　　　　　　　　　Alaa & Al-Rudainy

EFFECT OF ZEOLITE ON AMMONIA TOXICITY AND ON SOME OF BLOOD PARAMETERS IN COMMON CARP CYPRINUS CARPIO

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ABSTRACT

The current study was undertaken to investigate the efficacy of different levels of natural zeolite on ammonia and on some of blood parameters of *Cyprinus carpio*. The experimental treatments included adding zeolite as stones placed in the bottom of aquarium : Control(0 zeolite); T1, T2 and T3 zeolite stones were added in the water at concentrations of 6 , 8 and 10 mg/l respectively. Results of ammonia concentrations ranged between 0.17 – 1.25 mg/l in T2 and in control treatment respectively from the first week up to fourth week. In T1 values of ammonia were 0.18mg/l in first week with gradually increase from 0.48 mg/l in second week and 0.78mg/l in the third week, then decline to 0.66mg/l in the fourth week, same condition was found in T2 and T3. In the second week level of ammonia was increased highly in control treatment , compared to other treatments. In the third week the increasing level of ammonia was continuously especially in control treatment followed by T3,T2 and T1 respectively. In the fourth week the level of ammonia recorded 1.25 mg/l , but decrease in T3 which reached 0.62mg/l. No mortalities were recorded in the first week for all treatments during the experimental period, while, in the fourth week in control reached up to 100% mortality compared with the T1,T2 and T3 which recorded 50, 33.33 and 33.33% respectively . Generally results showed that ammonia and mortality were decreased with increased zeolite level in water. Results of RBC ranged between 2.05 – 2.33 $\times10^6$/mm^3 in control treatment and T3 respectively .The highest number of WBC in the control treatment which recorded 24.55 $\times10^3$/mm^3 , while the lowest number was document in T2 (23.01$\times10^3$/mm^3) . The highest PCV(%) was registered inT3(26.50%). For Hb content the highest mean was reported in T3 (9.70g/dl). T3 can be considered as better treatment which contained zeolite in blood parameters followed by T2.

Key words: common carp, clinoptilolite, hematology, minerals, mortalities

تاثير الزيولايت على سمية الامونيا وبعض المعايير الدمية في اسماك الكارب الشائع *Cyprinus carpio*

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المستخلص

نفذت الدراسة الحالية لمعرفة كفاءة الزيولايت على سمية الامونيا وبعض المعايير الدمية في سمكة الكارب الشائع *Cyprinus carpio*. وزعت المعاملات التجريبية باضافة صخور الزيولايت الى مياه الحوض بالشكل التالي: معاملة السيطرة (بدون زيولايت)؛ المعاملة الاولى والثانية والثالثة تم اضافة الزيولايت بنسبة 6 و 8 و10 ملغم / لتر .اشارت النتائج الى ان تراكيز الامونيا تراوحت بين (0,17– 1,25ملغم/لتر في المعاملة الثانية (الاسبوع الاول) ومعاملة السيطرة (الاسبوع الرابع).بلغت قيم الامونيا في المعاملة الاولى 0,18ملغم/ لتر في الاسبوع الاول وازدادت تدريجياً من 0,48 ملغم/ لتر في الاسبوع الثاني الى 0,78 ملغم/ لتر في الاسبوع الثالث ، ثم انخفضت الى 0,66 ملغم/لتر في الاسبوع الرابع ، نفس الحالة ظهرت في المعاملتين الثانية والثالثة . ازدادت قيم الامونيا بشكل سريع في الاسبوع الثاني مقارنة بالمعاملات الاخرى. استمرت قيم الامونيا بالارتفاع في معاملة السيطرة تلتها المعاملتين الثانية والثالثة ثم الاولى . وصلت قيم الامونيا في الاسبوع الرابع الى 1,25 ملغم/لتر ، مع انخفاضها في المعاملة الثالثة بقيمة 0,62ملغم /لتر . لم تسجل هلاكات في الاسبوع الاول من التجربة لجميع المعاملات. سجل هلاك في جميع الاسماك في الاسبوع الرابع في معاملة السيطرة مقارنة مع المعاملة الاولى التي سجلت نسبة هلاكات 50% في الاسبوع الرابع ، بينما سجلت نسبة هلاكات في المعاملتين الثانية والثالثة نسبة 33,33% لنفس الاسبوع. أظهرت النتائج بانخفاض قيم الامونيا ونسبة الهلاكات مع زيادة الزيولايت في الماء. تراوحت قيم كريات الدم الحمر بين 2,05– 2,33×10^6 ملم^3 في معاملتي السيطرة والثالثة على التوالي. سجلت اعلى قيم لكريات الدم البيض في معاملة السيطرة اذ وصلت 24,55× 10 ^{3} ملم^3، بينما اقل قيمة سجلت في المعاملة الثانية بلغت 23,01× 10 ^{3} ملم^3. بلغت اعلى قيمة لحجم كريات الدم المرصوصة في المعاملة الثالثة لتصل الى 26,50% ،بينما تراوحت اعلى قيمة للهيموغلوبين في المعاملة الثالثة لتصل الى 9,70 غم/100مل . يتضح من النتائج بان المعاملة الثالثة هي الافضل لقيم معايير الدم تلتها المعاملة الثانية.

