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EFFECTS OF ZEOLITE, DIATOMITE, AND LEONARDITE AS FEED ADDITIVES ON THE GROWTH PERFORMANCE OF GUPPY (*Poecilia reticulata*) FRY

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Abstract
This study examined how possible feed additions, such as zeolite, diatomite, and leonardite, affected aquarium fish growth and reproduction. The meals of the guppy (*Poecilia reticulata*) fry were supplemented with 4% zeolite, leonardite, and diatomite. Weight increase (g), specific growth rate (SGR, %), and survival rate (%) showed statistically significant differences ($p<0.05$) after 60 days, but feed conversion ratio (FCR) showed no statistically significant changes ($p>0.05$). The results of this study indicate that adding natural zeolite, leonardite, and diatomite to fish diets has beneficial effects on growth parameters, feed utilization, and survival rate, suggesting that these materials can be used as natural feed additives in aquarium fish culture. The water quality parameters measured at the start and at the end of the experiment did not differ significantly ($p>0.05$).

Keywords: *Poecilia reticulata*, zeolite, leonardite, diatomite, growth parameters

Introduction
Ornamental fish farming has been a popular pastime for the past century and is still very popular today (Peh et al., 2025). The industry contributes significantly to rural development, international trade and exports, employment creation, tourism, habitat restoration, and environmental management (Evers et al., 2019; Sajesh et al., 2021; Pailan et al., 2022). Over the past 40 years, advancements in aquaculture technologies have increased the overall production of both oviparous and ovoviviparous fish species. Guppies (*Poecilia reticulata*), platies (*Xiphophorus maculatus*), mollies (*Poecilia sphenops*), and swordtails (*Xiphophorus*

helleri) are some of the most well-liked livebearing ornamental fish. These species are very sought after in the aquarium sector because of their vivid coloring and ease of upkeep. Guppies, in particular, stand out due to their diverse fin shapes and coloration, which render them indispensable in the ornamental fish market. They can thrive under a wide range of environmental conditions and reproduce relatively easily. Optimal development occurs at temperatures between 22–24°C and a pH range of 7.0–8.0. Guppies reproduce frequently, with a single female producing approximately 20–30 fry every 4–6 weeks. To optimize reproduction and minimize stress on females, a sex ratio of one male to three to five females is recommended. Guppies consume a variety of food types, including live feeds such as bloodworms and *Daphnia*. The initial feeding of larvae is particularly crucial for ensuring high survival rates. Intensive aquaculture operations require the consistent provision of nutrient-rich diets and economically efficient feeds, contributing to the sustained growth of the ornamental fish industry (Patel et al., 2023). One of the major bottlenecks in ornamental fish production is the high mortality rate observed during the larval and early juvenile stages, primarily due to inadequate nutrition. At these developmental stages, the digestive systems of fish are not yet fully developed, requiring live and motile prey that can stimulate feeding behavior and meet their specific nutritional needs (Lim et al., 2003; Hill et al., 2020; Patil & Kamble, 2025). Moreover, various dietary additives have been reported to positively influence fish health, enhance stress tolerance, and improve disease resistance under intensive culture conditions (Dawood et al., 2018; Yadav et al., 2021; Prokešova et al., 2024). Feed additives are defined as substances added to diets or feed ingredients at low inclusion levels to enhance or maintain their functional and nutritional properties. n aquaculture, these additives are commonly employed to meet the physiological and metabolic needs of fish, including growth performance, reproductive capacity, and pigmentation. Furthermore, feed additives are essential for regulating immunological responses and reducing the negative impacts of stress on fish (Rasidi et al., 2023). Feed additives provide many benefits, including lowering the need for antibiotics and avoiding the expenses and side effects of using them (Onomu & Okuthe, 2024). The growth, reproduction, and general health of ornamental fish are all directly impacted by the quality of the water. Stress levels can be lowered, disease outbreaks can be avoided, and sustained ornamental fish farming can be accomplished with complete water management techniques (Patel et al., 2023). Recently, zeolites, particularly clinoptilolite, have gained widespread attention due to their chemical and physical properties. Many researchers have focused on the applications of zeolites in biochemistry. In aquaculture, zeolite has been examined in numerous studies for its role in maintaining water quality and improving feed quality, particularly within filtration systems (physical, chemical, and biological treatment) and live fish transportation (Danabas & Altun, 2011; Danabas & Dorucu, 2021; Tekeşoğlu & Ergün, 2021; Şahin et al., 2025a). The use of zeolite as a feed additive promotes fish growth and enhances resistance to diseases (Shlenkina et al., 2021). In recirculating aquaculture systems, zeolite-supplemented diets not only contribute to improved fish performance but also help maintain environmental conditions within desirable ranges (Obradovic et al., 2006; Cargnin & João, 2021; Basyuni et al., 2023; Verma et al., 2023; Farrag et al., 2024). Leonardite is frequently used as a soil amendment in agricultural applications. It is mainly made up of humic acids that come from the breakdown of organic waste. Natural humic substances have demonstrated antioxidant properties that can support animals during stressful periods, such as weaning. Additionally, their antimicrobial activity against pathogens has been shown to reduce the incidence of diarrhea and improve growth performance by regulating the animal's metabolism (Turan & Turgut, 2020). Diatomites, lightweight and porous fine-grained rocks, are primarily composed of the minute opaline skeletons or fragments of diatomaceous algae. Internationally, diatomite has found extensive applications as a sorbent in the petroleum, food, and chemical industries. Diatomaceous powders serve as excellent filters and are used for both coarse and fine (clarifying) filtration in several sectors of the food industry. Diatomite is an effective filtration medium for drinking and industrial water and has been successfully applied in the atomic industry for removing radioactive substances, particularly the radioactive isotope cesium from liquids. Diatomites and diatomaceous rocks have also become increasingly important as catalyst carriers due to their high pore surface area and strong retention capacity for deposited catalysts (Ivanov & Belyakov, 2008).

