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Effects of Dietary Natural Zeolite (Clinoptilolite) on Growth and Some Blood Parameters of Rainbow Trout (*Onchorynchus mykiss*, Walbaum 1792)

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Research Article

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Abstract

This study was performed to evaluate the effects of adding different levels of natural zeolite (clinoptilolite) to juvenile rainbow trout (*Onchorynchus mykiss*) diets about growth performance and some biochemical blood parameters. The experimental system was designed as a semi-recirculating aquaculture system and 20 rainbow trouts which weigh 7.91±0.03 g distributed randomly for each tank (300 L). Fish were fed to apparent satiation with diets containing 45% crude protein and 14% crude fat for 70 days which included no zeolite (Control), 0.5% zeolite (Z05), 1% zeolite (Z1), 2.5% zeolite (Z25) in triplicate. Finally, blood samples were collected from all fish tanks to analyze some biochemical blood parameters like cholesterol, triglycerides, LDL, HDL, and VLDL. At the end of the experiment, Z25 group showed negative results in final body weight, weight gain, and specific growth rate ($p<0.05$). Although Z05 group showed no significant difference, it showed the best results in final body weight, weight gain, specific growth rate, feed intake, feed conversion rate, and protein efficiency. In terms of blood parameters, all the groups had similar values with no significance ($p>0.05$) compared to the control group. As a result, there was a tendency towards decreased growth and feed utilization due to the addition of zeolite higher than 1% in diets. It could be concluded that limited usage of clinoptilolite in rainbow trout diets might have beneficial effects on growth parameters.

Keywords: Zeolite, trout, growth, blood parameters.

Diyetteki Doğal Zeolitin (Klinoptilolit) Gökkuşağı Alabalığının (*Onchorynchus mykiss*, Walbaum 1792) Büyüme ve Bazı Kan Parametreleri Üzerindeki Etkileri

Bu çalışma, genç gökkuşağı alabalığı (*Onchorynchus mykiss*) diyetlerine farklı seviyelerde doğal zeolit (klinoptilolit) eklenmesinin büyüme performansı ve bazı biyokimyasal kan parametreleri üzerindeki etkilerini değerlendirmek amacıyla yapılmıştır. Deney sistemi, yarı devridaim yapan bir kültür balıkçılığı sistemi olarak tasarlanmış ve her bir tanka (300 L) 7.91±0.03 g ağırlığında 20 gökkuşağı alabalığı rastgele dağıtılmıştır. tasarlanmıştır. Balıklar, %45 ham protein ve %14 ham yağ kompozisyonlu zeolit içermeyen (Kontrol),% 0.5 zeolit (Z05),% 1 zeolit (Z1),% 2.5 zeolit (Z25) içeren diyetlerle 70 gün boyunca doyana kadar beslenmiştir. Son olarak, kolesterol, trigliseritler, LDL, HDL ve VLDL gibi bazı biyokimyasal kan parametrelerini analiz etmek için tüm balık tanklarından kan örnekleri alınmıştır. Deney sonunda Z25 grubu son vücut ağırlığı, ağırlık artışı ve spesifik büyüme oranında negatif sonuçlar göstermiştir ($p <0.05$). Z05 grubu, anlamlı bir fark olmamasına rağmen son vücut ağırlığı, ağırlık artışı, spesifik büyüme oranı, yem alımı, yemden yararlanma oranı ve protein veriminde en iyi sonuçları göstermiştir. Kan parametreleri açısından tüm gruplar, kontrol grubuna göre anlamlı olmayan benzer değerlere sahiptir ($p> 0.05$). Sonuç olarak, zeolitin diyetlere %1'den fazla eklenmesi nedeniyle büyüme ve yemden yararlanmada azalma eğilimigörülmüştür. Gökkuşağı alabalığı diyetlerinde sınırlı klinoptilolit kullanımının büyüme parametreleri üzerinde faydalı etkileri olabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Zeolit, alabalık, büyüme, kan parametreleri.

