



## RESEARCH ARTICLE

# The Effect of Zeolite (Clinoptilolite) as a Feed Additive and Filter Material for Freshwater Aquariums

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**ABSTRACT**

Ammonia, which occurs as a natural result of aquaculture in production facilities, has a negative effect on the quality of aquaculture water and aquatic organisms. In this study, it was aimed to determine the effect of zeolite in fish feed and in water, which has the ability to adsorb ammonia, on ammonia removal. In the study, 12 different experimental groups were organized by creating 0, 2, and 10% ratios for fish feed (G1-G3), groups that zeolite only in water (G4, G5), and combinations (zeolite and/or in water/in feed) of 0, 7, and 20 g/L amounts to water (G6-G12). When NH<sub>3</sub> and TAN data of G1-G3 were examined, it was determined that although there was no statistical difference, it decreased proportionally with the increase in the amount of zeolite in the feed. The difference between water temperature, dissolved oxygen, pH, NH<sub>3</sub> and TAN values in G4 and G5 groups was found to be insignificant. The dissolved oxygen, pH, NH<sub>3</sub> and TAN values between the groups (G6-G12) were statistically different. As a result, it was determined that 10% addition of zeolite into the feed decreased the TAN values by 37%, and the addition of 10% into the feed and 20 g/L into the water decreased the TAN values by 45%. When the results are evaluated from another point of view, considering the economy and ease of use, it is concluded that 2% zeolite in feed and/or 7 g/L in water can be recommended for aquatic species with high tolerance to ammonia values.

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**1. Introduction**

Water quality is a critical factor in the breeding of aquatic organisms. Therefore, water quality should be monitored in order to achieve optimum production in the cultivation of aquatic organisms and to ensure the growth and survival of the organisms, to provide optimum conditions that vary according to the species. The concentration of total ammonia nitrogen (TAN) is the most important parameter limiting water quality in aquaculture (Shalaby et al., 2021).

Fish metabolism and the protein in unconsumed fish feed cause ammonia to build up in the water. The amount of ammonia in the water increases with water temperature and pH. Most of the NH<sub>4</sub><sup>+</sup> converts to NH<sub>3</sub> when the temperature and

pH are high (under alkaline conditions). According to Maulini et al. (2022), ammonia in the molecular form (NH<sub>3</sub>) is more harmful than the ionic form (NH<sub>4</sub><sup>+</sup>). To avoid harming aquatic creatures, ammonia (NH<sub>3</sub>) content should be lowered, especially in re-circulating aquaculture systems (RAS) like aquariums.

High organic matter content in the effluent of aquaculture facilities can promote or increase eutrophication and algae bloom and cause serious problems for the aquatic ecosystem. It is usually characterized by an increase in dissolved nitrogen and phosphorus content from unconsumed feed residues and metabolic wastes of fish. Ammonia is the main nitrogenous waste produced by fish through metabolism (Lazzari & Baldisserotto, 2008). More than 90% of waste materials in

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aquaculture pass into the water through unconsumed nutrients and fish feces.

As reported in YSI (2010), the main source of ammonia is simply fish excrement. stool rate; It is affected by the feeding rate and the amount of protein in the feed used. While the bait protein is broken down in the fish's body, some of the nitrogen is used to create energy and the unused portion is excreted through the gills as ammonia. If this ammonia in the water is too much, aquatic creatures will be damaged.

The main methods used to remove ammonium from wastewater are chemical, biological and physical treatment. Much literature on ammonium removal by adsorption has focused on natural minerals because of their high safety, good adsorption capacity, and cost effectiveness (Şahin et al., 2019; Öz et al., 2021). In addition, natural adsorbents are harmless for fish that can be used in aquaculture. There are many ways to reduce ammonia (NH<sub>3</sub>) levels in the aquatic environment, including interrupting the feed temporarily, adding new water, reducing fish density, and venting the aquaculture system (Maulini et al., 2022).

