



## Effect of Natural Adsorbents Addition into Fish Feed Rations on Water Parameters

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### Abstract

In this research, the effects of zeolite, leonardite, and diatomite, added into fish feed of 40 % protein value in different amounts, on water parameters were investigated. The experiment consisted of 10 groups with three replications (control (C), 2 % zeolite (Z2), 4 % zeolite (Z4), 8 % zeolite (Z8), 2 % leonardite (L2), 4 % leonardite (L4), 8 % leonardite (L8), 2 % diatomite (D2), 4 % diatomite (D4), and 8 % diatomite (D8)). When the experimental groups in the study were examined individually, it was determined that similar to the results in the zeolite groups and leonardite groups, there was a decrease in the ammonia and TAN values in the water as the amount of adsorbent in the feed increased. When the TAN values determined at the end of the experiment (14th day) were examined, the ideal group was determined to be L8 with a value of  $27.55 \pm 0.39$ , and the second group was determined to be the Z8 group with a value of  $27.58 \pm 0.32$ . When these results are evaluated, it is thought that the addition of adsorbent in greater amounts (e.g., > 8 %) than the amounts in this study could be significantly more effective.

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## INTRODUCTION

One of the most important subjects in aquaculture is the healthy growth of fish. Various feed additives and raw materials are used to maximise growth in fish feeding studies (Hasaan et al., 2020; Şahin, 2022). Feed additives are products used in feed to improve feed quality and increase the growth and overall performance of fish in a way that does not negatively affect water parameters, and therefore, fish health (Ebenezar et al., 2021). It is necessary to remove ammonium ( $\text{NH}_4^+$ ), which is harmful in excessive amounts, from the aquatic ecosystem (Jorgensen & Weatherley, 2003; Alshameri et al., 2014). Biological and physical and chemical treatment methods, such as adsorption, are used to balance excess  $\text{NH}_4^+$  in the environment (Gupta et al., 2011; Öz & Aral, 2023). Recently, due to their positive effects on ammonium and pH balance, natural adsorbents such as zeolite, leonardite, diatomite, and bentonite, which have chemical, biological, and physical filtration properties, are preferred for various reasons such as their non-toxicity, low cost, and wide application and usage areas, etc. (Öz et al., 2016; Öz et al., 2022; Şahin et al., 2018; Şahin, 2023).

Leonardite is formed as a result of the sedimentation of plant and animal remains on the bottoms of oceans, lakes, and swamps and is an organic material stratified as a result of the decomposition and humification of biological waste under high pressure, temperature, and anaerobic conditions. Leonardite contains high amounts of humic acid and fulvic acid. Leonardite is used in agriculture as a direct organic soil regulator or as the primary source of organic matter in organic products with organomineral fertilisers to which it is added (Chammui et al., 2014; Ausavasukhi et al., 2016).

Zeolites are materials with a basic composition of Al, Si, and O and can be synthesised or found in natural rock deposits. Clinoptilolite, a natural zeolite, is used as a fertiliser additive, soil improver, feed additive, and filter material in agriculture and husbandry (Cataldo et al., 2021).

Diatomite is light, finely porous rocks composed primarily of extremely small opaline skeletons (or their fragments) of diatomic algae (diatoms). Their characteristic features are the presence of amorphous active silicon dioxide on one hand and fine porous structure, lightness, and low thermal conductivity on the other. These properties make these materials highly chemically active, enabling their possible use as absorbers, dryers, catalysts, filters, thermal insulation materials, and filling material carriers (Ivanov & Belyakov, 2008; Ahmad et al., 2019; Bakr, 2010).

Zeolites have been used as feed additives in fish, cattle, pigs, horses, and poultry for many years, and they are a mineral substance that has been the subject of many studies, especially on water purification and feed additives (Alshameri et al., 2014; Öz & Aral, 2023) but studies on leonardite and diatomite are very limited (Öz et al., 2022; Şahin, 2022; Zengin, 2013).

In this research, the effects of zeolite, leonardite, and diatomite, added into fish feed of 40 % protein value in different amounts, on water parameters were investigated.

