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EFFECTS OF NATURAL ZEOLITE (CLINOPTILOLITE) LEVELS IN FISH DIET ON WATER QUALITY, GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF TILAPIA (*Tilapia zillii*) FRY

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ABSTRACT

In the present study, the influences of dietary zeolite levels on growth performance, nutrient utilization of *Tilapia zillii* and water quality parameters were investigated through-out a 45-days period. Each experimental diet was applied to triplicate groups of 10 fish (mean wet weight 1.08±0.01 g). Fish were fed, five times a day with diets containing 38 % (crude protein) and 8.5% (lipid) supplemented with 0 (control), 1.0, 2.0 zeolite (clinoptilolite). Results indicated that fish groups fed diets with zeolite had higher weight gain, specific growth rate ($p>0.05$), protein efficiency rate ($p<0.05$), and better feed utilization than those fed diet without zeolite ($p>0.05$). Total ammonia nitrogen (TAN) was found to be 0.34 and 0.36 mg/L, respectively, in the groups fed with 1% and 2% zeolite-added rations. TAN was the highest as 0.39 mg/L in the control group ($p<0.05$). Lower ammonia release is one of the most important matters for maintainable and environment-friendly aquaculture activities. It was concluded that zeolite may be a suitable feed additive for use in *Tilapia zillii* diets.

KEYWORDS: zeolite (clinoptilolite), growth performance, feed utilization, ammonia discharge, tilapia.

INTRODUCTION

Although the pollution released from aquaculture to hygrophilous ecosystem is at minimum levels compared to domestic, agricultural and industrial sectors, articles and criticisms written about the negative environmental effects of aquaculture are rising [1]. Releasing of the least pollution to the environment around the fish reared, doubtlessly, can be provided through well-balanced fish-feed ration. When the feed reached the fish-body, nitrogen compounds are released to the environment.

Ammonia is the main nitrogenous waste compound excreted by fish and crustaceans. Two fractions of total ammonia nitrogen (TAN) are ionized ammonia (NH_4^+) and un-ionized ammonia (NH_3). The un-ionized type of ammonia- nitrogen is enormously toxic to fish [23]. Total ammonia nitrogen can be fatal above 1 mg/L for tilapia cultures [14]. In minimizing nitrogen, which is one of the main polluters, a qualified feed which meets the physiological needs of the fish and raw-materials, which are highly digestible and comprising this feed, are in the lead. In addition to that, feed additives such as zeolite, may provide the feed taken by the fish heartily and may reduce the water pollution, that is arisen from feed and faecal matters.

Natural zeolites have three functions in aquaculture. These can be classified as removing ammonium from the hatchery, where aquaculture is done, pool and aquariums; enriching ventilating systems with oxygen in aquarium and fish carrying; using feed additives in feed rations [3]. Many natural zeolites may considerably contribute in removing nitrogen from aquaculture because of their ion exchange and selective adsorption features [4]. In aquaculture, zeolites are used for producing nitrite and nitrate, which are less poisonous than ammonia, by means of activating bacteria, which help decomposing protein in fish-feed that was not consumed [5]. Clinoptilolite is found abundantly in natural zeolites [6], and has the best selectivity for ammonia. Moreover, clinoptilolites have a fine ion exchange capability and can absorb ammonium easily [7].

Studies related to zeolite are mainly performed in the fields of poultry, cattle and porcine husbandries, and there are not many studies available related to its usage in aquaculture. In a research performed by Dias et al. [8], among different rations made by different feed additives in European seabass, they ascertained that, while the diet with 10%-20% zeolite addition did not have any negative influence on growth performance of the fish, it brought out values very close to control group in terms of feed evaluation ratio. Mumpton [3] affirmed that adding less than 2% zeolite to the portions makes no negative influence on the health of trout. Obradović et al. [9] carried out a research study on rainbow trout with feeds, with or without addition of 1% zeolite (minazel type). Fish fed with 1% zeolite added had a positive influence on growth of the fish and feed conversion. Besides, they found that the water

quality values (ammonia compounds) of the group, fed with zeolite-added feed, were insignificantly reduced. Eya et al. [19] indicated that incorporation of zeolite (bentonite or mordenite) into the diets of juvenile rainbow trout had no adverse effects on performance and whole-body composition. Ergül et al. [10] investigated ammonia excretion rates in zeolite levels of trout dietary. They concluded that the diets, which contained 2.5% zeolite, decreased ammonia discharge at about 24% compared to control group.