Dangerous short-term levels of toxic un-ionized ammonia which are capable of killing fish over a few days start at about 0.6 mg/L (ppm). Chronic exposure to toxic un-ionized ammonia levels as low as 0.06 mg/L (ppm) can cause gill and kidney damage, reduction in growth, possible brain malfunctioning, and reduction in the oxygen-carrying capacity of the fish (Durborow et al., 1997).

In aquaculture, it is reported that clinoptilolite-type zeolites are used to remove excess ammonia. The utilization of zeolite to regulate ammonia levels has the potential to enhance the efficacy of aquaculture practices. Furthermore, it is possible to mitigate the pollution issues arising from the incorporation of ammonia in fish discharge waters into the receiving water environment (Mumpton & Fishman, 1977; Terlizzi, 1996; Emadi et al., 2001; Peyghan & Azar Takamy, 2002; Karadağ et al., 2006; Sirakov et al., 2015; Skleničková et al., 2020; Öz et al., 2021).

The structural features and functions of clinoptilolite are affected by many factors (such as the characteristics of the mining area and the characteristics of the environment in which they will be used). Therefore, according to the area (environment) where it will be used; In order to evaluate the potential of the zeolite, much more detailed research is required to determine its technical properties and to provide the conditions for the purpose of use.

With this research, the effects of zeolite, which is used as a feed additive and filter material, in aquatic environments such as aquarium was determined. The addition of zeolite to water has been observed to result in a reduction of elevated ammonia levels, an increase in oxygen levels, and the restoration of pH balance. The addition of zeolite to fish feed has been found to

have a potentially beneficial impact on fish metabolism and the elimination of metabolic waste (Ghiasi & Jasour, 2012; Ibrahim et al., 2016). Presence of cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>2+</sup> in the feed solution reduced the clinoptilolite adsorption capacity to about 11.68 mg NH<sub>4</sub><sup>+</sup>/g/zeolite (Ashrafizadeh et al., 2008). In addition, at the end of this study, if the zeolite added to the feed and water has a negative factor in balancing the water parameters (such as the rivalry of sodium, calcium, potassium ions in the feed with ammonium), data on these can be obtained. With this study, it is thought that the zeolite added to the feed will reduce the ammonia release in the feed, and the water parameters will be kept at an ideal level for fish farming and at the same time, the waste water quality will increase. In this study, it was aimed to add zeolite to water and fish feed at different rates to provide suitable conditions in water parameters for aquaculture.

## 2. Materials and Methods

### 2.1. Feed Materials

Feed raw materials were obtained from a local feed company. Trial feeds were prepared from these raw materials. Zeolite (clinoptilolite) was not added to the control feed (G1), while 2% (G2) and 10% (G3) zeolite were added to the other two feeds, respectively. It was ensured that the chemical content of the feeds was the same in all groups. The amount of nutrients used in the study and the nutritional components of the feeds are given in Table 1.

**Table 1.** Formulation and chemical composition of experimental diets (g/100g).

	Experimental Feeds		
	Without zeolite (Control)	2% zeolite	10% zeolite
<b>Nutrients</b>			
<b>Fish meal</b>	24	24	24
<b>Soybean</b>	21	21	21
<b>Corn protein</b>	4.5	4.5	4.5
<b>Sunflower seeds</b>	22	23	26
<b>Semolina meal</b>	22.5	19.5	8.5
<b>Fish oil</b>	4	4	4
<b>Vitamin-mineral premix</b>	2	2	2
<b>Zeolite</b>	0	2	10
<b>Nutrient composition (dry matter %)</b>			
<b>Moisture</b>	9.94	6.82	8.72
<b>Crude protein</b>	40.47	40.30	39.75
<b>Crude oil</b>	6.66	6.91	6.96
<b>Crude ash</b>	6.45	8.37	10.43
<b>Crude cellulose</b>	1.78	1.85	2.01
<b>Starch</b>	16.19	12.28	10.34
<b>Nitrogen free extract</b>	35.27	34.18	31.09

## 2.2. Zeolite Material

The type of zeolite used as feed and filter material in the research is the West-Anatolian clinoptilolites, which are Turkey's most important zeolite source in terms of the size of the reserves and their potential for use.

