Growth Performance of Cultured African Catfish (C. Gariepinus) Fingerlings in the Presence of Nano and Macro CuO Feed Supplements

U C Onuegbu¹, A Agarwal² and N B Singh¹*

¹Research and Technology Development Center, Sharda University, Greater Noida, India
²Department of Chemistry, School of Basic Sciences & Research, Sharda University, Greater Noida, India

Received 02 January 2018; revised 15 June 2018; accepted 14 July 2018

Nanomaterials are having transformative effects on the world of science including animal feeds. The present study investigated the dietary effects of copper oxide nanoparticles on the growth and general health profiles of African catfish (Clarias gariepinus) fingerlings. Conventional catfish feed (initial copper content 0.83 mg Kg⁻¹) was supplemented with nano (n) and macro forms of CuO at levels of 0, 1, 3, 5, 7 and 10 mg Kg⁻¹ feed respectively. Fish were fed the diets for 7 weeks, after which significant (p < 0.05) weight differences were observed in fish fed 3-10 mg nCuO Kg⁻¹ feed and those fed 5-10 mg CuO Kg⁻¹ feed respectively. Highest percent weight gains attained were 620.20% and 491.63% at the supplementation level of 10 mg (nCuO & CuO) Kg⁻¹ feed respectively, against 322.55±3.19% for the control, suggesting that nCuO was more effective in impacting the health of C. gariepinus than CuO.

Keywords: Nanomaterial, Copper Oxide, Gariepinus, Transformation, African Catfish

Introduction

Minerals are very essential in the normal physiological functions in animals but they can also produce adverse effects if desirable limits of exposure exceeds. Copper (Cu), as an essential trace element in fish, plays big role in the biological processes of hemoglobin synthesis, bone formation, nervous system myelin maintenance and as primary component of enzymes like cytochrome oxidase¹, and is involved in oxidation reduction reaction². Dietary Cu enhances growth performance and immunological functions in some fishes, while both its deficiency and overload have serious health consequences, including death³. Dietary Cu supplementation in conventional animal feeds is important because these feeds fail to meet animal requirements. Cu nanoparticles (Cu-NPs), due to their antimicrobial and other properties, are among several other NPs which are recently being used in biological sciences and engineering⁴. This study investigates the effects of nano and macro CuO on the growth performance of African catfish, a major cultured fish species in African, and for which there is little or no such information.

Materials and methods

Materials

Copper oxide, CuO (molar mass: 79.545 g mol⁻¹: Density: 6.31 g cm⁻³), Copper sulphate CuSO4.5H2O (MW: 249.68: Density: 2.29 g cm⁻³) and Ammonia solution (SG: 0.088, MW: 1703) were taken from chemical shops from Onitsha International market, in Anambra state, African catfish (C. gariepinus) fingerlings with average weight 5.07 ± 0.26 g were purchased from the hatchery of a commercial fish farm, Aqua Farms, in Anambra state of Nigeria. Two extruded, pelletized and floatable conventional catfish feed types (1-mm diameter ‘Skretting Feeds’ and 2-mm diameter ‘Vital Feed’) were taken from livestock feed market in Ekwulobia town, Nigeria.

Methodology

Copper sulphate was dissolved in distilled water in the presence of few drops of acetic acid and sonicated at 50 Hz for 30 minutes. Liquid ammonia was gradually added to the solution till precipitation of cupric hydroxide was complete. The precipitate was filtered off, washed with warm distilled water and dried in an oven at 100 °C to produce cupric oxide, which was in turn Calcinated in a muffle furnace at 500 °C for 4 hrs to form dark-brownish copper oxide nanoparticles.

nCuO formed was characterized by X-Ray diffractometer (XRD), model ADX 8000 from Angstrom Advanced Inc., and scanning electron microscope (SEM), model FlexSEM 1000 at the laboratory of Moserbaer India Ltd, 66 Udyog Vihar, Greater Noida, UP, India. The average particle size was calculated using Scherrer’s equation.

