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IMPACT OF REPLACING INORGANIC COPPER AND ZINC IN CATFISH DIET WITH COPPER NANOPARTICLES AND ZINC NANOPARTICLES AT DIFFERENT LEVELS ON BODY PERFORMANCE AND BIOCHEMICAL PARAMETERS

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ABSTRACT

The current study was carried out to investigate the effect of adding zinc and copper oxide nanoparticles metals at different levels from the recommended requirements of fish on the growth performance, biochemical parameters and some antioxidant enzymes in catfish. Three hundred and fifty channel catfish (Clarias gariepinius), average weight (90 ±5 g) were divided into seventh groups. The first group (control group) received the basal diet that contains normal requirements (copper sulfate 12.5 mg / kg DM to provide 5 mg/kg DM copper, zinc oxide 25 mg / kg DM to provide 20 mg/kg DM zinc); the second group (Gr Cu 1) received the basal diet with normal dose requirement from CuO-NPs 6.25 mg / kg DM to provide 5 mg/kg copper; the third group (Gr Cu 2) received the basal diet with half requirement from copper (3.125 mg /kg DM CuO-NPs); the fourth group (Gr Cu 3) received the basal diet with quarter requirement from copper (1.562 mg /kg DM CuO-NPs); the fifth group (Gr Zn 1) received the basal diet with dose requirement from zinc (25 mg /kg DM ZnO-NPs); the sixth group (Gr Zn 2) received the basal diet with half requirement from zinc (12.5 mg/kg DM ZnO-Nps); the seventh group (Gr Zn 3) received the basal diet with quarter requirement from zinc (6.25 mg /kg DM ZnO-NPs). Better responses were reported to feeding nanoparticles at quarter dose than normal dose for both elements (ZnO – CuO). These results were supported by biochemical and the antioxidant enzyme results. We could conclude that the uses of quarter requirements from Zn and Cu nanoparticles were recommended in our work for better performance and growth.

Keywords: *Clarias gariepinus, Zinc oxide nanoparticles, Copper oxide nanoparticles, Growth performance, Biochemical Parameters, Antioxidant*

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INTRODUCTION

The life standards of human societies have been rigorously changed after introduction of modern technologies. One aspect of these new technologies is daily increase of emerging nanoproducts (Handy, 2012). Nanotechnology involves the synthesis of nanoscale particles that exhibit unique physiochemical properties like higher intestinal absorption, bioavailability and enhanced bactericidal and catalytic activities (Dube *et al.,* 2010). Aquaculture is more than a science in its infancy; it is now recognized as a viable and profitable enterprise worldwide. As aquaculture technology has evolved, the push toward higher yields and faster growth has involved the enhancement or replacement of natural foods with prepared diets (NRC 1993). Minerals are essential nutrients for normal body processes; mineral requirements vary depending on forms, interactions with other elements, water quality and fish itself (age, size and species) (Tawfik *et al.,* 2017). Copper is an essential trace element for vertebrates, including fish, for a large number of biological processes, mainly as a cofactor for some enzymes, such as cytochrome oxidase, superoxide dismutase, lysyl oxidase, dopamine hydroxylase and tyrosinase (Tan *et al.,* 2011). Zinc is one of the most important trace minerals needed for good health of animals (Onuegbu *et al.,* 2018). Zinc has both catalytic and structural roles in enzymes, and its antioxidant properties have been widely recognized (Maret, 2000), the objective of this study the effect of adding zinc and copper oxide nanoparticles metals at different levels on the recommended requirements of fish on the growth performance, biochemical parameters and some antioxidant enzymes in cat fish.

MATERIALS AND METHODS

Experimental Fish

A total number of three hundred and fifty (350) unsexed catfish (Clarias gariepinius (mean weight 90 ± 5 g) were obtained from a local commercial fish farm, Rasheed, Behara governorate, and were randomly distributed into seven circular tanks of 220 L capacity (50 fish per each tank) with similar initial weight. Fishes were fed daily (twice per day) with a feeding rate of (3% of their body weight, 135 g for each group) with commercial pelleted feed according to (NRC 1993).