The filter material (trade name FILTER-CLINO) and the zeolite used as a feed additive (trade name NAT-MIN 9000) are the same, the difference between them is the crushed sizes. Zeolites were obtained from the manufacturer in 100 microns to be added to the feed, and 1-3 mm in size as filter material.  $\text{NH}_4^+$  exchange capacity of the zeolite used in the research; is between 1.6-2.1 meq/g (1.8 meq/g on average) for natural products, Bet surface area 40.79  $\text{m}^2/\text{g}$  (Bilgin, 2017). The zeolites (1-3 mm) were washed with tap water until the turbidity was removed and dried at 105 °C (Şahin et al., 2018a).

The zeolite (100 microns) was added to the raw materials of pellets and mixed by hand until the ingredients were homogeneity. Each formulated diet was mixed to prepare each of the experimental diets. Warm water was added with continuous mixing. The paste-like passed through the meat mincer to produce pellets (1-2 mm in diameter), then the feeds were dried in the drying cabinet at 60 degrees for 10 hours. The experimental diets are: Use the commercial control diet (0% zeolite), adding a diet with 2% zeolite and adding a diet with 10% zeolite (Abdulathem & Al-Rudainy, 2021).

## 2.3. Experimental Design

In the research, 12 aquariums of 60x45x50 cm (three same division) were used. The volume of water used in aquariums is 20 liters. A hand-held field and laboratory device named YSI Professional Plus was used to measure the water parameters (Water temperature, ammonium, dissolved oxygen, pH) of the groups.

Three separate experiments were conducted in this study. In the first experiment, the effects of adding zeolite to the feed, adding zeolite to the water in the second experiment, and adding zeolite to the feed and/or water in the third experiment were investigated.

The experiment was carried out in three designs. In the first design, the effects of zeolite additives only in feed; in the second design, the effects of zeolite additives only in water and in the third design, the effects of zeolite additives in feed and/or water were determined.

In the first experiment, there were three groups; control without zeolite (G1), 2% dietary zeolite (G2) and 10% dietary zeolite in feed (G3).

In the second experiment, there were two groups with 7 g/L (G4) and 20 g/L (G5) zeolite in the water without feed.

In the other seven experimental groups in the third design, feed without zeolite and water without zeolite (G6), feed

without zeolite, 7 g/L zeolite in water (G7), 2% zeolite in feed, 7 g/L in water (G8), 10% zeolite in feed and 7 g/L zeolite in water (G9), feed without zeolite and 20 g/L zeolite in water (G10), 2% zeolite in feed and 20 g/L zeolite in water (G11), 10% zeolite in feed and 20 g/L zeolite in water (G12) were added. In the experiment, the effects of zeolite on water parameters were examined for a week.

24 hours before the start of the research, the aquariums of all groups were filled with 20 liters of water and aerated using an air stone, and all groups were aerated equally during the 7-day experiment. Aquarium water temperature values were obtained by heating the environment of the experimental setup with a room-type air conditioner. In the study, the contact surface of the zeolite with water was increased by dispersing the zeolites in the porous net bags on the aquarium floor in a way that they would not clump together. The amount of zeolite determined for the research groups was placed on the aquarium floor in a single bag.

After placing the zeolite bags in the amounts determined in the research plan within the aquariums, 10 grams of feed was distributed equally to all aquarium floors in a way that it would not clump together. The next day, water quality parameters such as oxygen, pH, temperature, and ammonium were determined, data were started to be collected and the measurements were repeated every day at 24-hour intervals.

As a result of the research carried out for seven days, the effects of the presence of zeolite in two different amounts of feed and/or water on the aquatic environment were determined.