الكلمات المفتاحية: كارب شائع، كلينوبتيلولايت، دم، معادن، هلاكات

INTRODUCTION

High concentrations of ammonia c caused by high feed loads and high fish densities, it is an important limiting factor for intensive aquaculture. Ammonia concentrations in water should therefore be kept below species-specific threshold levels (20). Concentrations of ammonia that are acutely toxic to fish may cause loss of equilibrium, hyper excitability, increased breathing, cardiac output, and oxygen uptake, and, in extreme cases, convulsions, coma, and death , cause a reduction in hatching success, reduction in growth rate and morphological development, and pathologic changes in tissues of gills, livers, and kidneys (22). The influence on the toxicity of ammonia to fish is impacted by both duration and frequency of exposure. Duration of exposure to ammonia has been demonstrated to be a critical factor affecting fish survival - and should therefore be incorporated (24).Zeolites are microporous crystalline hydrated aluminosilicates, which have found various applications because of their very unique physicochemical characteristics such as ion exchange and adsorption–desorption properties (20). Hematological parameters are important for the evaluation of fish physiological status. Their changes depend on the fish species, age, cycle of sexual maturity and health condition (18) and they are closely related to the response of the animal to the environment , an indication that the environment where fish live could exert some influence on the hematological characteristic (11) . The present study aimed to calculate the period in which zeolite affects ammonia and determine the best treatment of zeolite cause reduce ammonia, as well as the effect of zeolite in different levels on hematological parameters including: RBCs, WBCs count, Hb content and PCV%.

MATERIALS AND METHODS

Experimental Fish: The present study was conducted at the College of Veterinary Medicine, University of Baghdad, Fish diseases Laboratory. A total of 200 fingerlings of common carp *Cyprinus carpio* with average weight of 20g. were obtained from a commercial farm (Al-Messayyab, Babylon). Fish were briefly bathed in NaCl for 5 minutes to remove all of the external parasites if present. Two weeks of acclimation for fish before start the experiment. During this time, fish were stocked in two baths with dimensions of $150 \times 80 \times 50$ cm. Then healthy fish were randomly selected and distributed into eight glass aquariums at rate of 6 fish per aquarium (two replicates / treatment) were maintained for each of the four treatments. The experimental treatments include adding zeolite as stones placed in the bottom of aquarium according to the following Control (0% zeolite) ; T1,T2 and T3 zeolite were added in the water at concentrations of 6 , 8 and 10 mg/l respectively . Each of the four treatments fish was fed commercial diet twice daily at rate of 3% body weight during the experimental trail. Using de-chlorinated tap water filled the aquarium to reach 60L.Chemo-physical parameters of water such as temperature recorded daily.

Determination of Ammonia concentration

Total ammonia was measured using drops Kit: Hanna Ammonia test kit for fresh water : determines the ammonia concentration in water in several easy steps. The kit is portable and can be used in the field as well as in the laboratory.

