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Role of Natural Zeolite (Clinoptilolite) on the Biochemical Blood Parameters and Liver Enzymes of Common Carp (*Cyprinus carpio L.*) Reared in Recirculation Aquaculture System

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Abstract

The aim of conducting this study was to evaluate the use of added natural zeolite (clinoptilolite) in common carp fingerling (Cyprinus carpio L.) breeding ponds and to demonstrate its effect on biochemical blood parameters and liver enzymes, This study was conducted for 12 weeks with an initial weight of the fish reachin (25 ± 1) gram, The study divided into Seven treatments, each treatment was divided into 3 replicates, in each replicate (10 fish), the first treatment (control treatment) Without adding zeolite, Zeolite was added to the rest of the treatments in two ways, the first way was (add inside the filter) in proportions (5,10,15) g/L for the second, third, and fourth treatments, respectively, In the second way, add zeolite (inside the rearing ponds with plastic mesh bags) in proportions (5,10,15)g/L for the fifth, sixth, and seventh treatments, respectively, The results of the study showed The fifth treatment recorded the highest values for biochemical blood parameters (CHO, NHDL, Sugar, Cortisol) when compared to the rest of the experimental treatments The results of the study showed The fifth treatment recorded the highest values for biochemical blood parameters (CHO, NHDL, Sugar, Cortisol) when compared to the rest of the experimental treatments, The best ratios for adding natural zeolite (Clinoptilolite) were (5, 15) g/L zeolite in the biofilter and (10, 15) g/L zeolite in the fish tanks, as these ratios gave the best values for the blood biochemical indicators of the common carp that were used in the experiment (CHO, T.G, NHDL, sugar, protein, albumin, ALT and cortisol) compared to the rest of the Studied standards.

Keywards: Natural zeolite, *Cyprinus carpio L.*, Biochemical Blood parameters, Ammonia.

دور الزيوليت الطبيعي (Clinoptilolite) في مؤشرات الدم الكيموحيوية وأنزيمات الكبد لاسماك الكارب الشائع (Cyprinus carpio L) المستزع في نظام الاستزراع المائي المتداور المغلق.

الهدف من إجراء هذه الدراسة هو تقييم استخدام الزيوليت الطبيعي (كلينوبتيلوليت) المضاف في أحواض تربية إصبعيات الكارب الشائع (Cyprinus Carpio L) وبيان تأثيره على مؤشرات الدم الكيموحيوية وأنزيمات الكبد، أجريت هذه الدراسة في مختبر الأسماك التابع لقسم الإنتاج الحيواني – كلية الزراعة – جامعة الأنبار – العراق، لمدة 12 أسبوع بوزن ابتدائي للأسماك بلغ (25 ± 1) غرام، قسمت الدراسة إلى سبع معاملات تجريبية، قسمت كل معاملة إلى 3 مكررات في كل مكرر (10 أسماك)، المعاملة الأولى (معاملة السيطرة) دون إضافة للزيوليت وتم إضافة الزيوليت إلى باقي المعاملات بطريقتين، الطريقة الأولى كانت (الإضافة داخل المرشح) بنسب (5،10،15) غرام/لتر للمعاملة الثانية والثالثة والرابعة على التوالي، الطريقة الثانية اضيف الزيوليت (داخل أحواض التربية بأكياس شبكية بلاستيكية) بنسب (5،10،15) غرام/ لتر للمعاملة الخامسة والسادسة والسابعة على التوالي، أظهرت نتائج الدراسة أن المعاملة الخامسة سجلت أعلى قيم لمؤشرات الدم الكيموحيوية (CHO، NHDL، Sugar، Cortisol) مقارنة ببقية المعاملات، كانت أفضل النسب لإضافة الزيوليت الطبيعي (الكلينوبتيلولايت) هي (5 ، 15) غم/لتر زيوليت في الفلتر الحيوي و(10 ، 15) غم/لتر زيوليت في أحواض الأسماك، حيث أعطت هذه النسب أفضل القيم لمؤشرات الدم الكيموحيوية لأسماك الكارب الشائع (Cyprinus carpio L.) التي استخدمت في التجربة (CHO، T.G، NHDL، السكر، البروتين، الألبومين، ALT والكورتيزول) مقارنة ببقية المعايير المدروسة.

