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RESEARCH ARTICLE

Effect of a Natural Adsorbent Mixture (Zeolite and Leonardite) on the Reduction of Ammonia Caused by Fish Feed

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ABSTRACT

In this this research, the utilization of zeolite (clinoptilolite) and leonardite mix, which are the natural adsorbents that can be used to provide optimum water conditions for aquaculture, was investigated. Three groups with 3 replications were formed and a commercial aquarium fish feed having 47.5% crude protein was added as the ammonia factor in three different concentrations (0.2 g feed/500 ml tap water, 0.4 g/500 ml tap water, 0.6 g/500 ml tap water). Ammonia increases resulted from 3 different amounts of feed were monitored for 7 days. At the end of this period, the adsorbent mixture, which has water-regulating properties, was added to the experimental groups at a ratio of 1:2 (clinoptilolite:leonardite) to remove ammonia, which is harmful for aquatic organisms, and ammonia decreases was determined at regular intervals. NH_3 value reached its highest (0.7 mg/L) at the end of the stage where the ammonia values from the feed were measured. After this period, it started to decrease with the addition of natural adsorbents (mixed clinoptilolite-leonardite) and the lowest ammonia value was determined at the end of the 6th measurement (0.07 mg/L). As a result of this study, it was determined that the clinoptilolite:leonardite mixture has a positive influence on ammonia removal in freshwater aquariums.

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1. Introduction

Water parameters such as temperature, pH, dissolved oxygen, ammonia, etc. are the parameters that should be in optimal conditions for sustainable aquaculture (M. Öz et al., 2017; Ü. Öz et al., 2017). Among these parameters, ammonia exists in two forms in water as ammonia (NH_3-N) and/or ammonium ion (NH_4-N). Changes in the pH and temperature of the water affect the presence of ammonium and ammonia (Aly et al., 2016). In aquaculture systems, removal of excess nitrogenous compounds is a mandatory process. Because especially ammonia (NH_3) is harmful to many aquatic organisms (Lin et al., 2023). It is possible to remove this harmful factor from the environment with mechanical, biological, and chemical treatments. To achieve this, adsorbent materials such as zeolite, diatomite, bentonite, and leonardite, etc. are used (Şahin et al., 2018; Öz et al., 2022; Şahin, 2022).

Naturally abundant and mined zeolites are crystalline hydrated alumina silicates of alkali and alkaline earth metals. Clinoptilolite type zeolite can be used for many processes such as molecular sieve, cation exchange and adsorption (Rodrigues et al., 2007; Mazeikiene et al., 2008; Şalcıoğlu, 2022). Scientific studies have determined that zeolite has high efficiency in ammonia removal from wastewater and freshwater (Aly et al., 2016; Ghasemi et al., 2018; Skleničková et al., 2020). Furthermore, the use of zeolite as a dietary supplement or to remove ammonia from water has more useful

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for aquaculture (Öz et al., 2016; Şahin et al., 2019; Aly et al., 2020; Öz et al., 2021).

Leonardite emerges during coalification and has a humic acid content ranging in 40-85%. Leonardite is also referred to as a completely natural organic matter which contains carbon, macro and micro nutrients, in addition to high levels of humic acids, and has not reached the coal form. It is of significant economic value due to the high content of humic acids (İstanbulluoğlu, 2012). It is black-brown, appears like compacted soil, and can be easily crumbled by hand. Its pH varies between 3 and 5. It has a low solubility in water (Engin & Cöcen, 2012).

Unconsumed feed, fish excrement, and metabolic wastes discharged through gills in recirculated aquaculture units such as aquarium systems accumulate and cause ammonia formation in the water. Among the objectives of this study is to investigate the amount of ammonia that results from unconsumed feed. The other aim, on the other hand, is to investigate the use of natural adsorbent materials, i.e., zeolite (clinoptilolite) and leonardite, to maintain ammonia accumulation below the limit values.