## 2.4. Data Analysis

Total Ammonia Nitrogen (TAN) and  $\text{NH}_3$  values; were calculated using  $\text{NH}_4^+-\text{N}$ , water temperature and pH values which measured by YSI Professional Plus Multiparameter (Chow et al., 1997; EPA, 1999; Emerson et al., 1975; Jorgensen, 2002; YSI, 2007). In a previous study, it was reported that the results obtained from the Nessler method were similar and reliable to the results obtained from the traditional electrode method (Prajapati, 2014). The ammonia and TAN were calculated as given below (Purwono et al., 2017):

$$pK(\text{NH}_3) = \frac{2726.3}{273 + ^\circ\text{C}} + 0.0963 \quad (1)$$

$$\text{NH}_3\text{-N} = 10^{(pH - pK(\text{NH}_3))} \times \text{NH}_4^+\text{-N} \quad (2)$$

$$\text{TAN} = \text{NH}_3\text{-N} + \text{NH}_4^+\text{-N} \quad (3)$$

## 2.5. Statistical Analysis

Analysis of variance was performed to determine whether the difference between water parameters was significant at the beginning of the study and it was determined that the difference between the groups was not statistically significant ( $P > 0.05$ ).

Statistical analysis of the results obtained in the research was made with the "Minitab Release 15 for Windows" package

program. Parametric (ANOVA) tests were used in the data when the prerequisites of the analysis of variance were met, and non-parametric tests (Kruskal-Wallis) were applied when they were not. The results were given as mean±standard error (mean±SE) and the margin of error in the experiment was chosen as 0.05.

### 3. Results

The water quality parameters determined at the beginning and end of the study are presented in Table 2, Table 3, and Table 4.

When the data of G1, G2 and G3 presented in Table 2 are examined, the water temperature parameters are statistically indifferent. It was determined that the dissolved oxygen and pH values were statistically different and higher in the G3 supplemented with 10% zeolite than in the other two groups. When NH<sub>3</sub> and TAN data were examined, it was determined that although there was no statistical difference, it decreased proportionally with the increase in the amount of zeolite in the feed.

At the end of the experiment, the mean highest TAN concentration in the G1 group, which did not contain any

zeolite in the feed, was 5.99 mg/L, and the lowest TAN concentration was 3.77 mg/L in the G3 group. It was determined that 10% zeolite addition into the feed decreased TAN values by 37%.

The difference between water temperature, dissolved oxygen, pH, NH<sub>3</sub> and TAN values in G4 and G5 groups was found to be insignificant (Table 3) (P>0.05).

When the data of G6, G7, G8, G9, G10, G11 and G12 groups presented in Table 4 were examined, the water temperature parameters were statistically indifferent. It was determined that the dissolved oxygen, pH, NH<sub>3</sub> and TAN values between the groups were statistically different.

The pH values of the experimental groups were higher in all zeolite groups (except the G7 group) than in the G6 group without zeolite.

The average highest TAN concentration was 7.19 mg/L in the G6 group, which had no zeolite in its feed and water, and the lowest TAN concentration was 3.93 mg/L in the G12 group, which contained 10% zeolite in its feed and 20 g/L zeolite in its water at the end of the experiment. Adding 10% zeolite to feed and 20 g/L to water decreased TAN values by 45%.

**Table 2.** G1, G2 and G3 groups at the beginning and end of the study, mean temperature (°C), dissolved oxygen (mg/L), pH, NH<sub>3</sub> (mg/L) and TAN (mg/L) values (mean±SE).

Groups	Zeolite amount		Parameters									
			Temperature (°C)		Dissolved oxygen (mg/L)		pH		NH <sub>3</sub> (mg/L)		TAN (mg/L)	
			BE	EE	BE	EE	BE	EE	BE	EE	BE	EE
G1	-	-	22.00± 0.00 <sup>a</sup>	22.65± 0.08 <sup>a</sup>	6.71± 0.14 <sup>a</sup>	6.16± 0.11 <sup>a</sup>	8.44± 0.03 <sup>a</sup>	8.19± 0.05 <sup>a</sup>	0.04± 0.00 <sup>a</sup>	0.57± 0.11 <sup>a</sup>	0.34± 0.00 <sup>a</sup>	5.99± 0.93 <sup>a</sup>
G2	2	-	22.03± 0.03 <sup>a</sup>	22.74± 0.09 <sup>a</sup>	6.41± 0.09 <sup>a</sup>	6.34± 0.11 <sup>a</sup>	8.37± 0.01 <sup>a</sup>	8.28± 0.04 <sup>a</sup>	0.03± 0.00 <sup>a</sup>	0.50± 0.09 <sup>a</sup>	0.33± 0.00 <sup>a</sup>	4.71± 0.71 <sup>a</sup>
G3	10	-	22.00± 0.00 <sup>a</sup>	22.69± 0.08 <sup>a</sup>	6.69± 0.15 <sup>a</sup>	6.60± 0.21 <sup>b</sup>	8.35± 0.04 <sup>a</sup>	8.34± 0.06 <sup>b</sup>	0.03± 0.00 <sup>a</sup>	0.47± 0.08 <sup>a</sup>	0.33± 0.00 <sup>a</sup>	3.77± 0.54 <sup>a</sup>