In this study, we aimed to determine the influences of different natural zeolite (clinoptilolite) levels on growth performances of *Tilapia zillii* fries, feed utilization and water quality values.

MATERIALS AND METHODS

Tilapia zillii were caught from Köyceğiz Lagoon with the help of a small hand net. The fish were transferred to Muğla University, Faculty of Fisheries - Fish Feeding Unit, after being put in 50-L-buckets containing ventilating rocks. The fish were subjected to rearing conditions for 2 months of adaptation period. Then, 10 fish were randomly stocked into each tank, with 3 replications per treatment.

Protein and fat ingredients of the diets were formulised as 38% and 8.5%, respectively, and 3 different rations were formed for this research study. Natural zeolite (1-2%) was added to the diets, but the control group did not contain zeolite. While fish flour, soybean meals and fish oil constitute the main raw materials in the rations, vitamins, minerals, antioxidants and zeolite comprise the feed additives. The proximate compositions of the experimental diets are reported in Table 1. The chemical composition of the original zeolite (clinoptilolite) sample is given in Table 2. In order to powder granular zeolites, they were ground in a pestle for a while. The dry ingredients (weights taken as per ration formulation) were mixed for about 15 min. Hot water was added in order to guarantee the passage of the mixture through the laboratory-type pelleting machine and its viscous outlet. Diets pelleted (2 mm) were dried until approximately 10% water content. Feeds were cut into pieces small enough to be consumed by the fish. The fish were fed at 8% of their body-weight. Daily feed amounts were split into 5 equal pieces and given in 5 meal portions (at 9.00, 11.00, 13.00, 15.00, 17.00). Weights of the fish were measured at 15-days intervals.

Ammonium compounds (ammonia, ammonium), pH, and dissolved oxygen (DO) in the water were analysed from water outlet and inlet of the tank, once in a week. Temperature was measured daily. Water quality parameters were measured 3 h after the first feeding of the fish. Ammonium compounds were ascertained with the help of a Lovibond PC Multidirect photometer and by means of standard photometric method. DO was measured with an YSI 556 MPS model device and pH with a Hanna HI 9828 model device.

TABLE 1 - Proximate composition of the experimental diets.

Feed ingredients	Groups		
	Control	1% Zeolite	2% Zeolite
Fish meal	30	30	30
Wheat meal	31	30	29
Soybean meal	30	30	30
Fish oil	4	4	4
Zeolite	0.0	1.0	2.0
Vitamins	2.7	2.7	2.7
Minerals	2.2	2.2	2.2
Antioxidants	0.1	0.1	0.1
Proximate analyses			
Crude protein	38.33	38.22	38.12
Crude lipids	8.55	8.53	8.51
Crude ash	5.18	5.16	5.14
Moisture	8.13	8.16	8.00

TABLE 2- Chemical analysis[1] of Western Anatolia clinoptilolite used in the current study.

Elements	% (wt/wt)
SiO_2	71.0
CaO	3.4
Fe_2O_3	1.7
Al_2O_3	11.8
K_2O	2.4
MgO	1.4
Na_2O	0.4
TiO_2	0.1

[1] Mineralogic-Petrographic analysis is associated to the sample of Rota Mining Co. from Kalabak Damları district, Gordes-Manisa, Turkey

Data analysis results are expressed as means (±SD). Dietary treatments for adding zeolite or free-zeolite were compared by one-way ANOVA, and means were compared by Tukey's multiple range test, with $p < 0.05$ level. Statistical analyses were conducted using Minitab 13 for Windows.

RESULTS AND DISCUSSION

Growth performance and feed utilization

The growth performances of the fish and feed conversion rates have been determined and data are given in Table 3 for the experimental period of 45 days. In control group, average weight of fish was 1.08 g at the beginning of the experiments, whereas 1 and 2% zeolite diet had 2.65 g at the beginning but 2.95 g and 3.16 g, respectively, at the end of experiments. The difference between the fish fed containing different diets was not considered as significant in terms of growth performance ($p>0.05$). However, the growth performance of the fish fed with diets containing zeolite showed an increase compared to control group. The best value in terms of SGR (specific growth rate) was obtained to be 2.63, with 2% zeolite-added ration, but was found to be only 2.23 and 1.99, for 1%-zeolite and control group, respectively. There are no statistically significant differences between all groups ($p>0.05$).

In terms of feed conversion, the difference between groups with and without zeolite was found to be statistically insignificant ($p>0.05$), and FCR (feed conversion

TABLE 3- Growth performance of *Tilapia zillii* fed experimental diets.