2. Materials and Methods

2.1. Experimental Setup

The study was carried out in two different periods. Both periods were designed to consist of 3 groups with 3 replications. Crude protein values in the feeds of aquarium fishes vary between 28-30% and 45-50% depending on the nutritional requirement of the species (Khan & Maqbool, 2017). In experimental 1, commercial fish feed that contains 47.5% protein was used in 3 different concentrations (0.2 g feed/500 ml tap water, 0.4 g/500 ml tap water, 0.6 g/500 ml tap water) as the ammonia source (Şahin, 2022). The ammonia concentration resulting from unconsumed feeds in aquariums was determined in the first 7-day period. In the second period created subsequently, a mixture of clinoptilolite and leonardite (1:2 ratio), which has soil and water regulating properties, was added to suitable ammonia level, the excess amount of which is harmful to aquarium creatures. Table 1 shows the properties of the adsorbents investigated for this research. Analyzes were carried out by Central Research Laboratory, Kastamonu University. The pH was calculated following by according to Tokat (2019).

Table 1. Characteristics and chemical composition of clinoptilolite[a] and leonardite[b].

Clinoptilolite[a]			
	%		
SiO_2	78.41	SiO_2/Al_2O_3	5.67
Al_2O_3	13.83	BET Surface Area	34.316 m^2/g
MgO	1.646	pH	8.08
K_2O	2.372		
CaO	3.885		
Na_2O	1.042		
Fe_2O_3	1.414		
P_2O_5	0.058		
Leonardite[b]			
	%		
SiO_2	13.68	SiO_2/Al_2O_3	1.93
Al_2O_3	7.07	BET Surface Area	12.253 m^2/g
MgO	0.11	pH	3.15
K_2O	0.454		
CaO	0.323		
Na_2O	<0.014		
Fe_2O_3	1.238		
P_2O_5	0.055		

[a]Clinoptilolite was provided by Rota Mining Corporation, Manisa, Türkiye. [b]Leonardite was provided by Kütahya Chemistry, Kütahya, Türkiye.

In the second period, some water parameter values were determined as a result of 6 separate measurements conducted at regular intervals after the introduction of clinoptilolite:leonardite (1:2) mixture to the environment. Water criteria (dissolved oxygen, NH_4, pH, temperature) were determined by using a multiparameter instrument (YSI Professional Plus Series). A total of 3 g of water-regulating material was used in each replication in a way to contain 1 g of clinoptilolite type zeolite in 500 ml and 2 g of leonardite in 500 ml.

2.2. Data Analysis

NH_3 and TAN values in the study were calculated from NH_4^+, water temperature, and pH values (Emerson et al., 1975; Chow et al., 1997; EPA, 1999).

The TAN removal efficiencies (%) in the study were showed according to Alshameri et al. (2017):

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_e}{C_0}\; x\; 100 \quad (1)$$

Where; C_0 (mg/L): Initial ammonium concentration, C_e (mg/L): Equilibrium ammonium concentration.

Adsorption continues until an equilibrium is established between the concentration of the substance deposited on the adsorbent surface and the concentration of the substance remaining in the solution. Mathematically, this equilibrium is explained by adsorption isotherms. The most commonly used isotherms are the Freundlich and Langmuir equations (Fu et al., 2020).

The Freundlich parameters are calculated using as:

$$q_e = K_f C_e^{1/n} \quad (2)$$

Where; qe(mg/g) is the amount adsorbed at equilibrium, C_e: The remaining unadsorbed concentration of the adsorbate at equilibrium (mg/L), K_f: Freundlich constant, n: Constant (n>1).

The Langmuir parameters are calculated using as:

$$q_e = \frac{q_{max} K_d C_e}{1 + K_d C_e} \quad (3)$$

Where; q_{Max}: Maximum adsorbing capacity of the adsorbent (constant), K_d: Langmuir adsorption constant.

2.3. Statistical Data Analysis

Statistical analyzes were performed using Minitab Statistical Software version 17 on Windows operating system. Data are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) and subsequent Tukey's HSD post-hoc test were employed to compare different experimental groups. A 95% confidence interval was used.

3. Results

3.1. Experimental Period

Water temperature, dissolved oxygen, pH, and ammonium values were determined as 21.4±0.02 °C, 0.45±0.01 mg/L, 8.38±0.01, and 0.1±0.01 mg/L, respectively at the beginning of the experiments. It was determined that there was no difference between the groups (P>0.05).