## MATERIALS AND METHODS

### Experiment setup

In the study, three different proportions (2 %, 4 %, 8 %) of powder size (100 microns) zeolite, leonardite and diatomite were added to fish feed with 40 % protein value. The crude materials were kneaded with the feed in the determined proportions to ensure a homogeneous mixture. In order to preserve the nutritional values of the feeds were dried at 60 °C about 20 h and stored at + 4 °C.

Each experiment repetition received 500 ml of water and 0.5 g feed (Kibria et al., 1997; Öz et al., 2022). Water parameters were measured 14 times at the same time every day. The experiment was carried out at room temperature without any aeration or mixing. The water temperature, pH, and ammonium values obtained at the end of the experiment were recorded with the multiparameter (YSI Professional Plus Series instrument).

The experiment consisted of 10 groups with three replications (control (C), 2 % zeolite (Z2), 4 % zeolite (Z4), 8 % zeolite (Z8), 2 % leonardite (L2), 4 % leonardite (L4), 8 % leonardite (L8), 2 % diatomite (D2), 4 % diatomite (D4), and 8 % diatomite (D8)). Crude protein values in aquarium fish feed vary between 28-30 % and 45-50 %, depending on the nutritional needs of the target species (Khan & Maqbool, 2017). In the study, feed containing 40 % crude protein was used to be within this protein value range.

Zeolite was obtained from Rota Mining Corp. (Manisa, Türkiye), leonardite was obtained from Kütahya Chemistry (Kütahya, Türkiye), and diatomite samples were provided by Nanotech Corp. Chemistry Mining and Logistic (Türkiye).

### Evaluation of Data

TAN and  $\text{NH}_3$  data were computed from the pH, water temperature, and  $\text{NH}_4^+$  values. Purwono et al. (2017)'s following equations are employed to calculate TAN and ammonia values:

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

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

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

Tokat (2019)'s method was used to calculate the pH values. SiO<sub>2</sub> / Al<sub>2</sub>O<sub>3</sub> ratio was determined according to Chuan-hsia Liu (2000).

The BET (Surface area measurement instrument, Multi-point) and XRF (X-Ray fluorescence spectrometry) analyses of zeolite, leonardite, and diatomite. Analyses were carried out by Kastamonu University Central Research Laboratory. XRF analyses were determined with a Spectro brand Xepos II model instrument, whereas BET analyses were determined with a Quantachrome brand Nova Touch LX<sub>4</sub> model instrument. SEM-EDS (Scanning electron microscopy) analyses were assessed using the FEI brand Quanta FEG 250 model device in Kastamonu University Central Research Laboratory.

**Statistical Evaluation**

Statistical evaluation was made with Minitab Program Version 17 for Windows. The significance level was set to 0.05. Data are presented as mean ± standard error (SE). Obtained data were compared with one-way analysis of variance (ANOVA), and multiple comparisons were performed with Tukey's HSD post-hoc test.

**RESULTS**

**Morphological structure of zeolite, diatomite, and leonardite**

Table 1 shows BET (Surface area measurement instrument, Multi-point), XRF (X-Ray fluorescence spectrometry) and SEM-EDS (Scanning electron microscopy) analyses of zeolite, leonardite, and diatomite.

**Table 1.** Chemical compositions of zeolite, leonardite and diatomite

Zeolite<sup>a</sup>

	%		
SiO <sub>2</sub>	78.41	SiO <sub>2</sub> / Al <sub>2</sub> O <sub>3</sub>	5.67
Al <sub>2</sub> O <sub>3</sub>	13.83	BET Surface Area	34.316 m <sup>2</sup> / g
MgO	1.646	pH	8.31
K <sub>2</sub> O	2.372		
CaO	3.885		
Na <sub>2</sub> O	1.042		
Fe <sub>2</sub> O <sub>3</sub>	1.414		
P <sub>2</sub> O <sub>5</sub>	0.058		