Introduction

Aquaculture has flourished and is considered one of the most important and irreplaceable developing areas in animal food production sectors around the world [1,2]. This sector helps meet the important needs of providing food as well as food security [3,4]. Water quality is one of the most important factors in aquaculture, so water quality must be monitored to achieve optimal production in aquaculture and ensure their growth and survival, and in order to provide ideal conditions that vary depending on the type of fish, and the total ammonia nitrogen (TAN) concentration is the most important characteristic that limits the quality Water in aquaculture [5]. Ammonia in the molecular form (NH3) is more harmful than the ionic form of NH4, and To avoid these damages, the percentage of ammonia (NH3) in the water must be reduced, especially in recirculating aquaculture systems (RAS) (fish tanks)[6]. Recently, fish farmers have shown increasing interest in using zeolite as a water and sediment treatment compound in their aquaculture farms to remove toxic gases and increase water pH [7,8]. A number of researchers have so far identified zeolite as an environmentally friendly material [9,10,11]. There are recommendations regarding its use in improving water quality by removing gases and harmful substances such as (ammonia, nitrite) and heavy metals. [12,13]. Zeolite is classified as a wet crystalline aluminosilicate mineral and its source is volcanic rocks [14,15]. The porous structure of zeolite supports some special physical and chemical properties including ion exchange, adsorption, molecular screening and catalysis, making zeolite an effective agent in trapping and repelling aquatic toxic substances and also facilitating the control of water pH [16,17]. [18] Recommended the use of zeolite in aquaculture (Nile tilapia) which achieved excellent results in improving the quality of tank water by removing harmful gases (ammonia and nitrite), reducing the content of heavy metals, and improving growth performance and physiological condition. In fish farming, zeolite is considered a water treatment compound,The sediment is a relatively safe substance (inert substance) that has a short environmental life and does not cause harmful effects on aquatic organisms when used in the recommended dose. Adding zeolite to the water reduced high ammonia levels, raised dissolved oxygen levels in the water, and restored pH balance , Adding zeolite to fish feed was found to have a beneficial effect on fish metabolism, body composition, and metabolic waste disposal [19,20].

Materials and Methods

Experimental Treatment

Operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025 and College of Agriculture, University of Anbar, (120) common carp fish (Cyprinus carpio L.) were used in this experiment, These fish were distributed into seven experimental treatments (30 fish/treatment), and each treatment was divided into three replicates (10 fish/replicate), The tanks volume was (1) m3 and the water capacity was (1000) litre, The experimental fish were fed a commercial diet (pelt) and its components are shown in a Table 1.

Table 1 . Components of the commercial feed used to feed the common carp fish used used in the experiment.

Component	Percentage
Crude protein	32 %
Crude fat	4 %
Crude Fibers	4 %

In addition to minerals, vitamins, amino acids, antioxidants and antifungals.

Experimental Fish

The study continued for (90) days, in which fingerlings of common carp (Cyprinus Carpio L.) were used with an average weight of (25 ± 1) grams/fish. During the experiment period, weights were taken for all experimental fish individually every (15) days.

Statistical Analysis

A completely randomized design (C.R.D.) was used to analyze the experimental data statistically, as it included the effect of the experimental parameters on the studied traits, by following the general linear model and using the ready-made SAS statistical program, version 9.1 [21]. The presence of statistically significant differences between means was tested using Duncan's multinomial test [22] at the level of significance($P \geq 0.05$).

