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Effect of Clinoptilolite Zeolite to Prevent Mortality of Beluga (*Huso huso*) by Total Ammonia Concentration

Alireza Asgharimoghadam, Esmail Gharedaashi, Salar Montajami, Hamed Nekoubin, Mahan Salamroudi and Hojatollah Jafariyan

Department of Fishery,
Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: The experiments for determination of clinoptilolite efficiency to prevent mortality of beluga by lethal concentration form total ammonia have been studied. The fish weight was 9.5-21 g that exposed to four different concentrations (10, 15, 20, 25 mg/l) of total ammonia. A group of fish (13 fish) considered as control. Under stable condition, the lethal concentration of total ammonia was 25mg/l during 24 hours. In the lethal concentration of total ammonia different amount of 2, 5, 10, 13, 15 g/l zeolite were used. Application of 15g/l zeolite could prevent the mortality. Samples for histopathological examination were taken from gill, liver and kidneys. Histopathological findings showed that major lesion were hemorrhage, hyperemia, hyperplasia and epithelial cells necrosis. There were degenerated tubules of kidney, expansion of Bowman's capsule and hepatocytes necrosis by observations too.

Key words: Ammonia · Clinoptilolite · Beluga (*Huso huso*)

INTRODUCTION

Ammonia at relatively low concentration can have negative effects on fish tissues and physiological factors such as growth rate, oxygen consumption and disease resistance [1] and can restrict yields in intensive fish culture [2, 3]. The two principal methods for removing ammonia in water are: (1) nitrification and (2) ion exchange. Nitrification is a two-step oxidation of ammonia to nitrate by autotrophic bacteria and is an essential part of a recirculation fish culture system [4]. For nitrification, materials such as oyster shell, rock and, activated carbon, etc. are used to prepare a substrate for bacteria. Ion exchange is a process in which ions of an exchanger (synthetic or natural resin) are exchanged with certain ions in wastewater. Some natural resins, such as zeolite, are used in removing ammonia from wastewater culture systems. One of the best zeolites in ammonia removal is clinoptilolite [5, 6]. Zeolites are alumnosilicate clay minerals that are mined from natural deposits or synthesized from other clay minerals [6, 7]. Zeolite crystals consist of a framework of SiO^{-4} tetrahedral into which Al^{+3} has been substituted for some of the Si^{+4} [8]. The substitution of aluminum for silicon left unsatisfied negative charges on the surfaces of zeolite crystals. In nature, these charges were satisfied by attraction of cations from the surroundings [9]. However, the cat ions on zeolite readily exchange with other cations. These minerals have been widely used as cation exchange media for use in water softening and other applications [7]. For example, zeolite removes calcium from water by exchange with sodium as illustrated below:

Zeolite - 2Na + Ca^{+2} = Zeolite – Ca + $2Na^{+}$ [8]

When zeolite for water softening is saturated with calcium, it maybe backwashed with a sodium chloride solution to remove the calcium and used again. Zeolite can exchange cations on its surface for ammonium to lessen ammonia nitrogen concentration in water [10].

The present study aimed to assess the using of zeolite to prevent mortality of Beluga (*Huso huso*) by total ammonia concentration.

Corresponding Author: Alireza Asgharimoghadam, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

MATERIALS AND METHODS

The experiments carried out under stable conditions of temperature and pH (T= 16±1°C and pH= 7.7:7.8) by the water static method during 24 hours. 250 fishes (weight 9.5-21g) of Beluga Sturgeon were supplied of center of Institute of Aquaculture of the Marjani for Sturgeon, Golestan. Iran. They were kept in ponds to 25*2*0.8 meter size.

The stocking density was 13 fish per each experimental group. Ammonia concentration was produced by adding ammonium chloride (NH_4Cl) to water. After 24 hours, Total ammonia (N-NH_4) was determined with a spectorophotometric method. Zeolite used was clinoptilolite with pure 90%. For the preparation of zeolite, samples were crushed to different size from 0.125 mm to 0.15 mm diameter. Fishes were kept at different concentration of N-NH_4^+ (as 0, 10, 15, 20, 25 mg/l) at 5 groups with 13 fishes in each group. The determination of concentrations was according initial experiments, so fish mortality was from 0% to 100% in these concentrations. A group of fish was as a control.