Leonardite<sup>b</sup>

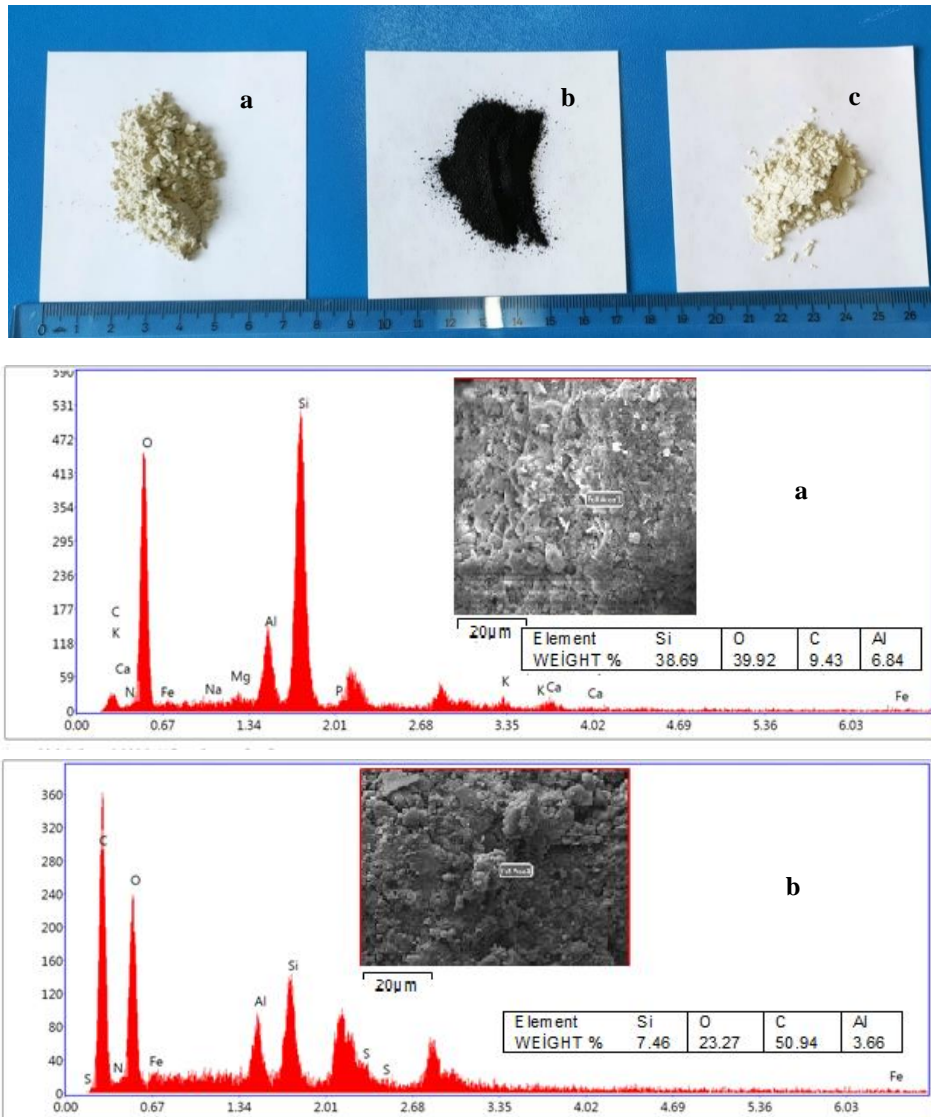
	%		
SiO <sub>2</sub>	13.68	SiO <sub>2</sub> / Al <sub>2</sub> O <sub>3</sub>	1.93
Al <sub>2</sub> O <sub>3</sub>	7.07	BET Surface Area	12.253 m <sup>2</sup> / g
MgO	0.11	pH	3.23
K <sub>2</sub> O	0.454		
CaO	0.323		
Na <sub>2</sub> O	< 0.014		
Fe <sub>2</sub> O <sub>3</sub>	1.238		
P <sub>2</sub> O <sub>5</sub>	0.055		

