Effects of dietary copper on survival, growth and biochemical parameters in juvenile common carp (Cyprinus Carpio L.) cultured at Indra-Sarovar of Makwanpur, Nepal

Pushpa Lal Homagain
Campus Chief, Amrit Science Campus, Institute of Science and Technology, Tribhuvan University, Thamel, Kathmandu, Nepal

Abstract
An outdoor experiment was conducted to evaluate the effects of dietary copper on survival, growth, and biochemical parameters in common carp Cyprinus carpio cultured in the Indra Sarovar lake of Makwanpur, Nepal. Altogether 375 fingerlings of Cyprinus carpio (9.43±14.09 g) were placed in 15 cages made of bamboo covered by nylon net and carps were introduced at the rate of 25 fish per cage on random basis. All of the 15 cages were grouped in five different treatments triplets as T1, T2, T3 T4 and T5 respectively. Treatment first of three cages (T1r1, T1r2, T1r3) was considered as the control since copper was not loaded in the dry diet while in the second (T2r1, T2r2, T2r3), third (T3r1, T3r2, T3r3), fourth (T4r1, T4r2, T4r3), and the last fifth (T5r1, T5r2, T5r3) cages were fed copper-loaded diets as (200 mg copper /kg), (400 mg copper /kg), (800 mg copper /kg) and (1600 mg copper/kg) dry weight feed, respectively for 90 days. This was then followed by a 21 days recovery period with all cages fed the control diet (no added copper) diet. At the end of feeding trials total weight gain, weight gain (%), SGR and FCR were done followed by protein profiles, hepatic enzyme profiles and liver histology and finally haematology. After the study of all the parameters it has been concluded that dietary copper 800 mg /kg found suitable and better for fish growth.

Keywords: Dietary copper sulfate, growth, Cyprinus carpio, Indra Sarovar, Nepal

1. Introduction
Copper (Cu) is an essential element involved in different biochemical process of animal metabolism such as: enzyme, co-enzyme catalytic reactions and plays a vital role in the physiology [1]. It is an essential micronutrient for vertebrate animals especially fish, and has numerous functions, in addition to the ones stated above, in cellular biochemistry including vital roles in cellular respiration, and a cofactor for over 30 different enzymes [2]. Copper deficiency leads to physiological disturbances like depression of growth, anaemia, spontaneous fractures, cardiac and vascular disorders and depressed reproductive performance including egg production [3]. Copper, though essential in fish diet, can be harmful when large single or daily intake occurs. The dietary effect of copper varies from species to species and has severally been reported for most temperate fish such as Salmon [4], but only little information on the dietary copper exposure and recovery in common carp has been reported. The common carp is a benthic omnivore native to Asia and reputed as a popular food fish and a highly cultivable species with year round breeding under tropical and subtropical conditions. It is the only exotic carp species that is known to breed naturally in lake due to its high fecundity and hatchability. Common carp Cyprinus carpio has been introduced into environments worldwide and can grow to a maximum length of 5 feet (1.5m), a maximum weight of over 40 kg and an oldest record age of at least 65 years [5]. This age longevity of common carp makes it good for chronic toxicity test. Thus, this present study will not only examine chronic dietary copper toxicity on common carp, but it will also establish threshold for dietary copper toxicity by investigating growth and ionic response, which has not been reported for the fish.
2. Materials and Methods

2.1 Experimental site and study area

Largest man-made lake also known as Indra-Sarobar is a beautiful famous lake for fishing, hiking, trekking and boating in Nepal and is located in Makwanpur district with an Area of 7 km². Thus, this experiment was conducted in the Indra-Sarobar using cage culture system made of bamboo and chemical analysis were done in the Department of Chemistry at Amrit Campus.

2.2 Selection of fish and maintenances

Around 500 Common carp Cyprinus carpio (9.43 ± 11.17 g) were procured from Government hatchery farm located in Machhapokhari of Kathmandu Valley and placed a cemented stock tank (300 l) filled with dechlorinated water inside the laboratory for acclimatization upto 10 days under natural environmental conditions. Carp were fed natural diet during this period and before starting the feeding trial they were left unfed in the first 24 hrs to adapt to the change in environment.