Feeding:

The experimental diets were formulated from commercially available ingredients. The basal ingredients of diets were soybean meal, corn, sunflower meal, fish meal. Diets were formulated to contain 32 % crude protein as minimum, 2800 Kcal/Kg diet.

Zinc and copper nanoparticles

The formulated fish diets were enriched with nanoparticles of zinc oxide and copper oxide. Copper oxide nanoparticles (nano powder <50 nm particle size (TEM) and zinc oxide nanoparticles (nano powder <100 nm particle size (TEM) from Sigma Aldrich company.

Table 1: Chemical composition and digestible energy value (Kcal/kg Diet) of the ingredients used in the experimental diets (as fed basis) (NRC 1993).

Ingredients	**DE (Kcal/kg)**	**Chemical composition, %**				
		DM	**CP**	**EE**	**CF**	**Ash**
Soybean meal	3.010	90	42	1.1	7.3	6.3
White Corn	2.200	88	8	3.6	2.3	1.3
Sunflower meal	2.870	93	33.96	2.9	11.7	7.5
Fish meal	4.060	92	61.42	9.6	0.7	19

*values of digestible energy (DE) were calculated according to NRC (1993)

Table 2: Chemical composition (%) of the experimental diet:

Item	Control	Gr Cu 1	Gr Cu 2	Gr Cu 3	Gr Zn 1	Gr Zn 2	Gr Zn 3
Soybean meal	40	40	40	40	40	40	40
White Corn	28	28	28	28	28	28	28
Sunflower meal	20	20	20	20	20	20	20
Fish meal	10	10	10	10	10	10	10
Slack	1.125	1.125	1.125	1.125	1.125	1.125	1.125
1- Calcium carbonate	0.7	0.7	0.7	0.7	0.7	0.7	0.7
2- Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3
3- Vitamin C	0.025	0.025	0.025	0.025	0.025	0.025	0.025
The rest of slack was completed from white corn							

Premix manually prepared to provide the following per kg of diet: vitamin A, 1000-2000 IU; vitamin E, 50 IU; vitamin D, 500 IU; thiamine, 1 mg; riboflavin, 9 mg; niacin, 14 mg; B6, 3 mg; folate, 1.5 mg; choline, 400 mg; vitamin C, 25-50 mg; pantothenic, 15 mg; magnesium, 0.04 %; phosphorus, 0.45 %; copper, 5 mg (inorganic or nanoparticles); iodine, 1.1E mg; iron, 30 mg; manganese, 2.4 mg; zinc, 20 mg (inorganic or nanoparticles); selenium, 0.25 mg.

Methods

Experimental groups:

The experiment was designed into seven groups each group contain 50 fish; The first group (control group) received the basal diet that contains normal requirements (copper sulfate 12.5 mg / kg DM to provide 5 mg/kg copper (copper sulfate contains 39.81% copper)), zinc oxide 25 mg / kg DM to provide 20 mg/kg zinc (zinc oxide contains 80% zinc)); the second group (Gr Cu 1) received the basal diet with normal dose requirement from copper (6.25 mg / kg DM CuO-NPs to provide 5 mg/kg copper (copper oxide contains 79.88 % copper); the third group (Gr Cu 2) received the basal diet with half requirement from copper (3.125mg /kg DM CuO-NPs); the fourth group (Gr Cu 3) received the basal diet with quarter requirement from copper (1.56 mg /kg DM CuO-NPs); the fifth group (Gr Zn 1) received the basal diet with dose requirement from zinc (25 mg /kg DM ZnO-NPs); the sixth group (Gr Zn 2) received the basal diet with half requirement from zinc (10 mg/kg DM ZnO-NPs); the seventh group (Gr Zn 3) received the basal diet with quarter requirement from zinc (5 mg /kg DM ZnO-NPs).This experiment was carried out according to the regulation of nutrition and clinical nutrition department at fish research lab, faculty of veterinary medicine, Sohag university, Sohag governorate and lasted 60 days from 2 November 2019 to 31 December 2019.