INTRODUCTION

Farmed rainbow trout (*Oncorhynchus mykiss*) production had increased by 19% from 2008 to 2017 (FAO, 2017). Most of the problems like environmental impact and sustainability in aquaculture are directly related to the origin of the diets which were used for carnivorous feeding (Tacon, 1997; Naylor et al., 2000). Most of the salmonid diets were used to be manufactured from animal originated

raw materials where plant-based raw materials and feed additives were only used as supplementary ingredients. To decrease the environmental impact and economical concerns, recent trends about fish feed manufacturing are to reduce protein cost, waste products, and marine originated raw materials (Papatryphon, 2004). Balanced diets having high digestibility rates had an important role in reducing the nitrogenous wastes which were excreted by the fish. Zeolites can also help to reduce ammonia excretion rates as well as using highly digestible raw materials. Zeolites were described as aluminosilicates of the alkaline and alkaline earth cations having an infinite, open and three-dimensional structure, capable of exchanging water and cations in the crystal structure without changing the crystal structure and containing water molecules (Mumpton and Fishman, 1977). Their general application areas were construction, water, wastewater treatment, adsorption, catalysis, removal of nuclear wastes, and agriculture. Animal nutrition and welfare, horticulture, removal of animal wastes, and aquaculture were some of its usage areas in agriculture. Zeolites were mainly used for; removal of ammonia from hatcheries, aquariums, transportation media, and feed additives for fish feed (Bernal et al., 1993; Mumpton, 1999) in aquaculture. Despite having this much broad application and there were many examples of dietary usage in terrestrial animals (Valpotić et al., 2017), there were limited experiments in aquaculture (De Silva and Anderson, 1995). Few trials were conducted on sea bass *Dicentrarchus labrax*, (Dias et al., 1998), sea bream *Sparus aurata*, (Kanyilmaz et al., 2015), rainbow trout *Onchorynchus mykiss*, (Obradovic et al., 2006; Demir and Aybal, 2004; Eya et al., 2008; Danabas, 2009; 2011; Alinezhad et al., 2017), coho salmon *Onchorhyncus kisutch*, (Edsall and Smith, 1989), snakehead murrel *Channa striata*, (Jawahar et al., 2016) and tilapia *Oreochromis niloticus*, *Tilapia zillii*, (Tore, 2006; Yildirim et al., 2009; Zahran et al., 2020), common carp *Cyprinus carpio*, (Kanyilmaz, 2008) and crayfish *Astacus leptodactylus*, (Aksu, 2016). The working mechanism of zeolites regarding growth is reported as selective adsorption of ions (Ames 1967; Mercer et al., 1970), changing feces profile and extending the passage time from the digestive tract (Lanari et al., 1996; Dias et al., 1998). Also, blood in terms of blood parameters, it was remarked (Ly et al., 2007; Kanyilmaz and Tekellioglu, 2016) that serum cholesterol and triglycerides values were affected by absorption of the short-chain fatty acids by zeolites. This study aimed to evaluate any effects of dietary natural zeolite (clinoptilolite) on rainbow trout diets on growth and some blood biochemical parameters.

MATERIAL AND METHOD

Experimental Design and Rearing

Rainbow trouts (*Onchornhychus mykiss*, n=360) obtained from a local commercial trout farm in Canakkale - Turkey were transported to the Faculty of Fisheries at Canakkale Onsekiz Mart University Research Unit. Before the start of the feeding trial, fish were fed a commercial diet (45% protein, 12% fat) for acclimatization. After the conditioning period, triplicate groups of 20 fish, which were initially averaging 7.91±0.03 g were stocked in cylindrical tanks (twelve tanks of 300 L), each filled with dechlorinated tap water. The experiment was carried out with 240 fish selected from 360, divided into 12 tanks (four groups and triplicate tanks). The water flow was 3 L/min, and all tanks were aerated by air stones. The semi-recirculating system used in the experiment consisted of eighteen 300 L tanks, a 300 L sump, 200 L sand filter, and a 300 L biofilter containing bio balls. The total volume of the system was 4400 L. One thousand liters of makeup water was exchanged daily corresponding to about 22,7% of the total volume. During the growth experiment, dissolved oxygen and water temperature daily and nitrite, nitrate and total ammonia) weekly monitored daily and all water quality parameters were in the optimal ranges. Through the experiment 70 days of feeding was and on every 15 days, the fish weighed. Twenty fish were randomly distributed to the experimental tanks from the stock tank and there were 12 tanks. To prevent weight and size inequality every fish was measured individually. In final weighing standard error was calculated. Total of four diets were prepared and given to the experimental groups three times a day to apparent satiation. Feed intake behavior and appetite were observed to prevent overfeeding. The amount of the feed was recorded every day to calculate feed intake.

Zeolite

Natural zeolite (clinoptilolite) used in the experiment was from Gordes Region in Manisa, Turkey. Chemical composition of the zeolite was given in Table.1.