Chemical reaction of kit : The ammonia level in mg/l, ammonia as nitrogen is determined by a colorimetric method . The nesssler reagent reacts with ammonia , under strong alkaline conditions, to form a yellow colored complex . an addition of reagent 1(EDTA solution) inhibits precipitation of calcium and magnesium ions due to the presence of the alkaline nessler reagent.The color intensity of the solution determines the ammonia concentration

method of the kit: 1.Remove the cap from the plastic beaker . rinse the plastic beaker with water sample before filling it up to the 10 ml mark. Add 2 drops of ammonia reagent 1 for freshwater, replace the cap and mix by carefully swirling the beaker in tight circles. Add 8 drops of nessler reagent, replace the cap and mix by carefully swirling the beaker. Then remove the cap and transfer the solution into the color comparator cube, wait for 5 minutes to allow color to develop. Finally determine which color matches the solution in the cube, and record the results in mg/l NH3 -N. 6. it is

better to match the color with a white sheet at about 10 cm behind the comparator.

Hematological parameters

Blood samples were collected from four fish randomly selected from each treatment. Samples were taken from caudal vessels puncture using 3 ml plastic syringe. Blood were transferred to eppendorf coated with lithium heparin that work as anticoagulant and used for measuring the hematological parameters. Dices fluid was used to dilute blood to count RBCs and WBCs by haemocytometer chamber. Pipette was filled it to the marker (0.5) and diluted with the Dacie's fluid was filled to mark (11) to make the dilution range 1:20, mixing well and remain for 5 min. The counting chamber was filled and left for about 5 minutes, then the WBCs were counted in the four corner primary squares of the chamber and the RBCs are counted in five secondary squares of the center primary square. The following formulae were applied to calculate the WBCs, and RBCs (9):

Total WBCs count = $\frac{N \, x \, 20}{0.1}$ **cell / μl**

N: number of the cells in 1 large square ,20: dilution factor,0.1: volume factor = (width x length x height).

Total RBCs count = $\frac{N \, x \, 20x5}{0.1}$ **cell / μl ,20: dilution factor,0.1: volume factor = (width x length x height).5: 5 squares equals**

Concentration of Hb was determined by using the standard cyanomethemoglobin method described by Dacie and Lewis (8). Value of PCV% was determined according to ase method described by Klontz (16).

Statistical analysis

The Statistical Analysis System- SAS (21) program was used to detect the effect of difference factors (Treatments and Period) in study parameters. Least significant difference –LSD test one and two analysis of variation(ANOVA) were compare the significant differences between means in this study.

RESULTS AND DISCUSSION

Results showed that the mean of ammonia concentrations during experimental period ranged between 0.17 – 1.25 mg/l in T2 (first week) and in control treatment (fourth week) (Tab. 1).Results of ammonia in control treatment recorded high variations ranged from 0.22 in the first week to 1.25 mg/l in

fourth week . In T1 values of ammonia were 0.18 in first week with gradually increase from 0.48 mg/l in the second week to 0.78mg/l in third week ,then decline to 0.66mg/l in the fourth week .Also T2 and T3 recorded 0.17 mg/l in the first week and 0.68 mg/l in the fourth week . In the second week the level of ammonia was increased highly in control treatment which reached up to 0.65mg/l while the lowest value of ammonia in T2 and T3 attained 0.47 mg/l. In the third week the increasing value was continuously especially in control treatment which reached 0.99mg/l followed T3,T2 and T1 respectively. In the fourth week the increasing value of ammonia recorded 1.25 mg/l , but decrease in T3 which reached 0.62mg/l(Tab.1). The values of ammonia decreased dramatically in T1 , T2 and T3 in fourth week .Statistical analysis of control treatment showed a significant difference (P ≤ 0.05) in fourth week compared with first and second weeks , but there was no significant difference (P >0.05) compared with third week. Results of T1 showed significant difference (P ≤ 0.05) in third and fourth weeks compared with other weeks. Same state found in T2 and T3. No significant different (p>0.05) among experimental treatments in the first week. Whereas in the second week showed a significant difference P ≤ 0.05 in control treatment compared with other treatments . The same phenomena were found in third and fourth weeks . There was no significant difference (P > 0.05) among T1 , T2 and T3 in the second and fourth week (Tab.1). It can be conclude that T3 is the better treatment which reached saturation point followed by T2. No mortalities were observed in the first week for treatments throughout the experimental period. This situation can be explained by the ammonia compound values within the safe limits, also no mortality noticed in the second week for T2 andT3, but recorded one dead fish in the second week represented in control treatment and T1 (Tab. 2). In the third week two fish died in control treatment, while one dead fish was in T1, T2 and T3. In the fourth week the rest of fish dead in control treatment reached 100% mortality rate, compared with the T1 that reached 50% mortality rate. Results of mortality rate recorded 33.33% in fourth week for T2 and T3

(Tab. 2). Generally results showed that ammonia and mortality were decreased level within increased zeolite in water.