Measurements:

The experiment was lasted 60 days (four periods 15 days each). In each period, we collected fish and serum samples for body performance tests ((body weight gain- feed conversion ratio) and for estimation some biochemical parameters (cholesterol-triglycerides -alanine aminotransferase-aspartate aminotransferase-alkaline phosphatase enzyme- -lactate dehydrogenase enzyme - creatinine- urea). Finally, at the end of the experiment after 60 days we estimated some serum Antioxidants (catalase-glutathione peroxidase-superoxide dismutase).

Feed Preparation

All feed ingredients were purchased from local market. All feed ingredients were grinded to form a feed pellets which let to dry at room temperature.

Proximate nutrient composition of experimental rations

The experimental feed was analyzed using the standard analysis method of the Association of Official Analytical Chemist (AOAC, 2001). They were analyzed as follows: The moisture content of the sample was determined using air oven at 105°C, crude protein was obtained by using Micro-Kjeldahl method (Behrotest inkjel). The ash content was determined using muffle furnace (Nabertherm) at 600°C for two hours until constant weight of ash was obtained. Crude fiber was determined by fiber analyzer (Ankom 2000) after the residue was ashed and loss in weight was recorded as crude fiber.

Growth Performance:

Growth performance parameters were carried out at a regular interval every 15 days for 60 days to measure Body weight gain (BWG), Feed conversion ratio (FCR).

Biochemical Parameters Analysis:

Frozen serum samples were analyzed for determination of the activities of aspartic aminotransferase (AST) and alanine aminotransferase (ALT) (Tietz *et al.,* 1994) as well as cholesterol (Allain *et al.,* 1974), triglyceride (Shephard *et al.,* 1986), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) (Marsh *et al.,* 1959), urea and creatinine (Tietz *et al.,* 1986) by using commercial kits (Chema Diagnostica and Spectrum) and determination of some antioxidants as catalase, glutathione peroxidase and superoxide dismutase (Aebi 1984) using commercial kits (Bio -Diagnostic) using spectrophotometer.

Statistical analysis

Experimental data subjected to several statistical analyses from which means ± standard errors was calculated using the Graph-Pad Prism (GraphPad Software, San Diego, CA, USA). Differences were testing for significance by one – way analysis of variance. Differences ($P < 0.05$) among treatment were tested using Tukey’ s Honest significant difference test.

RESULTS

Table 3: Effect of adding some nanominerals (copper and zinc) on body weight gain (g) of catfish (Clarias gariepinus).

GN	Experimental Time				
	From 1 - 15 Days	From 16-30 Days	From 31- 45 Days	From 46- 60 Days	Cumulatives
Control	29.2±2.02^{b}	34.2±1.2	31.35±1.5	34.66±1.1^{d}	129.41±3.64
Gr Cu 1	30.9±1.1^{b}	38.17±1.4^{c}	31.63±11	28.17±1.2	128.87±3.83
Gr Cu 2	33.38±1.1^{a}	35.21±1.4	36.98±1.6^{d}	35.27±1.8^{d}	140.84±4.45^{c}
Gr Cu 3	34.71±1.2^{a}	42.96±1.8^{b}	42.72±1.9^{b}	41.78±2.1^{b}	162.17±4.65^{b}
Gr Zn 1	28.45±0.4^{b}	32.33±1.9	30.41±2.1	34.66±2.21^{d}	125.85±3.5
Gr Zn 2	29.61±1.5^{b}	35.78±1.2	39.08±2.4^{c}	37.14±2.4^{c}	141.61±4.12^{c}
Gr Zn 3	33.33±0.8^{a}	45.5±1.6^{a}	45.24±1.7^{a}	44.21±1.5^{a}	168.28±3.23^{a}

Means in the same column with different superscripts are significantly different ($P \leq 0.05$)

Table 4: Effect of adding some nanominerals (copper and zinc) on feed conversion ratio of catfish (Clarias gariepinus).

