices including red blood cell (RBC), hemoglobin concentration(Hb), percentage of blood volume consisting of red cells and hematocrit (Hct) (Houston, 1990).

Conclusion

The present study provides further evidence that copper has a potentially deleterious effect on the fish organisms and is able to cause changes in biochemical and hematological aspects and enzymes of antioxidanta

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الجوانب الفسيولوجية في البلطي النيلي تحت تأثير سمية كبريتات النحاس

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الملخص العربي

في هذه الدراسة، تم توزيع البلطي النيلي 2 إلى ٣ جم على أربع مجموعات، مجموعة تحكم وثلاث مجموعات معالجة بكبريتات النحاس بتركيزات ٥ و ١٠ و ١٥ مل مول / لتر ، وفقًا لتصميم التجربة لأربع مجموعات كل مجموعة ثلاث مكررات (المجموعة الضابطة) تحتوي على ١٠ سمكة / ١٠٠ لتر بينما المجموعة الثانية والمجموعة الثالثة والمجموعة الرابعة واحدة بنفس الكثافة و تتعرض لكبريتات النحاس بتركيزات ٥ و ١٠ و ١٥ مل مول / لتر لثلاثة أشهر على التوالي. في نهاية التجربة ، تم أخذ عينات الدم لتحديد المتغيرات الفسيولوجية المختلفة. تأثرت معلمات النمو بكبريتات النحاس بتركيزات ٥ و ١٠ و ١٥ مل مول / لتر. أداء النمو المرتفع (WG. SGR و RGR) للبلطي ، الخاضع للمجموعة الضابطة والمجموعة المعرضة لكبريتات النحاس بتركيز ٥ مل مول / لتر ، على التوالي من المجموعات المعرضة لكبريتات النحاس بتركيز ١٠ و ١٥ مل مول / لتر ، على التوالي. تم الحصول على أفضل نسبة تحويل تغذية مع التحكم بالإضافة إلى تعرضه لكبريتات النحاس بتركيز ٥ مل مول / لتر على التوالي. كما تم الحصول على أعلى قيم لنسبة كفاءة البروتين وإنتاج البروتين مع التحكم بالإضافة إلى تعرضهم لكبريتات النحاس بتركيز ٥ مل مول / لتر على التوالي. تأثرت المتغيرات الفسيولوجية بما في ذلك عدد كريات الدم الحمراء (RBCs) ومحتوى الهيموجلوبين (Hb) وقيمة الهيماتوكريت (Hct) بشكل كبير بالتعرض لكبريتات النحاس بتركيزات أكثر من السيطرة على واحد. تشير النتائج الإجمالية المعروضة هنا إلى أن أفضل أداء لنمو البلطي تم الحصول عليه في أسماك التحكم.