The goal of the current study is to ascertain how the growth characteristics of guppy (*Poecilia reticulata*) fry are affected by zeolite, which has been thoroughly examined as a feed additive and water treatment material, as well as the less commonly studied leonardite and diatomite.

Material and Method

The study was conducted in a total of 12 aquaria, consisting of four different experimental groups with three replicates each. The experiment's guppy (*Poecilia reticulata*) fish were fed a control diet and diets containing 4% zeolite, leonardite, or diatomite for 60 days. A commercial aquarium fish feed with 48.5% crude protein, 6.7% crude fat, 1.9% crude fiber, and 10.4% ash made up the control diet.

The experiment started by adding powdered zeolite (clinoptilolite), leonardite, and diatomite to aquarium fish feed that had been soaked with warm water to create a uniform mixture. The created experimental diets were then dried at 60 °C.

From a stock tank of 300 fish, individuals weighing an average of 0.020 ± 0.01 g were chosen at random and placed in 10 L experimental aquaria at a density of 12 fish per tank. The fish were purchased from a local commercial source in Istanbul. Feeding protocols in aquaculture research typically involve either feeding to apparent satiation or rationing based on body weight. In juvenile fish culture, feeding to apparent satiation is commonly adopted to minimize stress caused by handling and weighing. (Choden et al., 2024; Edoziem et al., 2022; Gumus et al., 2016). Fish were fed to satiation at 9:00 and 15:00 during the trial, and *Artemia* sp. was given as a live feed once a week. Every week, the aquaria's leftover food and waste were siphoned off.

Under a 12 hour light:12 hour dark photoperiod, the aquaria's water quality was maintained with an average temperature of 23–25 °C, O_2 levels over 6 mg L^{-1}, pH between 7.5 and 8.5, and ammonium concentrations below 1 mg L^{-1} (Yusoff et al., 2024). A YSI Professional Plus device was used to measure the water's temperature, pH, dissolved oxygen, and NH_4^+ concentrations.

Zeolite used in this study was obtained from Rota Mining (Manisa, Türkiye), leonardite from Kütahya Kimya (Kütahya, Türkiye), and diatomite from Nanotech İnşaat Kimya Maden ve Lojistik San. Tic. A.Ş. The chemical compositions of the zeolite, leonardite, and diatomite used in the study are presented in Table 1 (Öz et al., 2022; Şahin, 2022). The BET (Brunauer, Emmett, and Teller) surface area analysis recorded as 34.316, 12.253 and 174.698 m2/g for zeolite, leonardite and diatomite, respectively.