In the present work, 3 different concentrations (0.2 g, 0.4 g, 0.6 g) of aquarium fish feed having 47.5% protein were added to 500 ml tap water for all experimental groups. At the end of the first period, mean water temperature (°C) and oxygen values (mg/L) were 19.14±0.23, 18.88±0.23, 18.83±0.23 and 0.19±0.01, 0.19±0.01, 0.17±0.01 for treatments a, b, and c, respectively (P>0.05). The pH values were determined as 7.81±0.05^a, 7.69±0.07^{a,b}, and 7.57±0.07^b for treatments a, b, and c, respectively (P<0.05). Difference in pH values increased depending on the feed amount. The level of NH_4 increase caused by different feed ratios in the groups at the end of the 1st period is shown in Figure 1. At the end of this period, TAN values (mg/L) were calculated and determined as 6.09±1.03, 9.08±1.55, and 9.62±1.74 for treatments a, b, and c, respectively (P>0.05). The increase in TAN values was directly proportional to the amount of feed.

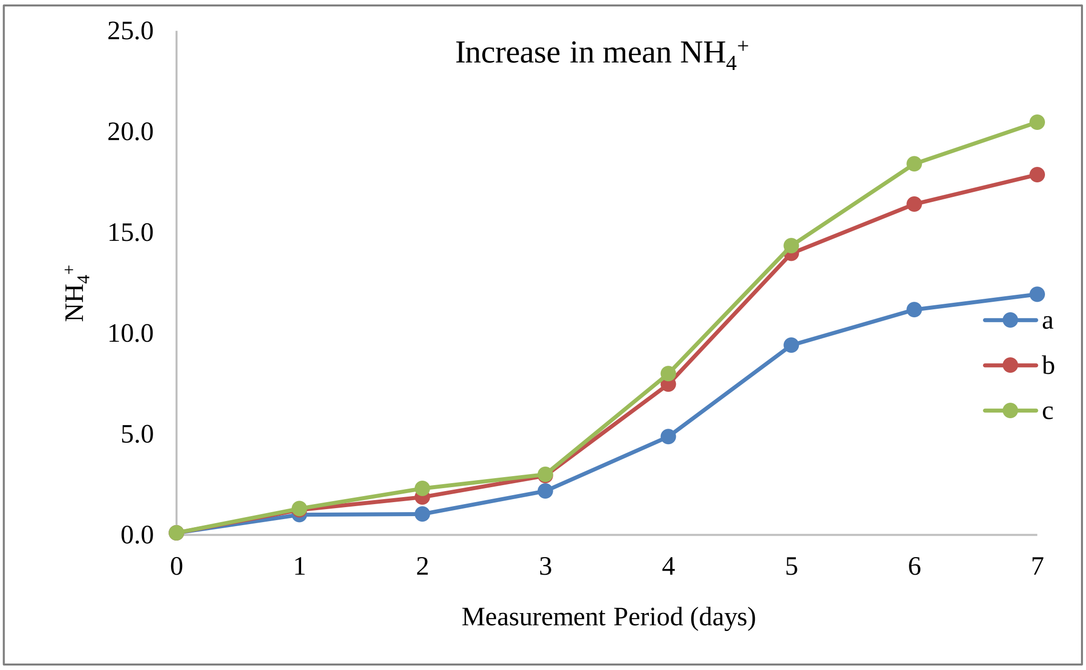

Figure 1. Increase in mean NH4+ at the end of the first period.

Water temperature (°C), dissolved oxygen (mg/L) and pH values obtained from measurements taken at regular intervals after the second experiment stage within a day were 20.31±0.18, 19.97±0.18, and 19.87±0.18; 0.23±0.01, 0.23±0.01, and 0.22±0.01; and 7.54±0.04, 7.48±0.03, and 7.52±0.02 for groups a, b, and c, respectively (P>0.05). NH_4 values (mg/L) were determined as 7.84±0.24[a], 13.07±0.40[b], and 16.62±0.42[c] for groups a, b, and c, respectively (P<0.05).

It was determined that TAN removal efficiency significantly differed between all treatments as shown in Figure 2 (P<0.05).