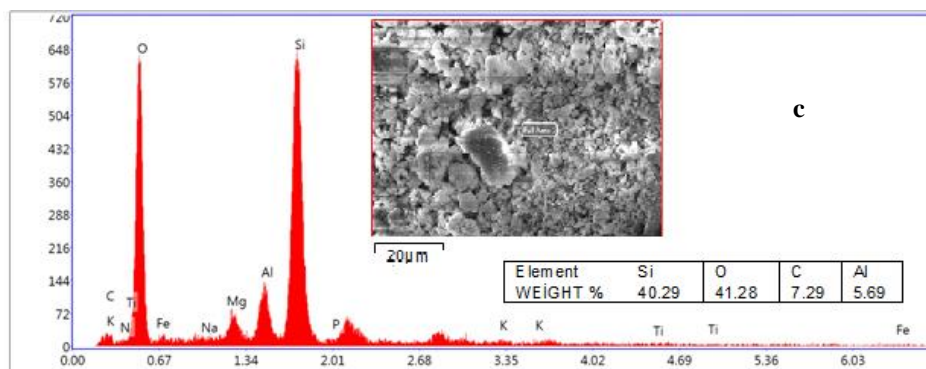
Diatomite<sup>c</sup>

	%		
SiO <sub>2</sub>	81.66	SiO <sub>2</sub> / Al <sub>2</sub> O <sub>3</sub>	8.149
Al <sub>2</sub> O <sub>3</sub>	10.02	BET Surface Area	174.698 m <sup>2</sup> / g
MgO	3.839	pH	7.00
K <sub>2</sub> O	0.99		
CaO	2.041		
Na <sub>2</sub> O	1.261		
Fe <sub>2</sub> O <sub>3</sub>	2.291		
P <sub>2</sub> O <sub>5</sub>	0.243		

<sup>a,b,c</sup> Zeolite, leonardite, and diatomite samples were obtained from Rota Mining (Manisa, Türkiye), Kütahya Chemistry (Kütahya, Türkiye), and Nanotech Construction Chemistry Mining and Logistic Ind. Trade. Inc. Co., respectively.

Zeolite (a), leonardite (b), and diatomite (c) used in the study were characterised by SEM images and EDS analyses (Figure 1).





**Figure 1.** Raw materials, EDS analyses and SEM

When EDS spectra were examined, it was determined that zeolite and diatomite contain high amounts of aluminium and silicon, while leonardite contains high amounts of carbon and oxygen.

#### *Effects of zeolite, leonardite, and diatomite on water temperature, pH, ammonium, and TAN*

The differences in initial water parameters between groups were statistically insignificant ( $P>0.05$ ) and were measured as  $19.7 \pm 0.01$  °C for water temperature,  $8.89 \pm 0.01$  for pH, and  $0.30 \pm 0.01$  mg/l for ammonium. The water parameter values obtained at the end of the 14-day experiment are given in Table 2. Differences in TAN and  $\text{NH}_4^+$  values were statistically significant at the end of the experiment ( $P<0.05$ ). On the other hand, no difference was found between groups in terms of pH and temperature values ( $P>0.05$ ).