2.3 Preparation of experimental diets

The control diet was prepared according to Labh et. al., [6] with slight modifications. First of all fish meal, wheat flour, cod liver oil and multivitamins were collected from the local market to prepare experimental diets. Fish meal was dried well and ground in a grinder and then sieved (mesh size: 500µ) to make the fine powder. The powdered fishmeal was mixed thoroughly with wheat flour and recommended multivitamin along with requires quantity of copper dust. Then lukewarm water was added in required amount for the formation of dough. Cod liver oil was added to this and mixed well so that all the ingredients were spread homogeneously. The prepared dough was passed through a feed maker using 1 mm die, the thread formed was air dried. The dried threads were further chopped into small pieces of required sizes of pellets through a blender and then passed through a sieve to obtain homogeneous particle size. The proximate composition of the experimental diets was determined following the standard methods of AOAC [7] (Table 1). Nitrogen free extract (NFE) was calculated by difference i.e., NFE = 100 – (CP + EE+ CF+ Ash).

The copper-supplemented diet was formulated by starch coating of the commercial feed with copper sulphate. In order to achieve a nominal copper concentration of 200 mgCupk-1 feed 0.234 g of CUSO4. 5H2O (Copper (II) sulphate pentahydrate, Hi-LRTM; product code-GRM630 from HiMedia, India) was dissolved in 35ml of deionised water with 1.2 g of starch to bind the copper to the food sticks. Similar processes were done for copper supplemented diet of 400 mgCu/kg, 800 mgCu/kg, and 1600 mgCu/kg in which 0.468, 0.937 and 1.875 g of CUSO4. 5H2O were added respectively in 35 ml of deionised water with 1.2 g of starch to bind the copper to the feed. The starch solution was gradually sprayed onto 300 g of the prepared diet and mixed in a container to ensure even mixing of the food. The starch coat dried within minutes, and the copper diet was stored in airtight containers at -20°C to prevent lipid oxidation. The control diet was similarly treated except that no copper was added. Thus, altogether five (40% crude protein) experimental diets T1, T2, T3, T4 and T5 containing 0, 200, 400 and 1600 mg copper.kg⁻¹ respectively had been used. Diet was stored at -20 °C until used.

2.4 Experimental design and set up

Altogether 375 fish (9.43±14.09 g) were placed in 15 cages made from bamboo with nylon net at the rate of 25 fish per cage on random basis. All of the 15 cages were grouped in five different treatments triplets as T1, T2, T3 T4 and T5 respectively. Treatment first of three cages (T1r, T2r, T3r) was considered as the control since copper was not loaded in the dry diet while in the second (T2r, T2r, T2r), third (T3r, T3r, T3r), fourth (T4r, T4r, T4r), and the last fifth (T5r, T5r, T5r) cages were fed copper-loaded diets as (200 mg cupper /kg), (400 mg cupper /kg), (800 mg cupper /kg) and (1600 mg cupper/kg) dry weight feed, respectively for 90 days. This was then was then followed by a 21 days recovery period with all cages fed the control diet (no added copper) diet. Throughout the experiment fish were fed to satiation twice a day in the morning (9 am) and evening (5 pm) at the rate of 4% of its body weight. Care was taken to ensure no uneaten food remained in the tanks during feeding and copper did not leach from the feed.

To achieve these objectives, water was constantly and completely changed daily with fresh well water added and uneaten food removed after satiation was noted. Daily feed intake was calculated by subtracting weight of feed plus container after feeding from feed plus container before feeding. Copper concentrations in the different aquarium were measured in the analysis of water quality. Growth and nutritional performance in the different treatments were monitored throughout the experiment and the fish randomly sampled from each tank after 15 day of copper exposure for histology and haemato-immunology. Fish were not fed the day before sampling times in order to empty the gut and to facilitate dissection.