GN	Experimental Time				
	From 1 – 15 Days	From 16-30 Days	From 31- 45 Days	From 46- 60 Days	Cumulatives
Control	1.40	1.58	2.21	2.41	1.92
Gr Cu 1	1.32	1.43	2.27	3.06	1.97
Gr Cu 2	1.22	1.59	1.94	2.51	1.83
Gr Cu 3	1.18	1.32	1.77	2.27	1.66
Gr Zn 1	1.4	1.66	2.24	2.36	1.94
Gr Zn 2	1.38	1.52	1.79	2.36	1.79
Gr Zn 3	1.22	1.23	1.69	2.19	1.61

Table 5: Effect of adding some nanominerals (copper and zinc) on Serum biochemical parameters of catfish (Clarias gariepinus):

GN	**Triglyceride (mg/dl)**	**Cholesterol (mg/dl)**	**Urea (mg/dl)**	**Creatinine (mg/dl)**	**ALT (IU/L)**	**AST (IU/L)**	**ALP (U/L)**	**LDH (U/L)**
Control	162 ± 11.91	149.67 ± 8.94^{a}	7.67 ± 1.36^{a}	0.37 ± 0.03^{b}	8.92 ± 0.13^{b}	129 ± 7.95^{bc}	45.67 ± 2.34^{b}	512.3 ± 23.06^{c}
Gr Cu 1	164.333 ± 11.91	168.67 ± 11.11^{a}	7.67 ± 1.43^{a}	0.37 ± 0.03^{b}	11.5 ± 0.21^{a}	140.33 ± 3.07^{a}	64.34 ± 4.16^{a}	360.24 ± 30.29
Gr Cu 2	139.67 ± 11.14	153.00 ± 5.41^{a}	6.33 ± 0.92^{ab}	0.52 ± 0.05^{a}	8.34 ± 0.31^{b}	135.67 ± 3.18^{b}	65.36 ± 3.27^{a}	612.33 ± 23.83^{a}
Gr Cu 3	138 ± 7.89	143.67 ± 7.47^{b}	5.67 ± 0.67^{b}	0.36 ± 0.03^{b}	8.6 ± 0.25	127.67 ± 9.91^{bc}	56.22 ± 3.22^{b}	623.80 ± 21.71^{a}
Gr Zn 1	141.67 ± 5.23	147.00± 6.79^{a}	6.33 ± 0.82^{ab}	0.33 ± 0.03^{b}	11.4 ± 0.29^{a}	132.00 ± 9.37^{b}	58.7 ± 3.18^{b}	632.33± 21.61^{a}
Gr Zn 2	117.67 ± 0.82	138.00 ± 7.74^{b}	6.67 ± 1.67^{ab}	0.31 ± 0.15^{b}	8.31 ± 0.11^{b}	127.33 ± 5.12	30 ± 5.25^{c}	353.47 ± 29.93
Gr Zn 3	102.33 ± 12.42	116.00 ± 5.48^{c}	5.07 ± 0.17^{b}	0.31 ± 0.02^{b}	8.03± 0.22^{b}	123.00 ± 7.79^{c}	29 ± 2. 55^{c}	584.66 ±23.73^{b}

Means in the same column with different superscripts are significantly different ($P \leq 0.05$)

Table 6: Effects of adding some nanominerals (copper and zinc) on antioxidant (U/L) of catfish (clarias Gariepinus).