Results and Discussion

During the experiment period, pH values ranged between (7.8 - 8.4), temperature between (24- 30) °C, dissolved oxygen concentration (4.2 - 6.3) mg L-1, ammonia concentration (0.21 - 0.38) mg L-1, nitrite. (0.16 - 0.49) mg/L, nitrates (18.64 - 93.89) mg L-1, these conditions are suitable for raising common carp (Cyprinus carpio L.), The results of our study are consistent with what was stated by [23,24], as he showed that the concentration of dissolved oxygen in the water suitable for the growth of common carp fish must be no less than (3) mg L-1. as he stated that the appropriate pH concentration for the growth of common carp fish must be no less than 6.4 and no more than 8.6, and The concentration of ammonia that is toxic to fish is (2.2) mg L-1. The presence of ammonia, nitrite and nitrate are a concern in closed aquaculture systems and must be monitored regularly as their production is directly related to nutrition, feed quality, feeding rate, fish weight and temperature[25].

The results of the statistical analysis of biochemical blood parameters showed that there were no significant differences at the level of significance ($P \leq 0.05$) between the experimental treatments in the values of (HDL, Globulin and AST), Treatment T5 was significantly superior to treatments T2,T4, T6, and T7 in CHO values, The values were (298, 201.66, 221.3, 222.6, and 166.3), respectively, where treatment T7 recorded the lowest values.

Treatment T3 recorded a significant difference over treatments T2,T4, and T7 in blood triglyceride (TG) values, The values were (560.6) for the third treatment and (445.33, 385.66, 182.6) for treatments T2,T4, and T7, respectively, as treatment T7 recorded the lowest values, Treatment T5 was significantly superior to treatments T2, T3, T4, T6, and T7 in the level of NHDL, and the values were (239, 145, 173.6, 164, 165.6, 123.3), respectively, while no significant differences were recorded between it and the control treatment.

As for blood sugar level, treatments T5, T6, and T7 were significantly superior to treatment T2, and the values were (158.66, 144, 140.60, 105.33), respectively, while no significant

differences were recorded between treatments T1, T2, T3, and T4 (136.33, 105.33, 136.30, 133.33) respectively in this standard.

As for blood proteins, T2 clearly outperformed the treatments T4, T6, and T7 with their values (74, 61.66, 60.33, 47.60), respectively, while significant differences between the treatments T1, T3, and T5 (74, 72, 69, and 62.33) prevail on the right, The control treatment was significantly superior to the T4, T5, T6, and T7 treatments in albumin values, and the values were (53.66, 42, 40.66, 39, 24.6), respectively, while no significant differences were recorded between them and the 2T and 3T treatments (53.66, 49.33, 48.33) respectively, Treatment T2 recorded an increase in the values of the liver enzyme ALT, as it was significantly superior to the rest of the experimental treatments and recorded the highest value, reaching (104.66), while the control treatment recorded the lowest values, reaching (14.86).

Treatment T5 was significantly superior at a significant level ($P \leq 0.05$) to the rest of the experimental treatments in cortisol values, as it recorded the highest value, which was (1454.51), while treatment T7 recorded the lowest value, which was (323.6), High Cortisol values in the blood of the experimental fish are evidence of stress, Therefore, the T5 treatment fish recorded the highest stress values compared to the rest of the experimental treatments, while the T7 treatment fish recorded the lowest stress values compared to the rest of the control treatments.

The serum activities of ALT and AST enzymes improved significantly when adding zeolite, which indicates that adding zeolite at different levels does not have a harmful effect on the health of Nile tilapia fish, The addition of zeolite to common carp breeding ponds also reduced the percentage of excess ammonia in the pond water[26]. This result is consistent with what was stated[27],[28],[29]. In addition, the presence of zeolite in fish tanks water played a role in lowering the pH[30]. These results are consistent with the results of our study and what was reported[31] For rainbow trout fish,[32],[33] Who added zeolite to the diet of Nile tilapia. Also explain[34] that the Serum proteins help produce more energy during stressful conditions (detoxification) to overcome this stress. The results of our study showed that with the increase in the inclusion of zeolite in the experimental treatments, in return, the values of blood proteins decreased, as it was confirmed[35] Zeolite and perlite supplements did not have a significant effect on total blood serum protein concentrations When adding zeolite and perlite in different proportions to common carp fish diets Cyprinus carpio L., The results of our study are consistent with this view.