Table 1. Mean values of ammonia in different treatments during the experimental period

Period / week	Mean ± SD of Ammonia(mg/l)				LSD value
	Cont.	T1	T2	T3	
1st	0.22 ± 0.06	0.18 ± 0.05	0.17 ± 0.05	0.20 ± 0.04	0.065 NS
	A c	A c	A c	A c	
2nd	0.65 ± 0.10	0.48 ± 0.08	0.47 ± 0.09	0.47 ± 0.06	0.277 *
	A b	B b	B b	B b	
3rd	0.99 ± 0.08	0.78 ± 0.09	0.74 ± 0.11	0.72 ± 0.11	0.236 *
	A a	B a	B a	B a	
4th	1.25 ± 0.13	0.66 ± 0.08	0.68 ± 0.09	0.62 ± 0.08	0.382 *
	A a	B b	B b	B b	
LSD value	0.388	0.290 *	0.217 *	0.368 *	----

Means with different small letters in the same column and capital letters in the same row are significantly different. (P≤0.05)

Table 2. Mortality rate in different treatments during the experimental period

Treatment	No. of dead fish (week)				Mortality rate %
	1st	2nd	3rd	4th	
Cont.	0	1	2	3	100
T1	0	1	1	1	50.00
T2	0	0	1	1	33.33
T3	0	0	1	1	33.33

Applying of zeolites was directly relative to removal of ammonia compounds in this study. The present study indicated that the most efficient removal rate of ammonia by zeolite was achieved when natural zeolite was applied at 10 g /l concentrations , this results is in agreement with Farhangi et al.[11] who used granulated zeolite at 12g/l. Chiayvareesajja and Boyd [6] showed that application 2 g /l of zeolite at 2 mg /l of N-NH4 concentrations could remove the N-NH$_4$ by 80-90%. Bergero [4] revealed that in water containing 10 mg /l of N-NH$_4$ around 80% of ammonia could be removed by adding 100 g zeolite per 45 L water, so finally concentrations was about 2.06 mg /l of N-NH$_4$. In the current experiment, mortality rate were recorded in the first week with a lower percentage, then increased in the following weeks, this could be due to the small amount of feed provided to fish, little of waste products and small size of fish as well as the good ventilation of aquariums led to the continuous distribution of dissolved oxygen.A regular response was increasing in mortality rate due to increased concentration of ammonia, it appears to have a direct effect on the growth of aquatic animals and can have a serious effect on the incidence of disease, especially under less optimum conditions of temperature, dissolved oxygen and ammonia[7]. Natural zeolite was used to prevent acute ammonia toxicity and increase survival rate in beluga *Huso huso* [11] and sturgeon *Acipenser persicus* [10], Whom reported that in lethal concentration of ammonia, application of 15 and 12 g /l of the zeolite could prevent the mortality rate in beluga and Persian sturgeon, respectively. In another study, natural zeolite was used to reduce ammonia and hardness level of water for rearing of freshwater aquarium fish, angel *Pterophyllum scalare* [12]. Haile and Nakhla [14] indicated that, the addition of zeolite to the artificial feed increased survival rates of tilapia. On the other side, Larmoyeux and Piper [17] mentioned that lower incidence of diseases and mortalities when zeolite was used. According to Peyghan [20] noticed that 10g/l of zeolite could prevent carp mortality after 24 h. , and mortality percentage of fish increase with increasing of ammonia concentrations.Values of survival rate ranged between 37.78% to 90% in results of Aly et al.[3], who clearly showed that, there was significant difference (P ≤ 0.05) among the treatments with zeolite compared to control treatment. Generally, results of clinical signs in present study showed a vertical and downward swimming patterns and sudden movements, the motion of fish became