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

Zeolite (%)				
SiO_2	Silicon dioxide	78.41	SiO_2/Al_2O_3	5.67
Al_2O_3	Aluminium oxide	13.83	pH	8.31
MgO	Magnesium oxide	1.646		
K_2O	Potassium oxide	2.372		
CaO	Calcium Oxide	3.885		
Na_2O	Sodium oxide	1.042		
Fe_2O_3	Ferric oxid	1.414		
P_2O_5	Phosphorus pentoxide	0.058		
Leonardite (%)				
SiO_2	Silicon dioxide	13.68	SiO_2/Al_2O_3	1.93
Al_2O_3	Aluminium oxide	7.07	pH	3.23
MgO	Magnesium oxide	0.11		
K_2O	Potassium oxide	0.454		
CaO	Calcium Oxide	0.323		
Na_2O	Sodium oxide	<0.014		
Fe_2O_3	Ferric oxid	1.238		
P_2O_5	Phosphorus pentoxide	0.055		
Diatomite (%)				
SiO_2	Silicon dioxide	81.66	SiO_2/Al_2O_3	8.149
Al_2O_3	Aluminium oxide	10.02	pH	7.00
MgO	Magnesium oxide	3.839		
K_2O	Potassium oxide	0.99		
CaO	Calcium Oxide	2.041		
Na_2O	Sodium oxide	1.261		
Fe_2O_3	Ferric oxid	2.291		
P_2O_5	Phosphorus pentoxide	0.243		

Evaluation of the Experimental Data

The initial and final body weights of the fish were measured using a KERN balance with an accuracy of 0.001 g. The following formulas were used to calculate growth performance, feed utilization, survival rate, and related parameters (Türkmen & Karadal, 2017):

$$\text{WG (g)} = \text{Wf} - \text{Wi}$$

where,
WG is weight gain in gram
Wf is for final live weight
Wi represent the initial live weight

$$\text{SGR } (\%\text{day}^{-1}) = \left(\frac{\ln(\text{Final weight}) - \ln(\text{Initial weight})}{\text{experimental period}}\right) \times 100$$

$$\text{FCR} = \frac{\text{Total live weight gain,g}}{\text{Total feed consumed,g}}$$

where,
SGR is specific growth rate in percent per day
FCR stands for feed conversion ratio

$$\text{Survival rate (\%)} = \left(\frac{\text{Number of fish at the end of the experiment}}{\text{Number of fish at the beginnig of the experiment}}\right) \times 100$$

Statistical Methods

All of the data was examined using Minitab statistical software for Windows (Version 17). The data's normality was assessed using the Anderson Darling, Ryan Joiner (which is comparable to Shapiro Wilk), and Kolmogorov Smirnov tests. Parametric analyses were performed when the ANOVA assumptions were satisfied; non-parametric tests (Kruskal-Wallis) were employed otherwise. All data are presented as mean±standard error (SE), and statistical significance was established at $p<0.05$ (Öz, 2024).

Results and Discussion

Water Parameter Values

The water parameter values of the experimental groups at the end of the study are presented in Table 2. Analysis of variance was used to compare the initial parameters, and no statistically significant differences ($p>0.05$) were found across the groups. Similarly, the water parameter values obtained at the start and end of the experiment did not alter significantly ($p>0.05$).

Table 2. Water quality parameters measured at the conclusion of the 60-day experiment (mean±SE)

	Experimental groups			
	Zeolite	**Leonardite**	**Diatomite**	**Control**
Water temperature (ºC)	24.42±0.04	24.47±0.01	24.32±0.1	24.45±0.04
Dissolved oxygen (mgL^{-1})	6.12±0.05	6.21±0.02	6.14±0.03	6.00±0.02
NH_4 ($mg\ L^{-1}$)	0.35±0.03	0.34±0.02	0.34±0.02	0.35±0.02
pH	8.53±0.04	8.58±0.04	8.51±0.01	8.53±0.01

Previous studies have consistently indicated that water parameters remained within the optimal range for guppy (*Poecilia reticulata*), regardless of dietary treatments (Patel et al., 2023; Müslim & Fitri, 2025). Supporting these findings, Danabaş and Altun (2011) reported that the addition of zeolite to trout feed did not result in statistically significant differences in water quality among experimental groups. Similarly, Şahin et al. (2025a) demonstrated that varying inclusion levels of leonardite in feed had no significant effect on water parameters. Collectively, these results suggest that the incorporation of these feed additives does not adversely affect the water conditions necessary for maintaining the health and well-being of the studied species, thereby supporting their suitability for use in aquaculture practices.