The results of our study showed that blood sugar levels decreased with the addition of zeolite in the experimental fish tanks, and this result is consistent with what was reported[36] Who reported that blood sugar levels decreased with increasing inclusion of zeolite in sea bream farming (Sparus aurata), The exact mechanism of the effects of zeolite on the biochemical properties of fish blood serum is currently unknown, and we have no explanation for this response.

Table 2. The effect of adding natural zeolite on the blood parameters of common carp fish used in the experiment.

	Treatment						
	T 1	**T 2**	**T 3**	**T 4**	**T 5**	**T 6**	**T 7**
CHO	259±21.12 ab	201.66±19.71 bc	231.3±11.20 abc	221.3±35.46 bc	298±16.50 a	222.6±22.1 bc	166.3±11.7 c
TG	475.33±23.69 abc	445.33±20.85 bc	560.6±33.76 a	385.66±64.8 c	541.6±29.79 ab	488.6±9.82 abc	182.6±5.04 d
HDL	56.33±5.36 a	56.66±4.09 a	57.6±4.33 a	57.33±2.18 a	59±4.72 a	57±5.29 a	43±2.30 b
NHDL	202.66±17.38 ab	145±17.89 bc	173.6±7.42 bc	164±37.64 bc	239±15.01 a	**165.6±17.7 bc**	**123.3±11.2 c**
Sugar	136.33 ± 4.80 ab	105.33± 4.63 b	136.3± 4.63 ab	133.33±15.62 ab	158.66±13.24 a	144± 14.57 a	140.6±7.85 a
Protein	72±3.60 ab	74±5.85 a	69±2.30 ab	61.66±1.85 b	62.33±3.17 ab	60.33±4.33 b	47.6±2.60 c
Albumin	53.66±1.76 a	49.33± 2.84 ab	48.33± 1.20 ab	42 ± 1.15 b	40.66 ± 6.43 b	39 ± 4.61 b	24.6 ± 2.60 c
Globulin	18.33±2.33 a	24.66± 8.11 a	20.66± 2.33 a	19.66± 0.88 a	21.66± 4.66 a	21.33±4.63 a	23 ± 4.50 a
ALT	14.86±0.40 c	104.66± 3.83 a	**43.53± 6.11 b**	17.86± 2.55 c	15.23± 0.61 c	15.66±0.99 c	**35.93±3.59 b**
AST	211.3±42.20 a	269± 42.33 a	228.3±17.9 a	188.6± 13.95 a	205.6± 20.34 a	210.6±4.63 a	195.3±18.0 a
Cortisol	1180.6±92.16 b	917.9± 43.95 c	**874.26±9.09 c**	718.1± 16 d	1454.5± 44 a	**1009.6±22.6 c**	323.6±56.6 e

T1: Control, T2: Add zeolite at a rate(5 g/L in filter) (90 day), T3: Add zeolite at a rate(10 g/L in filter) (90 day), T4: Add zeolite at a rate(15 g/L in filter) (90 day), T5: Add zeolite at a rate(5 g/L in tank) (90 day), T6: Add zeolite at a rate(10 g/L in tank) (90 day), T7: Add zeolite at a rate(15 g/L in tank) (90 day)

Conclusion

The results of this study showed that the best ratios for adding natural zeolite (clinoptilolite) were (5 , 15) g/L zeolite in the biofilter and (10 , 15) g/L zeolite in fish ponds, as these ratios gave the best values for the biochemical blood parameters of the Common carp fish (Cyprinus carpio L.) that used in the experiment (CHO, T.G, NHDL, Sugar, Protein, Albumin, ALT and Cortisol) compared to the rest of the experimental parameters.

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Conflict of interest

The authors declare that there are no

conflicts of interest.

References

[1] FAO, 2022. The state of world fisheries and aquaculture 2022. Towards blue transformation. Rome, FAO.

[2] Shen, Y., Ma, K., & Yue, G. H. (2021). Status, challenges and trends of aquaculture in Singapore. Aquaculture, 533, 736210.

[3] Belton, B., Haque, M. M., & Little, D. C. (2012). Does size matter? Reassessing the relationship between aquaculture and poverty in Bangladesh. Journal of Development Studies, 48(7), 904-922.