BE = Beginning of the experiment, EE = End of the experiment, G1 = No zeolite in feed - No zeolite in water, G2 = 2% zeolite in feed - No zeolite in water, G3 = 10% zeolite in feed - No zeolite in water. Values are expressed as mean ± standard error (n=3), Different letters on the same line indicate that the differences between groups are significant (P<0.05).

**Table 3.** G4 and G5 groups at the beginning and end of the study, mean temperature (°C), dissolved oxygen (mg/L), pH, NH<sub>3</sub> (mg/L) and TAN (mg/L) values (mean±SE).

Groups	Zeolite amount	Parameters									
		Temperature (°C)		Dissolved oxygen (mg/L)		pH		NH <sub>3</sub> (mg/L)		TAN (mg/L)	
		BE	EE	BE	EE	BE	EE	BE	EE	BE	EE
G4	7	21.53± 0.17 <sup>a</sup>	23.08± 0.25 <sup>a</sup>	7.80± 0.13 <sup>a</sup>	7.34± 0.15 <sup>a</sup>	8.34± 0.00 <sup>a</sup>	8.46± 0.01 <sup>a</sup>	0.02± 0.00 <sup>a</sup>	0.15± 0.01 <sup>a</sup>	0.22± 0.00 <sup>a</sup>	1.12± 0.05 <sup>a</sup>
G5	20	21.30± 0.00 <sup>a</sup>	23.02± 0.26 <sup>a</sup>	7.76± 0.11 <sup>a</sup>	7.33± 0.19 <sup>a</sup>	8.40± 0.05 <sup>a</sup>	8.44± 0.01 <sup>a</sup>	0.02± 0.00 <sup>a</sup>	0.17± 0.01 <sup>a</sup>	0.22± 0.00 <sup>a</sup>	1.37± 0.07 <sup>a</sup>

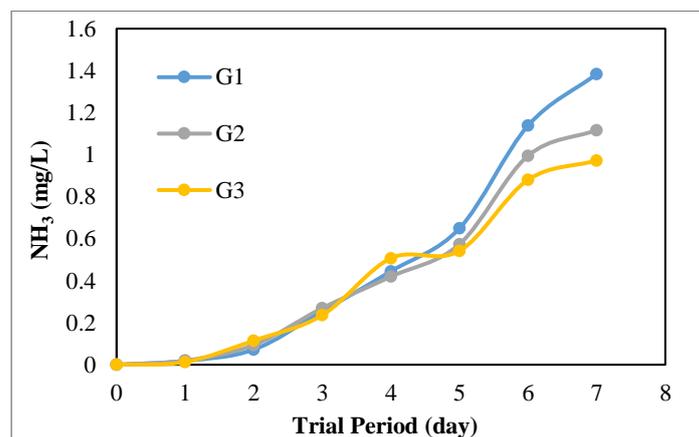
BE = Beginning of the experiment, EE = End of the experiment, G4 = No feed - 7 g/L zeolite in water, G5 = No feed - 20 g/L zeolite in water. Values are expressed as mean ± standard error (n=3), Different letters on the same line indicate that the differences between groups are significant (P<0.05).

**Table 4.** G6, G7, G8, G9, G10, G11 and G12 groups at the beginning and end of the experiment, mean temperature (°C), dissolved oxygen (mg/L), pH, NH<sub>3</sub> (mg/L) and TAN (mg/L) values (mean±SE).