Growth parameters	Control	1% Zeolite	2% Zeolite
Initial mean body weight (g)	1.08±0.01	1.08±0.01	1.08±0.01
Final mean body weight (g)	2.65±0.11[a]	2.95±0.09[a]	3.16±0.35[a]
SGR[1]	1.99±1.12[a]	2.23±1.08[a]	2.36±1.02[a]
FCR[2]	4.03±0.9[a]	3.26±0.33[a]	2.84±0.41[a]
PER[3]	0.79±0.05[a]	0.91±0.02[ab]	0.99±0.12[b]

[1]Specific growth rate, SGR (%) = [(ln final wet weight - ln initial wet weight) / days] x 100
[2]Feed conversion rate, FCR = Total feed intake / wet weight gain
[3]Protein efficiency rate, PER = Wet weight gain / protein intake
Values (means ± SD) with different superscripts in the same row are significantly different at the 5% level.

ratio) value was found to be best (2.84) for 2%-zeolite group and worst (4.03) for control group (Fig. 1). With regard to PER (protein efficiency ratio), the best group was the one that contained 2% zeolite (0.99), followed by 1%-zeolite group (0.91) and control group (0.79). According to the statistical evaluation performed above, the difference between the group fed with 2% zeolite and that of control was found to be significant ($p<0.05$). During the experimental period, no mortality was observed in any of the groups (survival rate was 100%).

Water quality parameters

Average temperature, DO, pH, total ammonia nitrogen values related to water quality parameters are listed in Table 4. During the experiment, the lowest temperature among the groups was measured as 23.1 °C, but the highest to be 26.7 °C. Average water temperatures were 24.56, 24.99 and 25.09 °C for control, 1 and 2% zeolite groups. Muir et al. [11] have stated that the suitable temperatures for tilapia culturing are between 10-35 °C. Our water temperature values for all groups were within this range.

Throughout the experiment, DO and pH were measured at their lowest levels as 5.27 ppm and 7.70, and their highest ones as 7.78 ppm and 8.56, respectively. Average DO and pHs in control, 1% and 2% zeolite groups were ascertained to be 6.72 ppm, 8.33; 6.56 ppm, and 8.18; 6.37; 8.14, respectively. Muir et al. [11] and Riche and Garling [12] suggest that DO concentration for tilapias should be higher than 3 ppm. Stickney [13] stated that water pHs between 6.5-9.0 are suitable for optimum growth. DO and pH values obtained during our experiment were within this range. The average total ammonia nitrogen, in the groups fed with diet plus 1 or 2% zeolite, was found to be 0.34 and 0.36 mg/L, respectively, and highest (0.39 mg/L) in control group (Fig. 1). Total ammonia–N discharge in the groups that were fed with zeolite-added rations was found to be relatively lower ($p<0.05$). Suresh [14] stated that the total ammonia should be less than 1 ppm. No fish mortality was observed throughout the experimental period. This situation can be explained by the ammonia compound values within the safe limits.

TABLE 4 - Physicochemical parameters of tank water during the experimental period.

Water parameters	Tank Entrance	Tank Discharge		
		Control	1% Zeolite	2 %Zeolite
Temperature (°C)	18.42±0.76	24.56±0.77[a]	24.99±0.93[a]	25.09±0.82[a]
Dissolved oxygen (mg/L)	8.39±0.78	6.72±0.66[a]	6.56±0.59[a]	6.37±0.62[a]
pH (units)	8.56±0.25	8.33±0.18[a]	8.18±0.23[a]	8.14±0.24[a]
Total ammonia (mg/L)	0.06±010	0.39±0.01[b]	0.34±0.01[a]	0.36±0.01[a]

Values (means ± SD) with different superscripts in the same row are significantly different at the 5% level

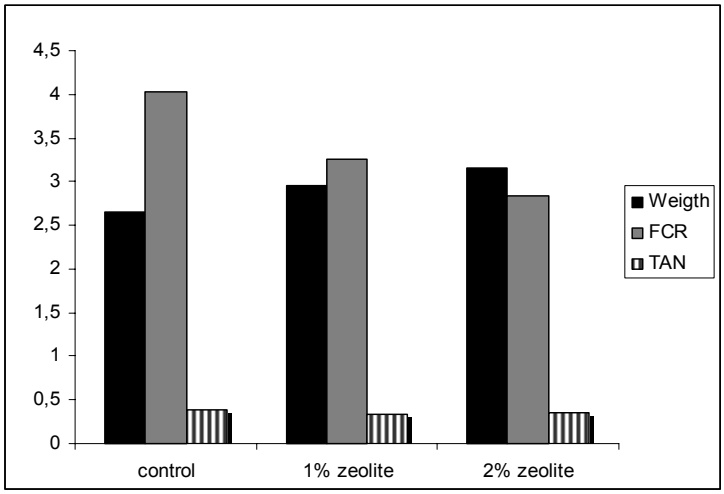

FIGURE 1 - Weight (g), Feed conversion rate and Total ammonia nitrogen (mg/L) after feeding *Tilapia zillii* with diets containing different levels of zeolite.