*Author for Correspondence
E-mail: nbsingh43@gmail.com
$D = (K \lambda) / (\beta \cos \theta)$

Where
- $D =$ Size of CuO
- $K =$ dimensionless shape factor with a typical value of about 0.94.
- $\lambda =$ X-ray wavelength
- $\beta =$ full width at half the maximum intensity (FWHM), denoted as $\Delta (2\theta)$.
- $\theta =$ the Bragg angle

Different amounts (0 mg, 1 mg, 3 mg, 5 mg 7 mg and 10 mg) of nCuO and CuO Kg$^{-1}$ feed were added to the basal (Vital) feed and 5 levels of the treatments (nCuO as T1 and CuO as T2) diets at 3 replications each. Fish fingerlings were acclimatized to the laboratory environment for 10 days, being fed the Skretting conventional feed brand. After acclimatization, they were starved for 2 days to increase their desire for feed intake. They were subsequently cultured in flow-through aquaculture units made of 120-liter capacity plastic tanks and fed experimental diets 3 times daily (at 7:00 am, 12:00 noon and 6:00pm) for 7 weeks. Care was taken, during feed administration, to minimize incidence of uneaten feed, and good records of daily feed administration was kept. Weights of the fish were taken every week with a weighing machine which could read up to the second place of a decimal. Water quality parameters were monitored with water quality kit, PONDLAB 200, from NT laboratories, Wateringbury, Kent, and dissolved oxygen status of the culture water was enhanced by bubbling air through the water by means of pneumatic pump. Culture tanks were drained weekly to permit weekly determination of fish weight, cleaning of the culture tank and total replacement of culture water. Fish were harvested after seven weeks of culture and subjected to analyses of hematological parameters as described elsewhere5. CuO accumulation in whole body/tissue was determined by using atomic absorption spectrometry6. Figure 1a and b are pictures of fish at the beginning and end of the 7-week experimental trial.

Statistical analysis
All results were analyzed by one-way ANOVA using online Free Statistics Calculator from StatPac Inc., 2017.

Results and Discussions

Materials characterization
The SEM image and X-ray diffraction (XRD) pattern of the synthesized material are shown in Figure 2 a & b. The average particle size was 15±1 nm.