In lethal concentration of ammonia used amount 0, 2, 5, 10, 13, 15 g/l of zeolite in the experiments. Air-stone has been used in treatment as an aerator. Samples were taken from gill, kidney and liver of fish and histopathological sections were prepared.

RESULTS

Results obtained in the trial are shown in Fig. 1. Mortality percentage of fish exposed to different concentration of N-NH_4^+ (0, 10, 15, 20, 25 mg/l) after 24 hours is shown in Fig. 1. All fish were survived in control. Mortality percentage of fish was 0, 15.5, 38.4, 61.38 and 100% respectively in different concentrations of ammonia (Fig. 1). The obtained results of Fig. 1 show that mortality percentage of fish increase with increasing ammonia concentration. The LC_{50} (median lethal concentration) was 15.77 mg/l N-NH_4^+ by used of linear regression and relationship between of mortality percentage and different ammonia concentrations (Fig. 2). The concentration of N- NH_3 (UN-ionized ammonia nitrogen) were calculated by N-NH_4 according below equation [11]:

$$N\text{-}NH_3 = N - NH_4 / 10^{(10.07 - 0.033\,T - PH)} + 1$$

In lethal concentration of N-NH_4^+ (25 mg/l) used amount of 2, 5, 10, 13 and 15 g/l of zeolite. Survival percentage of exposed to lethal concentration of N-NH_4^+ were 0, 20, 60, 90, 100% respectively after 24 hours (Fig. 3). Experiments indicated survival percentage of fish increase when zeolite concentration is increased too.

The mortality of fish was earlier time more. The most efficient removal rate of total ammonia by zeolite was achieved when zeolite was applied at 15g/l concentration.

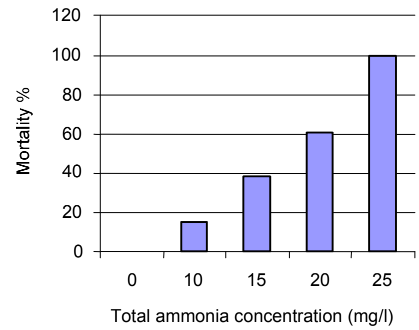


Fig. 1: Histogram showing the changes of fish mortality to different concentrations of ammonia under T= 16°C. DO=10-11mg/l, PH= 7-7, 7-8, water value = 50 l during 24 hours

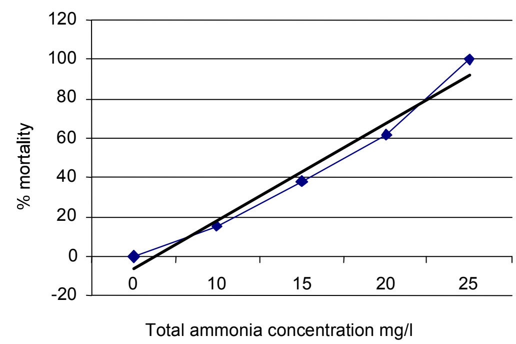


Fig. 2: Linear regression of fish mortality to different concentrations of ammonia under T= 16°C. DO=10-11mg/l, pH= 7-7, 7-8, water value = 50 l during 24 hours

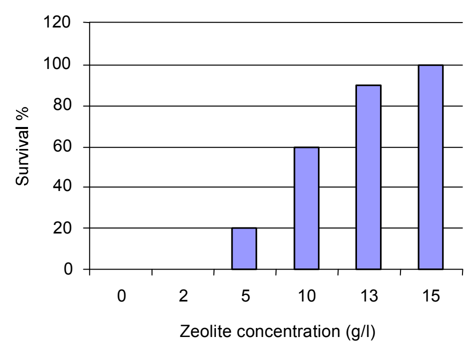
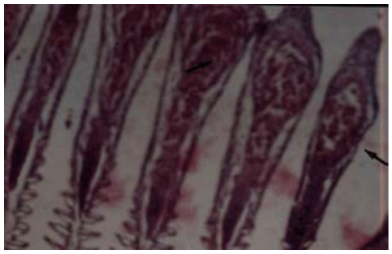
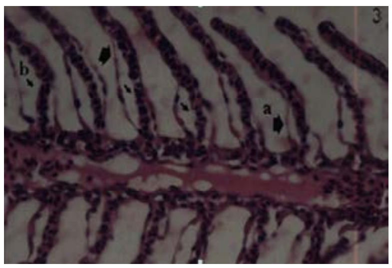