Fig 1: Satellite view of Indra-Sarobar lake, Makwanpur, Nepal

Fig 2: Common carp used during the experiment
2.5 Study parameters
Briefly, feed intake was calculated daily for each tank by weighing feed containers before and after feeding. All fish were individually weighed at the start of the experiment and the end of 90 days of exposure. The individual fish weight was used because the periodic sacrifice of fish during the experiment prevented nutritional parameters being calculated from cumulative tank biomass as follows:

2.5.1 Growth performance of fish
The growth performances of carp fingerlings were evaluated in terms of weight gain(g), weight gain (%), condition factor (%), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) by using following formulae:

- Weight gain(g)=final weight-initial weight
- Weight gain (%) = (Final weight- Initial weight) / Initial weight x 100;
- SGR = (ln(final weight in grams) - ln(initial weight in grams)) x100 / t (in days)
- FCR = Total feed given (dry weight) (g) / Weight gain (wet weight) (g);

2.5.2 Blood Collections and Biochemical analysis
At the end of the feeding trial, three fish in triplicates from each of the control and experimental groups were anaesthetized with tricaine methane sulfonate (MS-222) (5mg/l). Blood were collected from the caudal vein using a syringe with 25 gauge needle. The blood samples were then transferred immediately to eppendorf tubes and allowed to clot for a while then centrifuged for 5 min at 3000×g and thus collected serum was stored at -20°C for further analysis. Total Serum protein was estimated by Biuret and the Bromocresol Green (BCG) dye binding method [8] using total protein and albumin kit (Qualigens Diagnostics, Mumbai). Albumin was estimated by the BCG binding method [9]. The absorbance of standards and tests were measured against the blank in a spectrophotometer at 630 nm. Globulin was calculated by subtracting albumin values from total serum protein. The albumin/globulin ratio (A/G) was calculated by dividing albumin values by globulin values. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) were determined calorimetrically by using available kits [10].

2.5.3 Histological investigations:
For the histological investigations, small parts of the liver from the control and the treated animals were fixed in neutral formalin. After the fixation period, the tissues were washed in saline solution 0.9% NaCl, dehydrated through a graded series of ethyl alcohol, cleared in xylene, embedded in the parablast, sectioned at a thickness 5-6microns, mounted on the glass slides and stained with haematoxylin and eosin for general morphological studies. Sections were studied and the tissues were compared with the control [11, 12].

2.5.4 Haemato-immunological studies
For haematological parameters Blood samples were obtained from the caudal vein using a syringe and immediately transferred to eppendorf tubes containing EDTA powder and shaken gently to prevent haemolysis of blood. The bloods were diluted with appropriate diluting fluids for RBC and WBC counts and were determined using improved Neubauer haemocytometer and calculated. Replicated counts were made for each blood samples to minimize the error. The haemoglobin was determined by cyanmethemoglobin method. Haematocrit (PCV) was determined by the microhematocrit method. Mean Corpuscular Volume (MCV) was calculated according to. Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated [13].

2.5.6 Statistical analysis
Value for each parameter measured has been expressed as mean ± standard error of mean. The results were analyzed by one-way Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). Significance was tested at P<0.05 level. The software SPSS (version 20) was used to compare the target parameters.

3. Results
3.1 Growth performances
The growth parameters of the experimental carps of different groups at the end of feeding trials were well studied. Significant (p<0.05) growth rate found in Copper fed carp groups and highest weight gain, wt gain% and SGR were observed in the T3 group fed carp while inverse result noticed in the FCR of T3 group. The lowest FCR value (p<0.05) was detected in T3 followed by T4 and T2 group. The physiological effects of dietary copper on normal diet were studies throughout the entire length of the work. Growth performance like WG (%), SGR and FCR were investigated during the two phase of the experiment (exposure and recovery phase). The result showed that all parameters were within the range required and tolerated by common carp.

Common carp is one of the most cultured fish in the world. This fish is omnivorous, resistant and tolerant to wide variations of abiotic and biotic factors of the environment. A lot of different fish feed available on the market are used for culturing carp. Nutritional requirements for growth, reproduction and normal physiology are similar to the requirements of other domesticated animals. However, fish mainly differ from other animals in their demand for proteins, so usually feed with 25 to 45% of row proteins are used [14].

At the beginning of the experiment, the lowest weight of individual fish was 19.43±14.09 g and after 90 days of feeding the highest weight gain was 156.33±0.75dc g in T3 diet fed group (Table1). This indicates 7 time higher growth and the weight gain percentage was 802.39±0.11dc. Copper accumulations in fish tissue have severally been reported [15] and toxicity of metals may vary depending upon their permeability and detoxification mechanisms [16, 17]. In this study, copper accumulation in common carp also reflected the route of exposure, with large increase in copper content of the liver and intestine and is consistent with previous studies on rainbow trout.
Fig 4: Weight gain of *C. carpio* fed with diet containing varied doses of dietary copper.