extremely slow and they displayed behavioral anomalies such as capsizing in water and loss of balance, abnormal behavior included reduced food intake , finally the fish sank down to the bottom and became motionless. Fish exposed to ammonia were observed to be highly irritable and reduced swimming activity, their bodies were covered with thick mucus and finally died with mouths opened, lack of balance and severe reaction to external factors were observed in fishes. These results agreement are in with Farhangi et al. (11) and Tenalem (25) who recorded that increased activity and respiration, followed by uncoordinated movements; finally the fish lie flat on the bottom and die. The major patho-anatomic signs include hemorrhages on the skin and gills and in the internal organs. Results of RBC ranged between 2.05 – 2.33 $\times 10^6$/mm^3 in control treatment and T3 respectively (Tab. 3) . Results showed highest count of WBC in control treatment which reached up to 24.55 $\times 10^3$/mm^3, while the lowest number represented in T2 reached 23.01$\times 10^6$/mm^3. The values of PCV% in control treatment reached up to 23.75% which increase gradually reached highest value in T3 attained 26.50 %. Hb content ranged from 6.77 g/dl in control treatment to 9.70 g/dl in T3. Results of statistical analysis of RBC showed significantly different (P ≤ 0.05) in T2 and T3 compared to control treatment and T1. No significant difference was found (P> 0.05) between T2 and T3. Tab.3 showed significant difference in WBC between control treatment, T2 and T3 compared to T1. No significant differences (P > 0.05) was observed between T1 and T2. The values of PCV(%) showed significant difference (P ≤ 0.05) in T1, T2 and T3 compared to control treatment. Also the of Hb content showed significant difference (P ≤ 0.05) between T1, T2 and T3 compared to control treatment, but there was no significant difference (P >0.05) among T1, T2 and T3.The results indicated that T 3 can be considered as better treatment which contained zeolite (10mg/l) in blood parameters followed by T2 (Tab.3).

Table 3. Hematological parameters (Means ± SD) of *C. carpio* at the end of experimental period

Treatment	RBC cells$\times 10^6$/mm^3	WBC cells$\times 10^3$/mm^3	PCV (%)	Hb g/dl
Cont.	2.05 ± 0.15 d	24.55 ± 1.11 a	23.75 ± 1.45 b	6.77 ± 0.67 b
T1	2.13 ± 0.14 bcd	23.01 ± 1.12 b	25.75 ± 1.02 a	9.24 ± 0.67 a
T2	2.26 ± 0.08 ac	23.27 ± 1.10 ab	26.25 ± 1.36 a	9.41 ± 0.65 a
T3	2.33 ± 0.11 a	23.99 ± 1.08 a	26.50 ± 1.07 a	9.70 ± 0.36 A
LSD	0.16	1.22	1.50	1.20

Means with different small letters in the same column are significantly different (p≤0.05)

Results of higher count of RBC in present study in treatments than control, a reduction in RBC numbers below the normal range in fish acts as an indicator with subsequent result of inhibition of erythropoietin in the haemopoietic organisms (18). Leukocytes are defense cells of organisms,the species with a large number of leukocyte cells are able to fight with xenobiotics more effectively. The number of leukocyte cells is influenced by physiological and environmental factors. An increase of WBC showed in control treatment of present study, as response to stress in many fishes (19). A similar increase in the WBC count in response to nitrite was also reported in *Mirgala* sp., the changes in leucocytes counts after exposure to pollutants may be associated to a decrease in nonspecific immunity of fish (5). Çelik (5) showed that the reduction in WBC number is also an indicator of impairment of immunity. Hb, WBC and RBC count results obtained from the present study are in agreement with the study of

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gilthead sea bream (19). The level of low hemoglobin in control treatment indicates that the mechanism of iron synthesis in fish is impaired. This is thought to be due to the anemic state of the fish, the hemoglobin content being caused by low-level hemolysis, and the restriction of aerobic glycolysis, which interrupts the synthesis of hemoglobin (13). The results of present study are similar to findings of the studies in which the effects of different substances on the hematological index of different fish species were investigated (1, 2, and 23).

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