Table.1. Chemical composition of the natural zeolite (Clinoptilolite) used in the experiment

Ingredients*	%
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

*Mineralogic – petrographic report; was issued at İstanbul Technical University Mining Faculty to the samples which were obtained from Kalabak Damlari (Gordes – Manisa, Turkey) region.

Experimental Diets

The experimental diets were manufactured to contain 45% crude protein (CP) and 14% crude fat (CF). They were formulated to be isonitrogenic and isocaloric. The formulation of the experimental diets was given in Table.2. The wheat meal rate in the control group was decreased to add zeolite to the diet groups. At a rate of 0.5% (Z05), 1% (Z1), and 2.5% (Z25) zeolite were added to the other three experimental groups. The pre-weighed dry ingredients were carefully mixed using a laboratory food mixer, with separate addition of the fish oil and vitamin/mineral premix. The mixture was primed with water to yield a suitable mash. Moist diets were made into pellets of 2 mm diameter and dried at 40 °C in a fan-assisted drying cabinet. Formulation and nutritional composition of the experimental diets were given in Table. 2 and Table. 3.

Table.1. Formulation of the experimental diets

Ingredients (%)	Groups			
	Control	**Z05**	**Z1**	**Z25**
Fish Meal[1]	50	50	50	50
Wheat Meal[2]	14	13.5	13	11.5
Soy Meal[3]	24.5	24.5	24.5	24.5
Fish Oil[4]	10	10	10	10
Zeolite (clinoptilolite)[5]	0	0.5	1	2.5
Vitamin – mineral[6]	1.5	1.5	1.5	1.5

[1] Anchovy Meal, Can Kardesler Balık Unu Sirketi, Samsun - Turkey
[2] Kepez Un, Canakkale - Turkey
[3] Abalioglu Yem ve Gida Sanayi, Denizli - Turkey
[4] Anchovy Fish Oil, Can Kardesler Balık Unu Sirketi, Samsun - Turkey
[5] Clinoptilolite, Rota Madencilik, Manisa - Turkey
[6] Vit – Min. Vitamin per g: vitamin A: 342 IU; vitamin D3: 329 IU; vitamin E: 0.0274 IU; vitamin K3: 5.48 mg; vitamin B1: 2.05 mg; vitamin B2: 3.42 mg; vitamin B3: 20.5 mg; vitamin B5: 5.48 mg; vitamin B6: 2.05 mg; vitamin B12: 2.74 mg; vitamin C: 24.0 mg, Mineral per g: biotin: 0.411 mg; folic acit: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Kimya San. Ve Tic., Kocaeli -Turkey.

Table 3. Nutritional composition of the experimental diets

Parameter	Control	Z05	Z1	Z25
Crude Protein	44.96	44.90	44.84	44.86
Crude Fat	14.06	14.05	14.04	14.01
Crude Ash	8.1	8.6	9	9.6
Moisture	10.2	10.3	10.1	9.9

Sampling, Weighing, and Measurements

The weighing was done via a 0.1 g precision scale (Scaltec, Germany). At the beginning of the experiment, no anesthetics were used due to the small size of the fish but MS_{222} was used at a rate of 50 mg/L at the end of the experiment. Through the weight measurement, all the fish were extracted from the tank and weighted individually.

Nutritional Analysis of Experimental Feed

Sample diets were left to dry out in a drying oven for 24 at 105 ^{o}C until their weight was stabilized. A food processor was used for grinding the feed samples into powder form. Moisture, crude protein, crude fat, crude ash analysis of feed samples were done according to AOAC (2000) method.

Moisture Analysis

Five grams of samples were taken from the experimental diets. They were put into the previously tared aluminum holders and left to dry out in a drying oven for 24 at 105 ^{o}C until their weight was stabilized. Moisture percentage was calculated according to the formula below.

$Moisture\ (\%) =$

$(\text{Dry Sample} + \text{Tare (g)} - \text{Initial Sample Weight (g)}) / (\text{Initial Sample Weight (g)}) \times 100$

Crude Protein Analysis

Kjeldahl Method was preferred for protein analysis in experimental diets. Approximately 0.5 g of samples were used for analysis. The first catalyst tablet and 15 ml sulphuric acid (H_2SO_4) was added to the glass digestion tubes. Protein digestion was carried out at the Gerhard Kjaldelterm digestion block. Samples were incinerated at 250 ^{o}C for 30 minutes initially, following 75 minutes at 380 ^{o}C. Samples were taken to the Gerhardt Vapodest 3S distillation unit, neutralized with 40% NaOH solution, and diluted with distilled water. After distillation, inorganic ammonia was collected in 25 ml orthoboric acid solution containing BDH "4.5 " indicator. Samples were then titrated with 0.1 mol hydrochloric (HCl). The protein percentage was (P,%) calculated according to the formula below.