Fig 5: Weight gain percentages of *C. carpio* fed with diet containing varied doses of dietary copper.

Fig 6: Specific growth rate of *C. carpio* fed with diet containing varied doses of dietary copper.

Fig 7: Feed Conversion Ratio of *C. carpio* fed with diet containing varied doses of dietary copper.

Table 1: Growth performance of Common carp fed dietary copper in the cage culture at Indra Sarovar of Makwanpur, Nepal

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mn±SE</th>
<th>WG</th>
<th>WG (%)</th>
<th>SGR</th>
<th>FCR</th>
<th>Survival%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>123.57±0.51*</td>
<td>635.98±0.65*</td>
<td>2.49±0.011*</td>
<td>0.42±0.013*</td>
<td>81.99 ± 3.11*</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>130.27±0.77bc</td>
<td>670.46±0.06bc</td>
<td>2.54±0.059bc</td>
<td>0.41±0.060b</td>
<td>86.28 ± 4.28ab</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>156.33±0.75bc</td>
<td>802.39±0.11bc</td>
<td>2.72±0.024bc</td>
<td>0.34±0.023a</td>
<td>99.97 ± 4.76b</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>148.61±0.29abc</td>
<td>664.99±0.73abc</td>
<td>2.67±0.010abc</td>
<td>0.37±0.023abc</td>
<td>96.18 ± 2.18abc</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>139.17±0.82ab</td>
<td>716.58±0.84ab</td>
<td>2.61±0.020ab</td>
<td>0.38±0.023bc</td>
<td>85.32 ± 1.71b</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Biochemical Parameters
During biochemistry parameters a direct relationship was found between doses of copper and concentration of total serum protein (TSP) in blood of common carp. The concentration of TSP was significantly (*P*<0.05) higher in the blood sample of carp fed with diet T3 (32.38±1.48e µg/dl) followed by carp fed with diets T4 (28.42±1.22d µg/dl), T5 (23.87±0.69c µg/dl), T2 (18.93±1.98c µg/dl) and minimum was in control diet fed carp T1 (11.01±1.27a µg/dl). Serum protein level was 2.7 times higher in carp fed with T3 diet compared to that of control carp. The concentration of albumin in blood serum was significantly (*P*<0.05) high (19.87±0.476d µg/dl) in carp fed with diet T3. The ratio of albumin and globulin in blood serum in common carp fed with diet T3 (0.71±0.356) was 37.6% higher.

3.3 Histological Profiles
Microscopic examination of sections from the liver of control and treated group of carp showed normal histology, each liver lobe is seen to be made up of hepatic lobules. The lobules are roughly hexagonal and consist of plates of hepatocytes radiating from a central vein. The central vein joins to the hepatic vein to carry blood out from the liver. Between the hepatocyte plates are liver sinusoids as seen in plates, 1, 2, 3 and 4.
3.4 Haematological Profiles

The results of the haematological parameters of C. carpio fingerlings fed with different doses of copper showed significant enhancement with treated diet when compared with control. Carp fingerlings showed increased in RBC (3.27±0.15), WBC (8.40±18.76), Hb (13.61±0.69) while in PCV (19.27±1.10), MCV (49.33±4.67), MCH (67.35±1.65), and MCHC (36.30±0.61) in T3 diet fed group but on an average it has been clear that all the levels were at normal conditions so it can be concluded that no significant results observed in haematological parameters.

5. Discussion

Aquaculture has grown tremendously and has become an economically important industry in the world. Today, it is the fastest growing food-producing sector with the greatest potential to meet the growing demands of aquatic food [18]. There is an increase in fish production as a consequence of a rise in demand for fishery products. This means that the volumes of fish processing by-products and fish wastes have also increased. These fishery wastes are highly perishable, owing chiefly to the action of microorganisms that find the wastes to be an excellent growth medium. Fish wastes include viscera, scales, fins, frame bones and sometimes shells [19].