While this research is one of several studies about zeolites in aquaculture, it is the first one performed over *Tilapia zillii*. Several studies related to the usage of zeolites in aquaculture about sea bass [8] and rainbow trout [9, 10, 19] were already published. In a study, performed by Dias et al. [8], concerning sea bass fed with diet enriched by 10% zeolite, no statistical difference was found in terms of SGR and daily weight, with respect to control group fed without zeolite. On the other hand, the growth rate in 10% zeolite group was even a little better than in control group. Obradović et al. [9] reported that rainbow trout fed with 1% zeolite-enriched (minazel) feed had a positive influence on growth of the fish. Eya et al. [19] demonstrated that dietary zeolite (5, 10% bentonite, 2.5% mordenite) supplementation of trout feed enhanced weight gain, feed efficiency, and specific growth rate. Our growth parameters have similarities to the above-mentioned studies. FCR, measure of well or not converted feed given to the fish, and PER in control as well as 1 or 2% zeolite groups, were acquired to be 4.03, 3.26, 2.84 and 0.79, 0.91, 0.99, respectively. Both values were materialised better in zeolite-addition groups. These results were found to be compatible with those of Obradović et al. [9] and Eya et al. [19]. Feeding the fish with zeolite-containing diet influenced the growth and feed usage positively, and this was additionally underlined because with zeolite even little amounts of mineral substances (aluminium, sodium, potassium, magnesium etc.) were consumed.

Besides, zeolite consumption by fish led to change of pH and buffering capacity of gastrointestinal glands, and influenced the transport through intestinal epithelium. Moreover, fish fed diet supplemented with zeolite, showed a slower feed passage through the digestive system, thus possibly having better absorbed the nutrients [8, 19]. Zeolite has the ability to retain ammonium ions (NH_4) and ammonia gas (NH_3) in the digestive system [24]. It is emphasized by different researchers [6, 7, 15-17] that zeolites (clinoptilolites) can be used for removing ammonium from wastewater. TAN consisted of toxic, in other words, non-ionized (NH_3) and ionized ammonia (NH_4). In aquaculture, ammonium compounds are discharged into environment in 2 forms, non-consumed feed and faecal matter [2]. Proteins in the fish-feed are main sources of nitrogen, which is extracted through the gills of the fish [18]. In the present study, according to the groups (control, 1 and 2% zeolite), average ammonia nitrogen (NH_3-N) was determined to be 0.19, 0.17, and 0.17 mg/L, but average ammonium nitrogen (NH_4-N), respectively, 0.20, 0.17, and 0.19 mg/L. In the groups, which were fed with zeolite-supplemented diets, ammonia and ammonium were found relatively to be lower than in control group. Total ammonia nitrogen derivatives, depending upon the protein rate in feed, increased regardless of the protein source. Ergül et al. [10], in a study performed with trout, found that the diets containing 2.5% zeolite, decreased ammonia discharge to about 24% compared to control group. Obradović et al. [9] revealed that the diet containing zeolite decreased the release of ammonia. Ammonia compounds were found to be relatively lower than those in control group, according with the results of the above-mentioned authors. An important attribute of the zeolite is its capacity to effectively bind ammonia and, as a result, to reduce ammonia concentration by release into the environment [22]. Zeolite, as feed additive, can contribute removal of excess ammonia in the digestive system [20, 21].

As a result, usage of zeolite in diets at rates of 1-2% influenced the growth performance of the fish as well as feed conversion positively. In the groups fed with zeolite diet, total ammonium-N decreased but not totally. It is essential to know the ammonia compound excretion rates of the fish cultivated. In aquaculture operations, the ammonia discharge is at lower values, is one of the most important matters for maintainable and environment-friendly aquaculture activities. Therefore, in further researches, it would be beneficial that zeolite-containing feeds are evaluated for other fish species as well.

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