[4] Zhang, W., Belton, B., Edwards, P., Henriksson, P. J., Little, D. C., Newton, R., & Troell, M. (2022). Aquaculture will continue to depend more on land than sea. Nature, 603(7900), E2-E4.

[5] M Shalaby, A., K Khames, M., Fathy, A., A Gharieb, A., & A Abdel-Hamid, E. (2021). The impact of zeolite on ammonia toxicity, growth performance and physiological status of the Nile tilapia (Oreochromius niloticus). Egyptian Journal of Aquatic Biology

and Fisheries, 25(1), 643-663.

[6] Maulini, R., Sahlinal, D., & Arifin, O. (2022, April). Monitoring of pH, amonia (NH3) and temperature parameters aquaponic water in the 4.0 revolution era. In IOP Conference Series: Earth and Environmental Science (Vol. 1012, No. 1, p. 012087). IOP Publishing.

[7] Adhikary, R. K., Rahman, M., & Asif, A. A. (2018). Present status of aqua-medicines used in aquaculture at Jessore sadar upazila, Bangladesh. Asian Journal of Medical and Biological Research, 4(3), 288-297.

[8] Al Asif, A., Hossain, A., Hamli, H., Islam, S., & Kabir, S. L. (2021). Research trends of aqua medicines, drugs and chemicals (AMDC) in Bangladesh: the last decade's (2011-2020) story to tell.

[9] Mondal, M., Biswas, B., Garai, S., Sarkar, S., Banerjee, H., Brahmachari, K., ... & Hossain, A. (2021). Zeolites enhance soil health, crop productivity and environmental safety. Agronomy, 11(3), 448.

[10] Rani, P., & Srivastava, R. (2020). Comprehensive Understanding of the Eco-Friendly Synthesis of Zeolites: Needs of 21st Century Sustainable Chemical Industries. The Chemical Record, 20(9), 968-988.

[11] Lima, R.C., Bieseki, L., Melguizo, P.V., Pergher, S.B.C., 2019. Environmentally friendly zeolites. Eng. Mater. https://doi.org/10.1007/978-3-030-19970-8_1.

[12] Ahmad, R., & Mirza, A. (2018). Adsorptive removal of heavy metals and anionic dye from aqueous solution using novel Xanthan gum-Glutathione/Zeolite bionanocomposite. Groundwater for sustainable development, 7, 305-312.

[13] M Shalaby, A., K Khames, M., Fathy, A., A Gharieb, A., & A Abdel-Hamid, E. (2021). The impact of zeolite on ammonia toxicity, growth performance and physiological status of the Nile tilapia (Oreochromius niloticus). Egyptian Journal of Aquatic Biology and Fisheries, 25(1), 643-663.

[14] Wise, W.S., 2013. Minerals | Zeolites, in: Earth Systems and Environmental Sciences. p. 302.

[15] Passaglia, E., & Sheppard, R. A. (2001). The crystal chemistry of zeolites. Reviews in mineralogy and geochemistry, 45(1), 69-116.

[16] Aly, H. A., Abdel-Rahim, M. M., Sallam, G. R., Lotfy, A. M., & Abdelaty, B. S. (2020). Use of Natural Zeolites as a Detoxifier of Heavy Metals in Water and the Flesh of Reared European Seabass. Croatian journal of fisheries, 78(3), 121-132.

[17] Ghasemi, Z., Sourinejad, I., Kazemian, H., & Rohani, S. (2018). Application of zeolites in aquaculture industry: a review. Reviews in Aquaculture, 10(1), 75-95.

[18] Wang, S., & Peng, Y. (2010). Natural zeolites as effective adsorbents in water and wastewater treatment. Chemical engineering journal, 156(1), 11-24.

[19] Ghiasi, F., & Jasour, M. S. (2012). The effects of natural zeolite (clinoptilolte) on water quality, growth performance and nutritional parameters of fresh water aquarium fish, angel (Pterophyllum scalare). International Journal of Research in Fisheries and Aquaculture, 2(3), 22-25.