Fish meal with protein above 40% is generally to be of good quality [20]. Fish meal used in the present study was of good quality with protein content of 60.24%. The composition of other ingredients such as groundnut oil cake, rice bran and wheat gluten was within acceptable limits. Among the ingredients employed, fish meal had highest moisture (9.63%). Crude fat content was highest in ground nut oil cake (7.61%) and lowest in wheat gluten (0.80%). Dry matter content was highest in rice bran (94.88%) and lowest in fish meal (90.37%). Fibre content was highest in rice bran (27.28%), while carbohydrate content was highest in wheat gluten (42.22%). It may be concluded that the quality of all the ingredients used in the feed formulation was nutritionally adequate and suitable for feed formulation. Venkateswara [21] reported that carp grow better when fed on diets containing about 40% protein.

The quality of water is very important in the culture of freshwater fish, since use of feeds is known to have an influence on water quality, thereby affecting the species cultured. Maintenance of good water quality is essential for both survival and optimal growth of carp. Good water quality is characterized by adequate oxygen and limited levels of metabolites. A complete understanding of the relationship between water quality and aquatic productivity is a prerequisite for optimum growth and survival [22]. In the present investigation, important water quality parameters such as temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity and total ammonia-nitrogen were measured through the experimental period at fortnightly intervals and water was replenished in all the tanks to maintain good quality of water.

The results are in adverse with the findings of Kim and Kang that reported the reduced growth rate of rockfish (Sebastes schlegeli) due to Cu stress and there was an inverse relationship between growth and Cu exposure [23]. Labh et al. [24] observed reduced growth of Oreoichromis niloticus under different (0, 0.5, 0.3, & 0.5 ppm) water-borne Cu levels. In the fish, N. notopterus [26] serum enzymes such as SGPT and SGOT and alkaline phosphatase (ALP) were determined and found to be with a range of 15.15-17.20 U/L, 12.62 -16.70
U/L, 35.40–69.37 IU/L respectively. In the present study SGOT and ALP activities were found to be higher than those reported in Acipeps stellatus [16] whereas the SGPT activity is higher in the fish N. notopterus [23]. This variation has been also reported for common carp [28], channel catfish, Ictalurus punctatus [29]. Such variation in the activities of various enzymes in fish species may be due to sampling technique, analysis method, age of fish, habitat and diet [30].

Among all tissues, liver showed higher protein content which might be due to greater concentration of enzyme. Liver is the site of metabolism. The liver plays an important role in the synthesis of proteins. The impact of contaminants on aquatic ecosystem can be assessed by the measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination [31]. The intake of pollutants invariably affects the fish physiology and metabolism. The liver plays a primary role in the metabolism of xenobiotic compounds with biochemical alterations occurring in some toxic conditions [32], and it is a detoxification organ essential for the excretion of toxic substances in fish [33].

There is growing interest in the study of haematological parameters of fish blood cells regarded as important for aquaculture purposes. Blood parameters have been used as indices of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as transportation, handling, hypoxia and acclimation [34]. Haematological studies help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment. Haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the haematological characteristics [35]. These indices have been employed in effectively monitoring the responses of fishes to supplementation of diet and thus their health status under such adverse conditions.

The haematological characteristics of a number of cultivable fish species have been studied with the aim of establishing normal value ranges and any deviation from it may indicate a disturbance in the physiological processes [36]. Several of these studies were attempts to determine if significant variations from normal values of these parameters exist that could be attributable to some internal or external factors [37].

6. Conclusion

Present study has clearly indicated fingerlings species of Cyprinus carpio have different growth performance and different biochemical and Histopathological activities under the different concentration of dietary copper. Optimum dietary copper was necessary for higher net growth efficiency of the fish and approximately 400 mg copper Kg−1 are needed for the feed consumption, fast growth, protein content of Common carp. This new information on quantitative dietary requirement of Cyprinus carpio is useful in designing practical cost-effective diets for the intensive culture of this species.

7. Acknowledgement

The author is grateful to Amrit Campus of Tribhuvan University for providing all the essential equipments and lab facilities and is also thankful to Mr. Rajesh Mahaju, Campus Chief of Amrit Campus for his continuous inspirations.

7. References