$\text{Crude Protein } (\%) = [(B - S)\text{x N x } 1.4007 \text{ x F}] / W$

P: percentage of crude protein
F: protein factor (6.25)
B: ml, NaOH back titration of blank
S: ml, NaOH back titration of a sample
N: normality of NaOH
W: g, the weight of the sample
14.007: Molecular mass of Nitrogen

Crude Fat Analysis

The soxhlet extraction method was used for crude fat analysis. 3 g of dry matter was weighed and placed in the separation section of the instrument. The sample was then siphoned with 130 ml of petroleum ether for 40 minutes to collect the petroleum ether in the volumetric flask. Circulation was continued for 70 minutes after siphoning. After the end of the circulation process, siphoning was applied again and the remaining solution was removed by evaporation in the volumetric flask. The fat content of the dry matter was calculated by the following formula.

$\text{Crude Fat } (\%) = \text{Fat Accumulated (g)} / \text{Sample Weight (g)} \text{ x } 100$

Crude Ash Analysis

The dry matter was calculated by taking 0.5 g of sample and weighing by putting it into pre-tared porcelain crucibles. The crucibles were then fired in the incinerator at 525^{o}C for 12 hours. The ash content of the samples according to the weight change of the containers was calculated according to the following formula.

$\text{Crude Ash } (\%) = \text{Weight change in porcelain container (g)} / \text{Sample Weight (g)} \text{ x } 100$

Data Analysis

Growth indices were calculated according to the formula given below (Khodazanary et al., 2013; Jawahar et al., 2016).

$\text{Initial Body Weight (IBW)} = \text{Initial Weight (g)} / \text{n Fish}$

$\text{Final Body Weight (FBW)} = \text{Final Weight (g)} / \text{n Fish}$

$\text{Weight Gain (WG)} = \text{Final Weight (g)} - \text{Initial Weight (g)}$

$\text{Weight gain Percent (WG\%)} = [\text{Final Weight (g)} - \text{Initial Weight (g)}] / \text{Initial Weight (g)} \text{ x } 100$

$\text{Sp. Growth Rate (SGR)} = [\text{Ln (Final Avr. Weight (g))} - \text{Ln (Initial Avr. Weight (g))}] / \text{Day} \text{ x } 100$

Feed intake (g/fish/day) = Feed Consumption (g) / Day

$\text{Feed Intake (average)} = \text{Feed Consumption (g)} / \text{No of Fish}$

$\text{Feed Conversion Rate (FCR)} = \text{Feed consumed (g)} / \text{Weight Gain (g)}$

$\text{Protein Efficiency Rate (PER)} = \text{Weight Gain (g)} / \text{Protein Consumption (g)} \text{ x } 100$

Blood Sampling

All the fish were fasted for a day before blood sampling. Fish were anesthetized via 50 mg/L diluted MS_{222} and fixed with a dry towel to remove water, mucus from the body of the fishes. Approximately 1.5 – 2 ml of blood was collected from each fish to vacuette (Greiner Bio-One) gel containers via a single cut to the caudal vena. Immediately after the procedure samples were rushed to the laboratory and zanalyzed via auto analyzer (Olympus Optical Corp., Shizuoka-ken) (Kandemir et al., 2010).

Statistical Analysis

All the results were first subjected to analysis of variance (ANOVA) and later differences were evaluated by "Duncan's Multiple Range Test" was carried out in a 95% confidence interval.

RESULTS

Growth Trial

All the experimental groups showed no significant difference compared to the control group except Z25 group. Adding 2.5% zeolite had a statistically significant ($p<0.05$) negative effect on FBW, %WG, WG, and SGR. However, Z05 and Z1 groups showed a significant difference between each other on FCR also, the same observed between Z05 and Z25 ($p<0.05$). Growth and feed utilization values were presented in Table.4.