[20] Ibrahim, I. H., El-Bab, A. F. F., & El-Din, M. T. S. (2016). Evaluation of natural zeolite (clinoptilolite) as a feed additive in Nile tilapia fish diets and its influences on growth performance, genetic traits and fish health. Abbassa International Journal of Aquaculture, 9, 253-287.

[21] SAS Institute INC. 2002. SAS/STAT User's Guide: Version 9.1. (Cary, NC, SAS Institute, Inc.).

[22] Duncan, D. B. (1955). New multiple ranges and multiple F. Test Biometrics, 11, 1-42.

[23] Engelmann, B., & Massberg, S. (2013).

Thrombosis as an intravascular effector of innate immunity. Nature Reviews Immunology, 13(1), 34-45.

[24] Cuesta, A., Esteban, M.Á. and Meseguer, J., 2003. In vitro effect of chitin particles on the innate cellular immune system of gilthead seabream (Sparus aurata L.). Fish and Shellfish Immunology, 15(1), 1-11.

[25] Sánchez, I. A., Bastos, R. K. X., and Lana, E. A. T. (2018). Tilapia rearing with high rate algal pond effluent: ammonia surface loading rates and stocking densities effects. Water Science and Technology, 78(1): 49-56.

[26] Ali, B. A., Al Amir, M. A., & Eid, A. H. S. (2022). Effects of Dietary Natural Zeolite (Clinoptilolite) Levels on Water Quality, Growth Performance and Feed Utilization of Nile Tilapia (Oreochromis niloticus) Fingerlings. Journal of Animal, Poultry & Fish Production, 11(1), 65-73.

[27] Ghasemi, Z., Sourinejad, I., Kazemian, H., & Rohani, S. (2018). Application of zeolites in aquaculture industry: a review. Reviews in Aquaculture, 10(1), 75-95.

[28] Ghiasi, F., & Jasour, M. S. (2012). The effects of natural zeolite (clinoptilolte) on water quality, growth performance and nutritional parameters of fresh water aquarium fish, angel (Pterophyllum scalare). International Journal of Research in Fisheries and Aquaculture, 2(3), 22-25.

[29] Farhangi, M., & Hajimoradloo, A. M. (2011). The effect of zeolite (Clinoptilolite) in removing ammonia lethal concentration in Rainbow Trout (Oncorhynchus mykiss).

[30] Yıldırım, Ö., Türker, A., & Şenel, B. (2009). Effects of natural zeolite (Clinoptilolite) levels in fish diet on water quality, growth performance and nutrient utilization of Tilapia (Tilapia zillii) fry.

[31] Sheikhzadeh, N., Kouchaki, M., Mehregan, M., Tayefi-Nasrabadi, H., Divband, B., Khataminan, M., ... & Shabanzadeh, S. (2017). Influence of nanochitosan/zeolite composite on growth performance, digestive enzymes and serum biochemical parameters in rainbow trout (Oncorhynchus mykiss). Aquaculture Research, 48(12), 5955-5964.

[32] Nssar, K. M. (2019). Effects of dietary natural clay zeolite supplementation on growth performance, hematological parameters and body composition of Nile Tilapia, Oreochromis niloticus l. Fingerlings. Annals of Agricultural Science, Moshtohor, 57(3), 681-686.

[33] Zenhom, O. A., Refaey, M. M., Ayyat, A. N., & El-Sayed, H. G. (2020). Impact of natural zeolite on growth performance, feed efficiency, and carcass composition of Nile tilapia (Oreochromis niloticus L.) cultured at different stocking densities. Abbassa Int. J. Aqua, 13, 191-210.

[34] Khodanazary, A., Boldaji, F., & Dastar, B. (2013). Effects of dietary zeolite and perlite supplementations on growth and nutrient utilization performance, and some serum variables in common carp,(Cyprinus carpio). Turkish Journal of Fisheries and Aquatic Sciences, 13(3).

Kanyilmaz, M. (2012). The use of natural zeolite (Clinoptilolite) in gilthead sea brem (Sparus aurata) culture. Cukurova University (with english abstract, PhD Thesis).