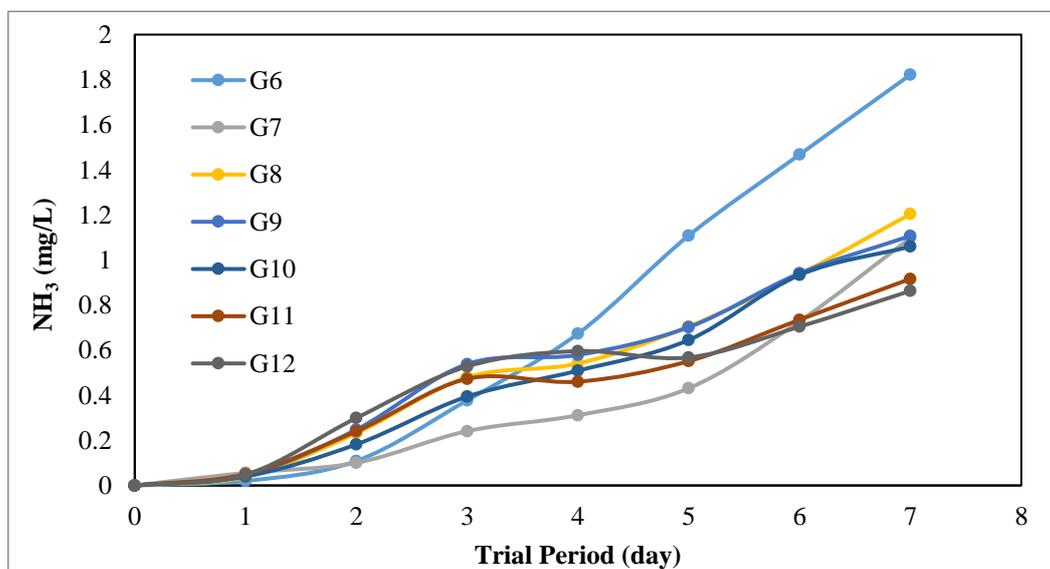
Zeolite amount			Parameters									
			Temperature (°C)		Dissolved oxygen (mg/L)		pH		NH <sub>3</sub> (mg/L)		TAN (mg/L)	
Groups	In feed (g/g)	In water (g/L)	BE	EE	BE	EE	BE	EE	BE	EE	BE	EE
G6	-	-	21.57±0.03 <sup>a</sup>	23.25±0.25 <sup>a</sup>	7.54±0.13 <sup>a</sup>	6.07±0.05 <sup>b</sup>	8.39±0.03 <sup>a</sup>	8.27±0.04 <sup>bc</sup>	0.02±0.00 <sup>a</sup>	0.79±0.15 <sup>a</sup>	0.22±0.00 <sup>a</sup>	7.19±1.16 <sup>a</sup>
G7	-	7	21.30±0.00 <sup>a</sup>	23.18±0.27 <sup>a</sup>	7.75±0.02 <sup>a</sup>	6.45±0.12 <sup>ab</sup>	8.39±0.02 <sup>a</sup>	8.23±0.03 <sup>c</sup>	0.02±0.00 <sup>a</sup>	0.42±0.08 <sup>b</sup>	0.22±0.00 <sup>a</sup>	4.83±0.71 <sup>b</sup>
G8	2	7	21.60±0.00 <sup>a</sup>	23.17±0.25 <sup>a</sup>	7.80±0.12 <sup>a</sup>	6.66±0.10 <sup>a</sup>	8.43±0.04 <sup>a</sup>	8.35±0.03 <sup>ab</sup>	0.02±0.00 <sup>a</sup>	0.59±0.09 <sup>ab</sup>	0.22±0.00 <sup>a</sup>	4.98±0.60 <sup>b</sup>
G9	10	7	21.63±0.20 <sup>a</sup>	23.16±0.25 <sup>a</sup>	7.92±0.06 <sup>a</sup>	6.89±0.15 <sup>a</sup>	8.46±0.04 <sup>a</sup>	8.38±0.03 <sup>a</sup>	0.03±0.00 <sup>a</sup>	0.59±0.08 <sup>ab</sup>	0.23±0.00 <sup>a</sup>	4.83±0.56 <sup>b</sup>
G10	-	20	21.37±0.09 <sup>a</sup>	23.07±0.26 <sup>a</sup>	7.78±0.10 <sup>a</sup>	6.57±0.11 <sup>a</sup>	8.42±0.02 <sup>a</sup>	8.28±0.03 <sup>bc</sup>	0.02±0.00	0.54±0.08 <sup>b</sup>	0.22±0.00 <sup>a</sup>	5.20±0.63 <sup>b</sup>
G11	2	20	21.53±0.07 <sup>a</sup>	23.21±0.25 <sup>a</sup>	7.55±0.03 <sup>a</sup>	6.77±0.11 <sup>a</sup>	8.45±0.02 <sup>a</sup>	8.37±0.03 <sup>a</sup>	0.03±0.00 <sup>a</sup>	0.49±0.07 <sup>b</sup>	0.23±0.00 <sup>a</sup>	4.09±0.42 <sup>b</sup>
G12	10	20	21.37±0.12 <sup>a</sup>	23.16±0.24 <sup>a</sup>	7.77±0.12 <sup>a</sup>	6.83±0.11 <sup>a</sup>	8.40±0.04 <sup>a</sup>	8.41±0.04 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.51±0.06 <sup>b</sup>	0.22±0.00 <sup>a</sup>	3.93±0.35 <sup>b</sup>