Growth Parameters

In this study, the growth of guppy (*Poecilia reticulata*) fry was examined following dietary supplementation with 4% zeolite, leonardite, or diatomite. Weight gain (g), specific growth rate (SGR, %), and survival rate (%) showed statistically significant differences between the experimental groups after 60 days ($p<0.05$). However, the feed conversion ratio (FCR) did not differ statistically significantly across the groups ($p>0.05$) (Table 3).

Table 3. Growth performance of guppy fry by the end of the 60-day experiment (mean±SE)

	Zeolite	Leonardite	Diatomite	Control
Final weight (g)	0.17 ± 0.01^{b}	0.21 ± 0.01^{a}	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}
Weight gain (g)	0.15 ± 0.02^{ab}	0.19 ± 0.02^{a}	0.13 ± 0.01^{b}	0.13 ± 0.01^{b}
SGR (%)	3.56 ± 0.16^{ab}	3.99 ± 0.13^{a}	3.37 ± 0.10^{b}	3.33 ± 0.12^{b}
FCR	0.95 ± 0.05^{a}	0.89 ± 0.11^{a}	0.81 ± 0.09^{a}	0.87 ± 0.07^{a}
Survival rate (%)	97.7 ± 2.33^{a}	100 ± 0.00^{a}	92.9 ± 4.12^{a}	83.7 ± 2.33^{b}

*Different letters within the same row are significantly different ($p<0,05$).

When the growth parameters were examined, the positive findings obtained were consistent with those reported by Paritova et al. (2013), Şahin et al. (2025a), and Turan and Turgut (2020). Paritova et al. (2013) investigated the biochemical and histological effects of diets supplemented with different levels of zeolite in rainbow trout (*Oncorhynchus mykiss*) and reported that a diet containing 4% zeolite was optimal. Şahin et al. (2025b) supplemented the diets of goldfish (*Carassius auratus*) juveniles with 0%, 2%, 6%, and 10% leonardite, and at the end of the study, they determined that weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) were optimal in the group receiving 10% leonardite, whereas the survival rate was highest in the group supplemented with 6% leonardite. According to Turan and Turgut (2020), when leonardite was added to the diet of goldfish (*Carassius auratus*) at levels of 2%, 2.5%, and 5%, the group that received 5% leonardite had the best SGR, FCR, and growth performance, while the group that received 2.5% leonardite had the highest survival rate.

No studies have been identified that have investigated the effects of diatomite supplementation in the diets of ornamental fish on growth parameters. Similarly, Efsa Feedap et al. (2025) reported that while the use of diatomite as a feed additive was evaluated in poultry, small ruminant, and pig diets, no research had been conducted on aquatic animals. Growth metrics did not differ statistically substantially between the diatomite-supplemented group and the control group in the current study ($p>0.05$); nevertheless, the diatomite-treated group had a considerably higher survival rate ($p<0.05$).

Conclusion

Since zeolite has been more extensively studied and utilized as a feed additive than leonardite and diatomite, it was regarded as a second control group in this study after the evaluation of the data collected at the end of the investigation. Leonardite has been examined in a very limited number of studies, while diatomite was investigated for the first time in this study as a fish feed additive. Compared to the control group, all three feed additives yielded markedly better results in terms of growth performance and survival rates. Among these three additives, the best results were obtained with leonardite supplementation. Unlike zeolite and diatomite, leonardite is rich in humic acid content, and this difference may be attributed to humic substances. The results of this study showed that adding natural zeolite, leonardite, and diatomite to the diet improved fish growth indices, feed consumption, and survival rate. In addition to *Poecilia reticulata*, other economically significant ornamental fish species should be studied in future research, and the more specific effects of the natural feed additives looked at in this study should be further explored.

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Ethical approval
The SINOP UNIVERSITY ETHICS COMMITTEE approved the study protocol (Decision No. 2024/9-13; approval date: October 2, 2024). Every experimental procedure was carried out in strict accordance with international standards governing the care and use of laboratory animals, as well as the ethical rules and regulations set forth by SINOP UNIVERSITY.

Informed consent
Not available.

Data availability statement
The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing this study.

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Contribution of authors
Dilek Şahin: Project administration, Funding acquisition, Conceptualization, Resources, Supervision, Validation, Visualization, Data curation, Formal analysis, Writing original draft, Review, Editing.
Meryem Öz: Investigation, Methodology, Data curation, Formal analysis, Writing original draft, Review, Editing.
Ünal Öz: Investigation, Methodology, Data curation, Formal analysis, Writing original draft, Software.
All authors have read and agreed to the published version of the manuscript.

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