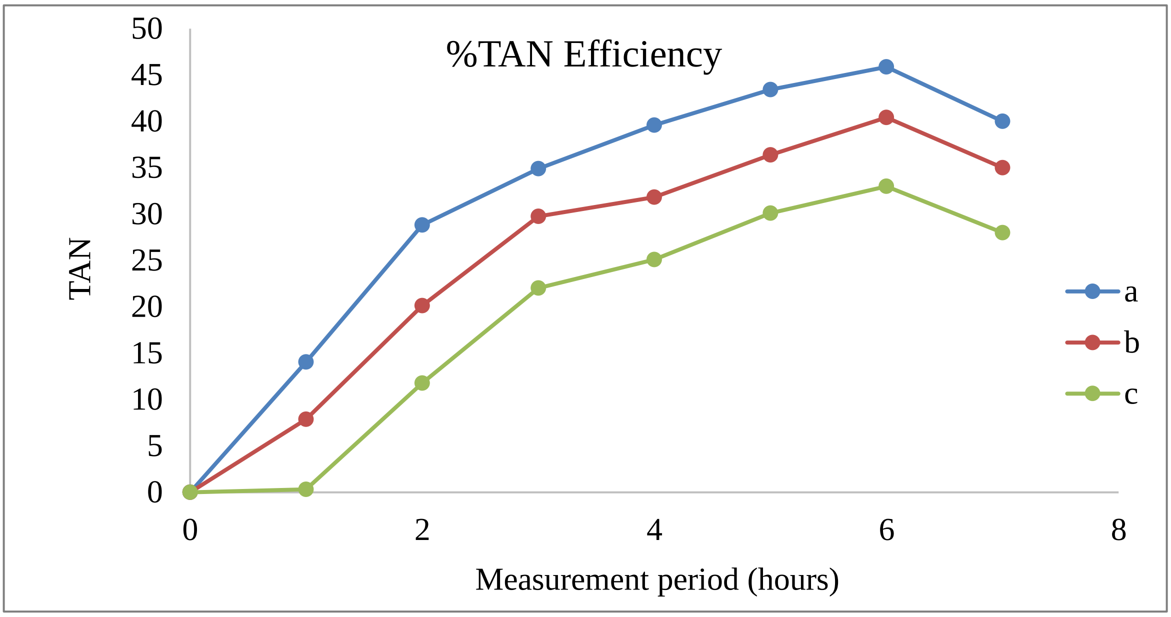

Figure 2. TAN removal efficiencies in groups a, b, and c throughout 6 measurements.

This experiment showed that clinoptilolite and leonardite can beneficially improve aquaculture water conditions by reducing increased levels of NH_3 and TAN.

With the increase in the amount of feed and the amount of TAN resulting from the feed, the retention efficiency of the adsorbents also increased.

In this study, it was shown that NH_3 values reached their peak at the end of the first period in which the ammonia values originating from the feed were determined, and started to decrease with the addition of clinoptilolite and leonardite to the water in the second period (Figure 3).