BE = Beginning of the experiment, EE = End of the experiment, G6 = No zeolite in feed - No zeolite in water, G7 = No zeolite in feed - 7 g/L zeolite in water, G8 = 2% zeolite in feed - 7 g/L zeolite in water, G9 = 10% zeolite in feed - 7 g/L in water zeolite, G10 = No zeolite in feed - 20 g/L zeolite in water, G11 = 2% zeolite in feed - 20 g/L zeolite in water, G12 = 10% zeolite in feed - 20 g/L zeolite in water. Values are expressed as mean ± standard error (n=3), Different letters in the same column indicate that the differences between groups are significant (P<0.05).

When NH<sub>3</sub> daily change values were examined, it was determined that after the 5<sup>th</sup> day in G1, G2 and G3 groups, it was lower in zeolite groups compared to the control (G1) group and ammonia values decreased as the amount of zeolite in the feed increased (Figure 1). When the daily NH<sub>3</sub> change in the groups with zeolite in both feed and water was examined, it was determined that after the 4<sup>th</sup> day, the experimental group without zeolite contained higher NH<sub>3</sub> values than the experimental groups with different ratios of zeolite in their feed and/or water. On the last day of the experiment, this difference was determined with the highest value in G6, which did not contain any zeolite, and the lowest value in the G12 group, which contained 10% zeolite in feed and 20 g/L zeolite in water (Figure 2).



**Figure 1.** Daily change in NH<sub>3</sub> values of G1, G2 and G3 groups.



**Figure 2.** Daily change in NH<sub>3</sub> values of G6, G7, G8, G9, G10, G11 and G12 groups.

#### 4. Discussion

In this study, it was determined that pH values increased in most of the groups (G2, G3, G4, G5, G8, G9, G10, G11 and G12) with zeolite added to the feed and/or water in comparison with the groups without zeolite addition (G1 and G6). This finding regarding pH is supported by other studies in which the ammonium retention capacity of clinoptilolite in various concentrations is investigated (Mazeikiene et al., 2008; Zabochnica-Swiatek & Malinska, 2010; Öz et al., 2017; Şahin et al., 2018a; Şahin et al., 2018b; Şahin et al., 2019).