GN	Control	Gr Cu 1	Gr Cu 2	Gr Cu 3	Gr Zn 1	Gr Zn 2	Gr Zn 3
Catalase	315.67 ± 1.94^{c}	162.33 ± 5.23^{d}	306.33 ± 2.44^{c}	322.33 ± 3.25^{b}	324.83 ± 6.64^{b}	337.67 ± 6.97^{b}	353.67 ± 9.63^{a}
SOD	74.6667 ± 1.63^{e}	84.1667 ± 2.69^{e}	204.33 ± 2.57^{d}	208.00 ± 3.56^{d}	230.67 ± 3.44^{c}	254.67± 2.06^{b}	277.67± 1.22^{a}
Glutathione	422.67 ± 9.23^{d}	154.33 ± 4.89^{f}	379.33± 9.62^{e}	510.67 ± 6.22^{c}	320.33 ± 9.21^{e}	525.00 ± 9.01^{b}	542 ± 6.35^{a}

Means in the same column with different superscripts are significantly different ($P \leq 0.05$)

DISCUSSION

Nutritionally balanced diets are necessary for culture system; therefore, micronutrients must be supplied at adequate levels in the prepared diets to support optimal growth and production efficiency (Swain *et al.,* 2016). The normal growth performance of cultured fish species requires a balanced diet containing all the necessary nutrients, including copper (Cu) (Mohseni *et al.,* 2014; El Basuini *et al.,* 2016).

In our work, growth performance and feed utilization parameters (tables 3, 4) were affected significantly by both CuO-NPs and ZnO-NPs supplementations, which is in agreement with the previous studies demonstrated that the implementation of Cu and vitamin C supplementations to enhance growth rates, feed utilization and immunity response of several fish species (Mohseni *et al.,* 2014). Also, Swain *et al.* (2016), confirm the improvement in growth in fishes with using nanoparticle minerals.

In agreement with our results, El Basuini *et al.* (2016) reported that enhanced growth performance and feed utilization were generally observed in fish fed diets supplemented with Cu-NPs compared to fish fed basal diets. Onuegbu *et al.* (2018), observed that there was sustained increased in fish weight across all supplementation levels of both Cu-NPs and CuO during the culture period.

The positive effects of using the Cu-NPs could be attributed to 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 implied that supplementation Cu-NPs is safe and unharmful to fish fingerlings (Onuegbu *et al.,* 2018).

However, no significant differences have been reported as a result of Cu supplementation on weight gain and feed efficiency in case of channel catfish (Gatlin & Wilson., 1986).

In contrast, Chen *et al.* (2013), reported that the growth suppression in the high dose of Cuo-NPs could be the cause of the reduction in growth performance which was most likely due to two reasons: first, Cu exposure caused increased metabolic expenditure for detoxification and maintenance of homeostasis; second, higher Cu exposure reduced feed intake, which would in turn lead to reduced growth.

Also, growth suppression could attributed to, loss of appetite in rainbow trout (Lanno *et al.,* 1985) Nile tilapia, Oreochromis niloticus (Shaw & Handy 2006) and juvenile yellow catfish (Tan *et al.,* 2011), when exposed to excessive dietary copper (Mohseni *et al.,* 2014).

The growth performance resulted by using Zn-NPs as feed supplements, confirmed an improvement with using half and quarter of the recommended dose of Zn, by (NRC, 1993) of fish.

Obtained results of using Zn-NPs are supported by findings of Khan *et al.* (2016), who was reported that zinc nanoparticles enhance the growth rate in fishes.

Similarly, the improvement in growth rate and FCR as reported in this experiment has also been recorded by Ahmed *et al.* (2012) who also indicate the scope of nanotechnology for the enhancement of healthy fish production.

The fish fed Zno-NPs form showed a higher growth rate followed by CuO-NPs nanoparticle group at quarter and half dose then the control group, while less growth was observed in groups offered dietary ZnO nanoparticle supplementation at normal dose.

In agreements with our results, Faiz *et al.* (2015) observed a significantly higher gain, growth rate and improved FCR of fish fed ZnO-NP supplemented diet compared to ZnO enriched diet at the same level may be due to small size (50-60 nm) of nanoform of ZnO.

The better performance of fish could be attributed to a higher intestinal absorption, bioavailability and catalytic activities (Alishahi *et al.,* 2011). Also the positive effect of Zn nanoparticles on growth performance may be attributed to somatic growth by stimulation of DNA and RNA synthesis and cell division (Siklar *et al.,* 2003).