Water quality
No adverse effects of the treatments were observed on the monitored quality parameters of the culture water throughout the experimental period as their values (ammonia (0-0.25g/l), ammonium nitrate (0-0.1mg/l), pH (7-7.5), dissolved oxygen (5.5 -7.0) were...
all within permissible limits for catfish culture. Fish survival rates were also not affected by the dietary treatments in the course of the experiment. Fish weight gain with time (weekly) percent weight gain in the cultured fish in the presence of copper oxide nanoparticles (nCuO) is shown in Figure 3a while Figure 3b shows the data for fish treated with macro copper oxide (CuO) diet. From the figures it is observed that there was sustained increase in fish weight across all supplementation levels of both nCuO and CuO during the culture period. Fish final percent gain after 7 weeks increased with supplementation levels in both treatments. The highest final weight gains of 620.20% and 491.63% were obtained with the highest supplementation level of 10 mg Kg⁻¹ in both nCuO and CuO respectively while the lowest values were achieved with the least supplementation level of 1 mg Kg⁻¹. The results also showed that fish growth, as measured by weight increase, became more pronounced and significant from the supplementation level of 3 mg Kg⁻¹ and 5 mg Kg⁻¹ of nCuO and CuO respectively. Generally better results were obtained with copper supplemented diets compared to the control diet. In Table 1 and Figure 4, fish final weight gains (percent basis) are compared for different supplementation levels of the two treatments and the control. At supplementation level of 1 mg Cu Kg⁻¹ feed, there were no significant weight differences (p < 0.05) among fish fed the experimental diets (Control, T₁ and T₂). Table 2 shows Cu accumulation in fish whole body, liver and muscle at the end of the 7 weeks culture period. In all the treatments, Cu accretion was significantly higher in both the liver and whole body of fish compared to the muscle. Similarly, in the tissues studied, copper concentration was highest in fish fed nCuO supplemented feed and lowest in those fed the control diet. After 7 weeks, red blood cells (RBCs) and white blood cells (WBCs) counts, hemoglobin (Hb) and hematocrit (Hct) profile of the cultured fish are shown in Table 3. All studied parameters were enhanced by the presence of copper oxide as feed additives, with the effect being more pronounced with nCuO against CuO. There were no significant weight differences (P <0.05) among fish fed control diet and those fed diets
supplemented at levels of 1-5 mg CuO Kg$^{-1}$ feed and 1-3 mg nCuO Kg$^{-1}$ feed. However, significant differences (P < 0.05) existed among these groups at higher supplementation levels of 5-10 Kg$^{-1}$ and 3-10 mg Kg$^{-1}$ for CuO and nCuO respectively. This could mean that, in as much as dietary copper supplementation enhanced growth performance of C. gariepinus fingerlings, the fish were unable to meet their minimum Cu requirement below these supplementation levels. It further suggests that CuO is more effective in the nano than in the macro form in enhancing growth in the fish and in promoting material use efficiency. This may be on account of the small size and larger surface area of the particles of nCuO, and the higher assimilation, bioavailability and better interaction with other substances associated with them, compared to the larger particles of bulk CuO. Researchers have shown that copper supplementation has enhanced the growth in various species of fish other than catfish$^{9,10}$. It has also been shown that nano performs better than macro forms of minerals as dietary supplements$^{9,11}$. It can be inferred that nCuO impart better palatability and other sensory appeals to feed causing fish in that group to feed more and grow faster. The sustained increase in weight gain across all levels of dietary CuO supplementation (1-10 mg Kg$^{-1}$ dry feed) and the absence of mortality during the experiment may imply that the dietary Cu requirement of African catfish (C. gariepinus) is higher than 10 mg Kg$^{-1}$. This would mean that this level of dietary Cu had no adverse effects on the fish. Earlier studies on the dietary requirement of channel catfish, using CuSO$_4$.5H$_2$O as dietary Cu source, had estimated minimum Cu requirement of the fish at 5 mg Kg$^{-1}$ with a report that supplementation levels of 16 and 32 mg Cu Kg$^{-1}$ dry feed caused growth suppression in the fish$^{12}$. Similarly, the dietary Cu requirement of Cynoglossus semilaevis has been estimated at 11-12 mg Kg$^{-1}$, irrespective of dietary source$^{13}$. Growth suppression has also been reported in the yellow croaker fish (Larimichthys croceus) at dietary Cu supplementation level of 25.78 mg Kg$^{-1}$ and above, while supplementation levels of 13.65 and 3.67 mg Kg$^{-1}$ were considered medium and low respectively$^{2}$. On the contrary, a much lower supplementation level (2 - 4 mg Kg$^{-1}$) has been recommended for the enhancement of growth, immune response and antioxidant defense systems in red sea bream (Pagrus major) with Cu-NPs as source of dietary copper$^{4}$. Higher Cu content observed in the liver, compared to whole body and muscle of fish may be attributed to the fact that metabolized Cu, as other minerals, are first stored in the liver from where it is distributed to other tissues as required$^{1}$. Naturally, therefore, there should be higher levels of it in the liver in contrast to other tissues of the body, all things being equal. Similar trends in tissue Cu attrition have been reported for red sea bream (Pagrus major)$^{1}$, the prawn$^{15}$ and channel catfish$^{12}$. The significantly (p < 0.05) higher copper concentration in tissues of fish fed Cu supplemented diets against those fed the control diet would be due to higher uptake, metabolism and assimilation of Cu from supplemented diets. This trend of result is in agreement with those reported for channel catfish and red sea bream (Pagrus major) respectively$^{12}$. Hematological parameters are good tools for studying, measuring and indicating the health status of animals. In fish, analysis of the blood parameters is used to predict the toxic stress and general health of the animal. In the present study, the improved hematological parameters may be an indication of the important role of copper in iron metabolism in fish which, itself, is a key factor in blood synthesis. Copper oxide in nano form, on account of its small particle size, would possibly pass the gastrointestinal barriers, get into the blood stream more easily and impact more effectively on the blood compared to macro CuO. It may also imply that supplementation level of 10 mg CuO Kg$^{-1}$ feed is safe and unharmful to C. gariepinus fingerlings. Earlier study$^{14}$ on rainbow trout (Oncorhynchus mykiss) exposed to CuO-NPs for a period of 96 h indicated similar enhancement in these blood parameters, whereas study with silver carp (Hypophthalmichthys molitrix)$^{16}$ indicated decrease in same parameters and attributed to either anemia or erythropoiesis disorder.

**Conclusion**

This study has shown that growth and health in African catfish (Clarias gariepinus) can be enhanced through dietary Cu supplementation with different forms of copper oxide (CuO). It further revealed that nano form of copper oxide is more effective than the bulk form not only in impacting on the growth and health of the fish but also in bringing about economy of material, as less quantity of the nano form is used to achieve same or even greater effects than that of bulk form. Finally, the study showed that C. gariepinus can tolerate dietary Cu supplementation up to 10 mg Cu Kg$^{-1}$ feed, as both nano and macro forms of CuO, without adverse effects. This study is apparently the first attempt to investigate the effects of CuO-NPs, as feed supplement, on the performance
of African catfish. Consequently, it has provided a launching pad for further investigations. As the highest supplementation level (10 mg Kg$^{-1}$) employed in the current study did not produce any known side effect, it is recommended that this study be carried further with higher supplementation levels to determine the optimal level of supplementation of dietary Cu (in both nano and bulk forms) for African catfish (C. gariepinus).

References