Temperature and pH values of the water environment are the most important factors affecting the  $\text{NH}_3/\text{NH}_4$  ratio. One unit increase in pH values increases the availability of  $\text{NH}_3$  10 times (Durborow et al., 1997). In this study, when the effect of pH increase in zeolite groups on  $\text{NH}_3$  change, which is important for aquaculture, was examined (Table 3), it was determined that it was not at a level that would adversely affect the reduction of  $\text{NH}_3$  values. It was founded that  $\text{NH}_3$  remained at lower values in zeolite groups, especially in increasing ammonia concentrations (after the 4<sup>th</sup> day). Similar to this result, Zhang and Perschbacher (2003) found in their study that although the pH ratio increased by 0.10 units (from 7.85 to 7.95) in the zeolite experimental group, there was no statistical difference compared to the control group. Although the pH values in the experimental groups were affected by the addition of zeolite, they remained within the limits suitable for fish farming in all experimental groups.

As a result of this study, when the daily changes of  $\text{NH}_3$  values were examined, it was determined that there was a significant decrease in ammonia values in the groups with zeolite added after the 4<sup>th</sup> day compared to the control groups (G1 and G6). It was found to be similar to Zain et al. (2018) study. The inclusion of 2.5% natural zeolite in the diet reduced the ammonia excretion rate of rainbow trouts by 24% compared to the control group.

Dissolved oxygen concentration was higher in G4 and G5 groups than in all other groups. This difference is due to the fact there is no factor, such as unconsumed feed, that will cause oxygen consumption in the environment. The lowest value of dissolved oxygen was determined from the G1 and G6 groups that did not contain zeolites in their feed and water (Tables 2 and 4). As the zeolite content in feed and water increased, the oxygen ratio generally increased. As a result, dissolved oxygen was positively affected by the addition of zeolite into water, feed or both feed and water. In this respect, the results were found to be similar to Berka (1986), Bower and Turner (1982), Sing et al. (2004), Zain et al. (2018), Shalaby et al. (2021). The sum of the  $\text{NH}_3$  and  $\text{NH}_4^+$  values in equilibrium depending on the pH and temperature values in the water is expressed as TAN. The use of zeolites in feed, water or both feed and water contributes to an effective reduction of TAN values in water. Among the zeolite groups, TAN values are lower as the amount

of zeolite increases. However, the lack of statistical difference between the groups showed that even low zeolite amounts in the study could be used effectively.

Despite the beneficial and promising effects of zeolite as an additive in various animal feeds (Valpotić et al., 2017), there is enough research on its use for aquaculture. It seems more likely that clinoptilolite could be used as a filter, for transporting live fish, and as a fish feed additive, especially to remove ammonia (Zhou & Boyd, 2014; Ghasemi et al., 2018).

When these results are evaluated, ammonia tolerance values of fish species are under the influence of many factors and variables. In the case of much more sensitive aquatic species, it may be recommended to prefer higher values such as 10% zeolite addition to the feed and 20 g/L zeolite into the water where lower ammonia values are determined. When the results are evaluated from another point of view, considering the economy and ease of use, it is concluded that 2% zeolite in feed and/or 7 g/L in aquarium water can be recommended for aquatic species with high tolerance to ammonia values. Similar to this study, Ibrahim et al. (2016) evaluated the effect of natural zeolite (clinoptilolite) as a feed additive in fish diets on the growth performance, genetic characteristics and health status of freshwater Nile tilapia fish (*Oreochromis niloticus* L.). In the study, in parallel with the amount of zeolite added to the feed, an increase was determined in the ash values in the feed, similar to the previous studies (Ibrahim et al., 2016; Tekeşoğlu & Ergün, 2021; Al Amir et al., 2022). The amount of ash is related to the mineral matter content, and the zeolite (clinoptilolite) also has mineral substance content. This may be a factor that positively affects the quality of fish feeds. By increasing the nutritional value of the feed, it can be effective both on fish growth and on the protection of water conditions.

As a result, it has been determined that the addition of zeolite to the feed or water has a positive effect for the control of important water parameters such as pH, oxygen and TAN in freshwater aquaculture systems.

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#### Compliance with Ethical Standards

The study protocol was approved by ethics committee of Sinop University (Decision number 2007/13) and experiments were carried out in accordance with the ethical guidelines and

regulations declared by the Sinop University and the international principles of laboratory animal use and care.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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