Tawfik *et al.* (2017) reported that fish fed diet supplemented with Zn in nano-form showed significantly ($p<0.05$) high %WG, besides their observation that ZnO-NPs supplementation enhanced the growth rates more than conventional ZnO, which were sometimes double the weight gain. While, Rather *et al.* (2011) reported that reduction of macromolecule to nanoscale changed their properties and increased their application.

Blood biochemistry parameters are one of the important indicators for fish general health condition as well as the physiological stress response (Dawood *et al.,* 2015). Metabolic enzymes such as aspartate aminotranseferase enzyme (AST) and alanine aminotranseferase enzyme (ALT) are often used for the evaluation of liver function as they are released into the blood during injury or damage to the liver cells (Dawood *et al.,* 2016). Moreover, Kim & Kang (2004) showed that Cu exposure increased ALT and AST serum concentrations with increasing time and dose in juvenile rock fish.

These results are in agreements with the findings of Abdel-Khalek *et al.* (2015) showed that ALP, AST and ALT concentrations in Oreochromis niloticus increased after 96 hours of exposure to CuO-NPs at higher levels.

While Bakalli *et al.* (1995) claim that copper addition contributes to a decreased level of triglycerides and reduced cholesterol synthesis in blood plasma and tissues of animals.

The increased activity of AST and ALT in tissues of ZnO-NPs treated fish may indicate the increased rate of proteins metabolism in cells (Taheri *et al.,* 2017).

ALP plays a significant role in phosphate hydrolysis and in membrane transport and it also acts as a good biomarker of stress in biological systems (Murray *et al.,* 2003). Moreover, the administration of ZnO NPs caused a significant decrease in ALP activity in liver of fish (Taheri *et al.,* 2017).

An increase in zinc level in liver can account for a reduced ALP activity because high levels of zinc can have deterrent effects on ALP activity (Farah *et al.,* 2012). Increased ALP activity in kidney may be due to the effects of ZnO NPs on transphosphorylation activity as well as a metabolic dysfunction in cells (Taheri *et al.,* 2017).

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme that catalyses the inter conversion of pyruvate and lactate (Swain *et al.,* 2019). Cells release LDH into the bloodstream after tissue damage or red blood cell haemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissues and cells (Taheri *et al.,* 2017).

In contrary to our result, the significantly increased creatinine level in the nano group suggested that the renal dysfunction be most likely caused by nano zinc administration (Najafzadeh *et al.,* 2013). Moreover, it was found that administration of high levels ZnO NPs was accompanied with signs of toxicity, including an increased level of LDH enzyme activity (Mohamad, 2013).

Oxidative stress is a state of abundance of reactive oxygen species (ROS), which interferes with biological processes by disturbing or damaging homeostasis. The activity of oxidative stress enzyme GSH, and GST indicates the alteration of normal homeostasis.

Also, in agreement with our results, administering low concentrations of

ZnO-NPs may enhance total antioxidant capacity of the cell by increasing the activity level of enzymatic (SOD and CAT) and non-enzymatic (protein antioxidants and glutathione) antioxidant system, reducing the ROS level and inhibiting the activity of nitric-oxide synthase and NADPH oxidase (Prasad, 2014). Furthermore, Zn inhibits the influence of lipid peroxidation products on the cellular antioxidant system (Prasad, 2014). Moreover, serum SOD activity was found to have risen in nanoparticles-treated groups due to adaptation of the fish to the nanoparticles (Sedeño-Díaz and López-López., 2012).

Previous study reported that SOD and CAT activity and gene expression fluctuated with the concentrations of ZnNPs, SOD activity and gene expression were increased at lower ZnNPs concentrations and reduced at the highest concentrations (Saddick *et al.*, 2017). The same result was reported by Xiong *et al.* (2011) who proved the inhibitory effect of ZnONPs on liver SOD in fish exposed to 5 mg/L ZnONPs.