Table 4. Growth Parameters and Feed Utilisation Values

Parameters	Control	Z05	Z1	Z25
IBW (g)	7.94±0.01^{a}	7.93±0.06^{a}	7.89±0.03^{a}	7.86±0.04^{a}
FBW (g)	75.53±2.43^{a}	81.06±2.69^{a}	74.13±5.61^{a}	62.36±0.31^{b}
WG (g)	67.59±2.44^{a}	73.13±2.68^{a}	66.24±5.64^{a}	54.50±0.30^{b}
% WG	850.55±31,20^{a}	921.96±34.11^{a}	839.50±74.54^{a}	692.97±4.83^{b}
SGR	3.00±0.04^{a}	3.09±0.04^{a}	2.98±0.10^{a}	2.76±0,00^{b}
FI $^{g/fish/day}$	0.86±0.05^{a}	0.87±0.03^{a}	0.86±0.08^{a}	0.75±0.02^{a}
Av. FI $^{d/n}$	65.09±3.76^{a}	65.49±2.73^{a}	65.13±6.37^{a}	56.68±1.51^{a}
FCR	0.96±0.005^{abc}	0.89±0.009^{c}	0.98±0,01^{ab}	1.04±0.01^{a}
PER	2.26±0.01^{a}	2.48±0.02^{a}	2.26±0.03^{a}	2.13±0.03^{a}

The values of the experiment groups with different exponential letters in the same row are different from each other ($p<0.05$) *± SE: Standart Error

Blood Parameters

At the last step of the experiment, some biochemical parameters were studied. Biochemical blood parameters were presented in Table. 5. Comparing with the control group there were no significant differences were observed among cholesterol (CHO), high-density lipoporteins (HDL), low-density lipoproteins (LDL), and blood urea nitrogen (BUN) ($p>0.05$). However, Z1 and Z25 showed a significant difference between them regarding triglycerides and very low-density lipoproteins (VLDL) ($p<0.05$).

Table 5. Biochemical blood parameters at the end of the trial

mg/dl	Control	Z05	Z1	Z25
Triglycerides	211.00±57.51^{ab}	161.00±20.30^{ab}	234.67±16.50^{a}	136.67±34.59^{b}
Cholesterol	186.33±70.30^{a}	143.33±12.01^{a}	180.00±6.08^{a}	133.67±24.50^{a}
HDL	68.00±30.64^{a}	49.33±3.79^{a}	58.33±1.53^{a}	52.67±8.50^{a}
LDL	76.00±28.79^{a}	61.67±4.51^{a}	74.67±3.21^{a}	53.67±9.61^{a}
VLDL	42.33±11.50^{ab}	32.33±4.16^{ab}	47.00±3.60^{a}	27.33±6.65^{b}
BUN	3.80±0.52^{a}	3.96±0.85^{a}	4.03±0.87^{a}	2.10±0.70^{a}

The values of the experiment groups with different exponential letters in the same row are different from each other ($p<0.05$) *± SE: Standart Error

DISCUSSION

It was reported that due to their probable role in efficient use of nutrients (Olver, 1989) and/or detoxifying role, incorporation of natural zeolites to animal diets resulted in increased growth and development of living organisms (Harvey et al., 1993; Parlat et al., 1999; Ortatatli and Oguz, 2001; Rizzi et al., 2003). There were mixed results reported in terms of dietary usage of zeolites on growth