**Table 2.** Temperature, pH,  $\text{NH}_4^+$  and TAN values in groups at the end of the experiment (mean $\pm$ SE)

Water Parameters				
Experimental Group*	Temperature (°C)	pH	$\text{NH}_4^+$ (mg/l)	TAN (mg/l)
Control	$19.80 \pm 0.12$	$8.37 \pm 0.07$	$16.62 \pm 1.63^a$	$19.56 \pm 1.99^a$
Z2	$19.81 \pm 0.11$	$8.39 \pm 0.07$	$13.84 \pm 1.43^{ab}$	$16.36 \pm 1.76^{ab}$
Z4	$19.79 \pm 0.12$	$8.34 \pm 0.08$	$13.57 \pm 1.38^{ab}$	$15.89 \pm 1.68^{ab}$
Z8	$19.80 \pm 0.11$	$8.35 \pm 0.08$	$12.69 \pm 1.32^{ab}$	$14.89 \pm 1.61^{ab}$
L2	$19.80 \pm 0.12$	$8.31 \pm 0.07$	$12.93 \pm 1.47^{ab}$	$15.02 \pm 1.78^{ab}$
L4	$19.79 \pm 0.12$	$8.33 \pm 0.07$	$12.11 \pm 1.34^b$	$14.41 \pm 1.68^b$
L8	$19.80 \pm 0.11$	$8.35 \pm 0.07$	$12.34 \pm 1.28^b$	$14.40 \pm 1.55^b$
D2	$19.77 \pm 0.12$	$8.35 \pm 0.07$	$14.55 \pm 1.56^{ab}$	$17.05 \pm 1.89^{ab}$
D4	$19.80 \pm 0.12$	$8.36 \pm 0.08$	$14.09 \pm 1.43^{ab}$	$16.45 \pm 1.74^{ab}$
D8	$19.80 \pm 0.12$	$8.33 \pm 0.08$	$12.18 \pm 1.36^b$	$14.25 \pm 1.65^b$

\*Difference superscript letters in a column indicate significant ( $p<0.05$ ) differences between experimental groups. Means were compared by ANOVA and subsequent Tukey's multiple range test.

When the TAN values determined at the end of the experiment (14<sup>th</sup> day) were examined, the ideal group was determined to be L8 with a value of  $27.55 \pm 0.39$ , and the second group was determined to be the Z8 group with a value of  $27.58 \pm 0.32$ . When evaluated in terms of the amount of inclusion into the feed, it was observed that as the amount increased, TAN values decreased.

## DISCUSSION

The use of adsorbents to regulate ammonia levels is applied for the treatment of drinking and wastewater, as well as aquaculture (Skleničková et al., 2020; Öz et al., 2021). These natural adsorbents are used as water filtration material (Öz & Aral, 2023), feed additive (Tekeşoğlu & Ergün, 2021), or substrate material (Öz et al., 2016) in aquaculture. Due to its physical and chemical properties, it has been reported in previous studies that it offers various benefits, including especially regulation of water

parameters and growth and disease resistance in fish (Aly et al., 2016; Alinezhad et al., 2017). In this study, the effects of using zeolite, which has been the subject of many studies, as well as leonardite and diatomite, which have been the subject of very limited studies, in fish feed were examined.

When the TAN values obtained in the study were examined (Table 2), it was observed that the TAN values in the two trial groups (Z8 and L8) containing zeolite and leonardite were very proximate to each other, and these two groups were significantly ( $P < 0.05$ ) lower than the control group. In this study, it was determined that TAN values decreased as the amount of 2, 4 and 8 % adsorbent added to the feed increased ( $P < 0.05$ ). When  $\text{NH}_3$  values, which are important for aquaculture, are examined, it approached the limit values for sensitive aquatic species in the control group (0.11 mg/l) on the fourth day and remained at low values in experimental groups as 0.03 mg/l in L2, 0.04 mg/l in L4, and 0.04 mg/l in D8. When similar studies were examined, it was observed that there are limited studies examining the effects of zeolite and leonardite supplementation into feed on water parameters (Ghiasi & Jasour, 2012; El Gendy et al., 2015; Öz & Aral, 2023; Turan & Turgut, 2020), while there is no study in aquaculture with diatomite supplemented feeds. When the experimental groups in the study were examined individually, it was determined that similar to the results in the zeolite groups and leonardite groups, there was a decrease in the ammonia and TAN values in the water as the amount of adsorbent in the feed increased. When these results are evaluated, it is thought that the addition of adsorbent in greater amounts (e.g.  $> 8\%$ ) than the amounts in this study could be significantly more effective.

### Compliance with Ethical Standards

#### a) Authors' Contributions

DŞ: designed the study. Performed the experiment and data collection. Wrote the work

MÖ: Performed the experiment and data collection

ÜÖ: Performed the data analysis.

All authors read and approved the final manuscript.

b) **Conflict of Interest:** The authors declare that there is no conflict of interest.

c) **Ethical approval:** Formal consent is not required.

d) **Data availability statement:** All data generated or analyzed during this study are included in this article.

e) **Acknowledgment**

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