CONCLUSION

From the present study we concluded that using of Cu and Zn nanoparticles in the aquaculture diets could be used as minimal as quarter dose for both inorganic copper and zinc; The beneficial effects for both nanoparticles elements copper and zinc on biochemical and antioxidant parameters do not decrease by decreasing amount but it become better. We need further works to investigate its action on digestibility, digestive enzymes and histopathology on liver, kidney and intestine.

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تأثير استبدال النحاس والزنك الغير عضوى فى عليقة اسماك القراميط بالنحاس والزنك فى الصورة المتناهية الصغر على الاداء والقياسات الكيميائية

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أجريت هذه الدراسة لتقييم تأثير إضافة بعض المواد متناهية الصغر من اكسيد النحاس و اكسيد الزنك على الوزن وبعض التغيرات البيوكيميائية وإنزيمات مضادات الأكسدة في اسماك القراميط. تم استخدام ٣٥٠ سمكة من اسماك القراميط بمتوسط وزن (٩٠ ± ٥ جم) وتم تقسيمهم إلي ٧ مجموعات. المجموعة الأولى: تم تغذية الأسماك علي العليقة الغذائية الضابطة التي تحتوي علي الإحتياجات الأساسية (6.25 ملجم كبريتات النحاس/كجم من المادة الجافة و ٢5 ملجم اكسيد الزنك / كجم من المادة الجافة)؛ المجموعة الثانية (Gr Cu 1): تم تغذية الأسماك علي العليقة الغذائية الضابطة التي تحتوي علي الإحتياجات الأساسية بالإضافة إلي(6.25 ملجم اكسيد النحاس متناهي الصغر/كجم من المادة الجافة و ٢5 ملجم اكسيد الزنك متناهي الصغر/ كجم من المادة الجافة) ؛المجموعة الثالثة (Gr Cu 2): تم تغذية الأسماك علي نفس العليقة الغذائية الضابطة ولكن بإضافة نصف الإحتياجات فقط من النحاس 3.125 ملجم / كجم من العلف جزيئات اكسيد النحاس متناهي الصغر؛ المجموعة الرابعة (Gr Cu 3): تم تغذية الأسماك علي نفس العليقة الغذائية الضابطة ولكن بإضافة ربع الإحتياجات فقط من النحاس 1.56 ملجم / كجم من العلف جزيئات اكسيد النحاس متناهي الصغر؛المجموعة الخامسة Gr (Zn 1): تم تغذيتها على نفس العليقة الغذائية الضابطة ، بالإضافة إلى 25 ملجم / كجم من العلف جزيئات أكسيد الزنك متناهية الصغر، بدلاً من أكسيد الزنك الغير عضوى؛ المجموعة السادسة (Gr Zn 2): تم تغذيتها على نفس العليقة الغذائية الضابطة بإضافة نصف الإحتياجات فقط من الزنك 12.5 ملجم / كجم من العلف جزيئات أكسيد الزنك متناهيه الصغر بدلا من اكسيد الزنك الغير عضوى؛ المجموعة السابعة (Gr Zn 3): تم تغذيتها علي نفس العليقة الغذائية الضابطة بإضافة ربع الإحتياجات فقط من الزنك 6.25 ملجم / كجم من العلف جزيئات اكسيد الزنك متناهي الصغر بدلا من اكسيد الزنك العير عضوى. لقد أظهرت النتائج أفضل زيادة في الوزن في المجموعات التي تم تغذيتها علي ربع الأحتياجات الغذائية من اكسيد النحاس وأكسيد الزنك متناهي الصغرمقارنة بإستخدام الأحتياجات الطبيعية. تم تدعيم النتائج بإجراء بعض الأختبارات علي بعض التغيرات الكيميائية وانزيمات مضادات الأكسدة. نستخلص من هذه الدراسة أن استخدام كمية صغيرة من النحاس والزنك في الصورة المتناهية الصغر في علائق الأسماك يحقق نتائج أفضل. تأثير النحاس والزنك في الصورة المتناهية الصغر علي التغيرات الكيميائية ومضادات الأكسدة لا تتغيرسلبا بإستخدام كمية صغيرة ولكنها تعطي نتائج افضل.