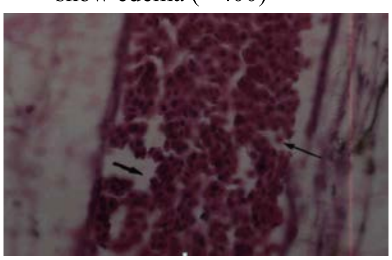
Fig. 3: Histogram showing the changes of fish survival to zeolite amount under of stable conditions of T= 16 °c. DO=10-11mg/l, pH= 7-7, 7-8, water value = 50 l during 24 hours



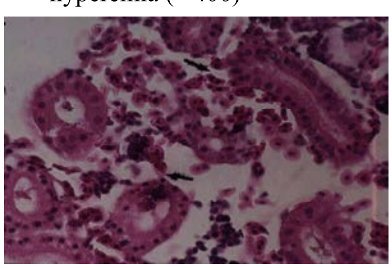
Photograph 1: Histological section of gills that exposed to lethal ammonia toxicity. Arrows show distention of secondary lamella (* 200)



Photograph 2: Histological section of gills that exposed to 20mg/l of ammonia toxicity. Arrows show edema (* 400)



Photograph 3: Histological section of gills that exposed to lethal ammonia toxicity. Arrows show hyperemia (* 400)



Photograph 4: Histological section of kidneys that exposed to lethal ammonia toxicity. Arrows show hemorrhage (* 200)

This means, that application 15g/l zeolite could prevent mortality of fishes in lethal concentrations of total ammonia after 24 hours. Acute ammonia toxicity can cause behavioral abnormalities such as slowly gill ventilation, air gulping, bending muscles, increase of opercular and buccal movement and hyper excitability.

The studied histological samples (Photographs 1-4) showed that, the common lesions of fish gill exposed to ammonia lethal concentration were hyperplasia, edema, hyperemia, hemorrhage, expansion of secondary lamella and epithelial cells necrosis of gill and inflammation. In control group and zeolite without ammonia group less hyperplasia in tip of gill filaments and edema observed too. The major lesions in kidney were such as expansion of Bowman's capsule, hemorrhage, hyperemia, degenerated tubules of kidney, epithelial cells necrosis of kidney and a lot of monocellular. In control fish, kidneys are absorbing any lesions. The lesions in liver were hyperemia, hemorrhage, inflammatory cells infiltration and hepatocytes necrosis. In control group and zeolite without ammonia group absorb any lesions. The fish exposed to lethal concentrations of $N\text{-}NH_4^+$ adding clinoptilolite zeolite the lesions were observed just less toxicity of ammonia.

DISCUSSION

The efficiency of clinoptilolite zeolite to prevent fish mortality by ammonia was studied. Many experiments are run on toxicity of ammonia in fish culture system [12-16]. Since, the ammonia toxicity is important for fish and shrimp that were reported by several authors [2, 3, 17, 18].

Chen [13] indicated that, levels of $N\text{-}NH_3$ from 0.01 to 1.0 mg/l reduced growth of freshwater fish. He reported maximum acceptable toxicant concentration (MATC) for shrimp is below 5 mg/l of $N\text{-}NH_4$ or 0.35 mg/l of $N\text{-}NH_3$. Loyd [19] reported for the rainbow trout the toxicity ratio of dissolved ammonia, zinc salt, lead and copper, as well as, phenols begin to increase markedly below 60% oxygen saturation. Wickens [20] reported that a level above 0.1 mg/l of $N\text{-}NH_3$ significantly reduced growth of *macrobranchium rosenbergii* post larvae to 60-70% in salinity of 0.5 -4 ppt for 6 weeks of exposure.