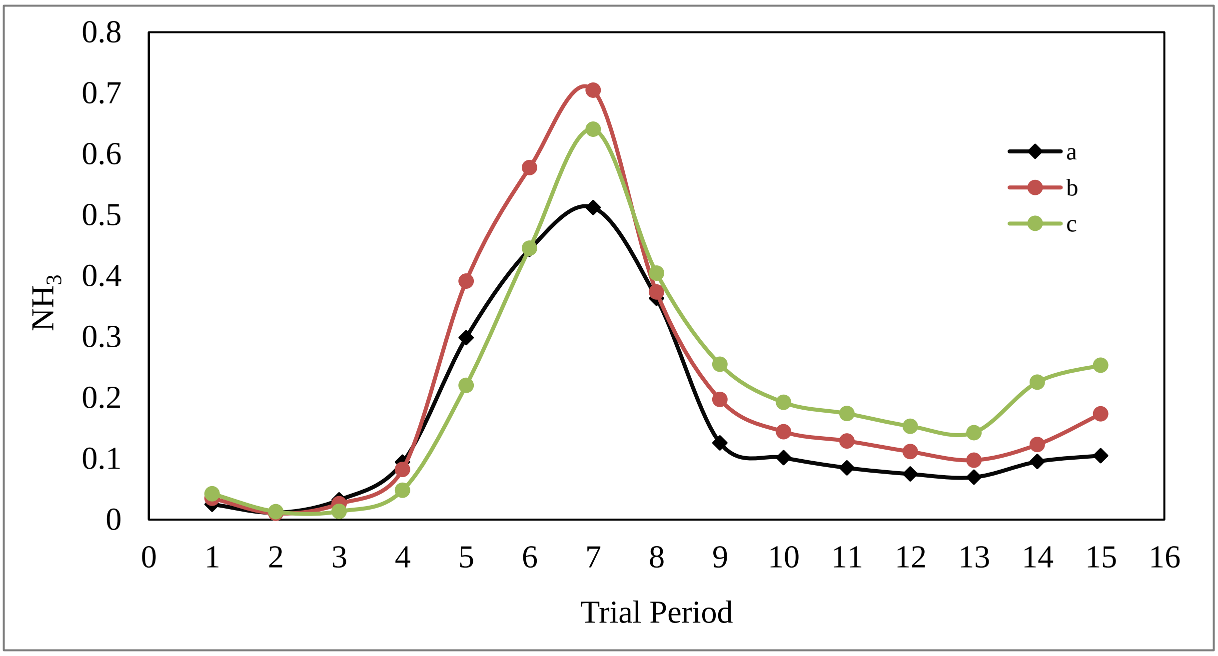

Figure 3. Changes in NH_3 values throughout the 15 measurements.

In the study examining the ammonia removal from different feed amounts, it was determined that the correlation coefficient value (R^2=0.984) found for Freundlich in the ideal group a, was higher when compared to the R^2 value (0.937) found for Langmuir. This showed that the experimental data were suitable for the Freundlich isotherm.

4. Discussion

Fish feed and excrement are the main wastes in aquarium waters. The majority of nitrogenous wastes in aquaculture also stems from unconsumed feed and excrement (Manguti et al., 2021). In this study, clinoptilolite and leonardite were used at high ammonium concentrations to keep ammonia levels at the desired level. At the end of the first period of the experiment, NH_4 values resulting from 47.5% protein feed for 7 days in treatments a, b, and c reached 12.2, 18.4, and 20.6 mg/L, respectively. Similar to this study, Kibria et al. (1997) reported that the amount of NH_4 was approximately over 20 mg/L after using 45% protein feed for 7 days. Şahin (2022) determined that the NH_4 values resulting from the feed that contains 35% protein were 3.53, 5.40, and 6.13 mg/L at the end of 7 days. The difference between the present study and Şahin (2022)'s study can be attributed to different amount of protein content of the feed.

Molecular ammonia (NH_3) and ammonium ion (NH_4^+), both of which are forms of soluble ammonia, coexist in equilibrium in water. Their relative concentrations are affected by pH and temperature. Higher pH and temperature values favor the formation of toxic molecular ammonia. Moreover, higher pH values result in more ionized ammonia, which raises toxicity (Purwono et al., 2017). The clinoptilolite used in this research can effectively adsorb NH4 from water. At the end of the experiment, in which the use of leonardite (another natural adsorbent) together with zeolite has a positive influence on ammonia removal, agrees with the studies of Öz et al. (2016) and Şahin et al. (2019). Chammui et al. (2014) and Terdputtakun et al. (2017) demonstrated that leonardite is more beneficial in removing unwanted compounds in aquatic environments. Furthermore, similar to this study, it was reported by Zengin (2013) and Şahin (2022) that the use of a clinoptilolite-leonardite mixture was effective.

Since nitrogen is one of the fundamental components of life, nitrogen molecules are necessary for the survival of living things (Şahin et al., 2019). There are two types of ammonia in water: unionized ammonia (NH_3) and ionized ammonium (NH_4^+). Ammonia (NH_3) is particularly toxic to fish. In aquaculture, NH_4^+ concentrations typically range from 1 to 5 mg/L. Aquaculture water has substantially lower NH_4^+ concentrations than municipal water (Jorgensen, 2002). When the NH_3 values observed in this study were examined, it was found that ammonia values from feed increased up to 0.77 mg/L and the highest value was reached in group c with the highest amount of feed, at the end of 7 days. With the addition of adsorbent, this value decreased to 0.1 mg/L, which is an acceptable value for aquaculture.

In this study, clinoptilolite and leonardite, which are utilized both as filtration material (Zengin, 2013; Şahin et al., 2018; Öz et al., 2021) and feed additive (Kanyılmaz et al., 2015; Turan & Turgut, 2020; Şahin, 2022), were used.

As a result of this research, it was concluded that it is possible to maintain nitrogenous compounds, which accumulates in the environment as natural wastes, within the desirable limits for aquaculture by using natural materials; zeolite and leonardite. Overall, when the effects of zeolite and clinoptilolite are evaluated together, it was determined that it does not have a harmful influence and the water parameters were within the appropriate values for aquaculture.

Conflict of Interest

The author declares no conflict of interest.

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