The present experiments show, addition of NH_4Cl salt to trials as an $N\text{-}NH_4$ causes to appear some nominal signs on fish behavior. So, the experiments showed at high ammonia concentration (>10 mg/l $N\text{-}NH_4$) signs were happened such as slowly gill ventilation, air gulping, bending muscles, increase of opercular and buccal movement and hyper excitability. Similar results were also obtained by [15, 16]. Various reasons are found for fish mortality as following:

- Degenerated tubules of kidney and glumroles, they cause to disorganize kidney function [11, 16].
- Disorient of osmoregulation conclusion gill harm [15].

- Declination of high and special of ammonia to brain, that it causes convulsion [11].
- Ability reduction of oxygen transfer by blood on the hand and increasing of plasma ammonia on the other [15, 18].

Similar results were obtained about the present experiments. The obtained ammonia lethal concentration was 25 mg/l of $N\text{-}NH_4$ after 24 hours for Beluga Sturgeon. Under the experimental conditions, 24h-LC_{50} was 15.77 mg/l of $N\text{-}NH_4$ or 0.28 mg/l of $N\text{-}NH_3$ (Fig. 2). The most mortality was happened at earlier hours. Svoboda and Vykusova [11] reported that 24h-LC_{50} was 0.05-0.08 mg/l of $N\text{-}NH_3$ for rainbow trout. Muir [21] reported the threshold effective levels of $N\text{-}NH_3$ on growth for rainbow trout was 0.130mg/l. According Knoph [15] 48h-LC_{50} was 59.4 mg/l of $N\text{-}NH_4$ (0.34 mg/l of $N\text{-}NH_3$) for smolts salmon. The obtained concentration of the present experiment condition was the best condition, in fish culture system. The various factors exacerbate ammonia toxicity, such as CH_4, H_2S and low oxygen and high temperature maybe, they are reasons to different kinds of experiment. The most efficient of removal rate of $N\text{-}NH_4$ by zeolite was achieved when zeolite powder was applied at 15g/l concentration, so application of 15g/l of zeolite at lethal concentration of ammonia could prevent the mortalities. According Peyghan findings [16] application of 10 g/l of zeolite concentration could prevent carp mortality at lethal concentration of total ammonia (150 mg/l) after 24 hours. However, application of zeolite to reduce ammonia toxicity on fish was demonstrated. It may be prevented late or it can be affect growth and fish physiological functions.

Chiayvareesajja and Boyd [22] and Mokarami and Emadi [18] demonstrated, finely zeolite is more effective than coarser zeolite for ammonia removal. Therefore zeolite powder was used in trials. Tests revealed that zeolite quickly reduced $N\text{-}NH_4$ concentration after the first hours. Amount of $N\text{-}NH_4$ decreased drastically more with increasing of zeoite concentration. However, total ammonia concentration was about 0.6 mg/l finally. This means, that $N\text{-}NH_4$ concentration only decreased by 80-90%. In order to, the complete removal of $N\text{-}NH_4$ wasn't achieved even when zeolite was applied at 15 g/l concentration. These finding are in agreement with studies of others [16, 18, 22]. Chiayvareesajja and Boyd [22] showed that application 2g/l of zeolite at 2mg/l of $N\text{-}NH_4$ concentration could remove of $N\text{-}NH_4$ by 80-90%. Brgero [5] revealed that in water containing 10mg/l of $N\text{-}NH_4$ around 80% of ammonia could be removed by adding 100g zeolite pre 45L water, so finally concentration was about 2.06 mg/l of $N\text{-}NH_4$. Application of 15 g/l of zeolite doesn't seem to be essential on fish ponds, because it is expensive. In second, the present lethal concentration isn't available in fish ponds at all. Therefore we just suggest applying about 2-5 g/l of zeolite to prevent fish mortality. However, the use of zeolite seems promising in intensive aquaculture systems.

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