parameters of different species in aquaculture. Reinitz (1984) reported that adding %5, 10%, and 15% bentonite to rainbow trout diets harm the weight gain of the animals. Leonard (1979) stated that there was no significant difference between the live weights of the trout fed with 2% clinoptilolite feed (Pond and Mumpton, 1984). Edsall and Smith (1989) reported that adding 5% and 10% of clinoptilolite to the diets of coho salmon did not cause any effect on growth rate. Dias et al. (1998) investigated the effect of different feed additives (silica, cellulose, and natural zeolite) on protein digestibility, growth, FI, and feed transition time in seabass by adding 10% and 20% natural zeolite to feeds and reported that there was no effect between SGR, FCR, FBW values. In a study carried out by Demir and Aybal (2004) where the change in growth parameters of different rates (1-2-3-4-5-6%) of clinoptilolite added to rainbow trout diets evaluated, it was reported that there was no difference between the control group and the experimental groups in terms of FBW and FCR. Although there was no statistical significance, there were fluctuations between values that did not follow any trend. In this experiment, although it was not significant, a trend in growth parameters could be easily seen in parallel to the quantity of the zeolite added to the diet. Alinezhad et al. (2017) reported that in rainbow trout juveniles, adding different rates of nano-structured zeolite (0.5-1%) and aflatoxin B1 (5 mg) combination had no significant differences in growth parameters like FW, WG, BWI, SGR, FCR and condition factor. This experiment showed parallelism with the aforementioned trials. However, Lanari et al. (1996) reported that adding zeolite to rainbow trout diets at a rate of 2.5% and 5% increased weight gain and feed efficiency. Obradovic et al. (2006) conducted a trial with rainbow trout reported that adding 1% zeolite (Minazel) to pellets and certain rates of zeolite (Ambizel-V) in the test ponds had positive effects on growth parameters. The positive effect seen as a result of 1% zeolite addition was similar to the positive effect seen in the Z05 group in this study. It might be interpreted as different rates of zeolite addition cause similar effects, depending on the different starting weight of the fish, the production system, and the type of zeolite used. Eya et al. (2008) reported that adding two different zeolites (bentonite and mordenite) varieties at the rates of 2.5- 5% and 10 to rainbow trout diets had a positive effect on many growth parameters. Adding 2.5% mordenite and 5% bentonite to feeds had a positive effect on WG, SGR, FCR. These values were the values that cause the maximum positive effect, and other bentonites and mordenite ratios have been reported to cause a positive effect compared to the control group in certain amounts. Although this study (Eya et al., 2008) showed the positive effects of zeolites on growth and feed conversion parameters, the results of the Z25 group which were observed in the current study did not coincide with the negative effect on growth and feed conversion. In another study, it was reported that (Kanyilmaz, 2008), adding natural zeolite to carp feeds at different rates (1-2-3-4%) did not affect parameters such as FI, FCR, SGR, PER. However, in the current study adding 2.5% zeolite to the diet had a significant negative effect on WG, FCR, and SGR. From this aspect, the two studies did not overlap in terms of the results seen by adding high levels of zeolite to the feed. The use of 1% zeolite, although it was not statistically significant, had a very low negative impact on parameters such as FCR, SGR, PER, FI. The study carried out in this respect shows parallelism with the Z1 group in the current study. Danabas (2009) reported that the addition of 1% clinoptilolite into rainbow trout diets showed remarkable effects on the growth and feed utilization values. In terms of FBW, groups fed with feeds containing 1% and 2% zeolite showed the best growth. This study was in line with the work done by us in terms of the positive contribution of zeolite to growth. It was reported by the researcher that FBW values in fish fed with 3% zeolite added diets were similar to the control group. However, in this study, it was concluded that the Z25 group showed the lowest growth. Kanyilmaz et al. (2015) reported that the addition of zeolites at a rate of 1-2-3-4% to gilthead seabream diets had a significant increasing effect on FBW, SGR, FCE, and SGR. Researchers found that around 2.7% inclusion rate was optimal for seabream juveniles however, in this experiment 2.5% inclusion rate had a significant limiting effect on rainbow trout juveniles. A trial was done by Jawahar et al. (2016) showed that the incorporation of different rates (2-4-6%) of zeolite had positive effects on FBW, SGR, FCR, PER, and average daily WG of snakehead murrell against *Aphanomyces invadans*. Danabas (2009) stressed that beneficial effects of natural zeolites could be originated from the resistance of zeolite minerals to liquids (Mumpton, 1999; Ivkovic et al., 2004) which played an important role in the digestion of undigested nutrients through the intestines (Mumpton and Fishman, 1977; Dias et al., 1998; Meisinger et al., 2001). Moreover, it has been suggested that during the movement of the zeolite in the digestive system, it may increase the protein efficiency by causing better synthesis by binding the ammonia in the environment and releasing the

nitrogen within the protein synthesis (Ayvaz, 2004; Hargreaves and Tucker 2004; Ivkovic et al., 2004; Danabas, 2009). However, in this study, the fact that there was no change in the PER values of all groups did not suggest an improvement due to protein metabolism. Due to the natural structural feature of clinoptilolite, the selectivity to the ions such as Cs, Rb, K, NH_4, Ba, Sr, Na, Ca, Fe, Al, Mg, Li (Ames, 1967; Mercer et al., 1970) might cause lack of mineral substances in animals and thought to had negative effects on growth and weight gain. The use of non-digestible silicate materials (zeolites, kaolin, bentonite) as feed additives had changed the feces profile in some fish species such as trout and sea bass (Lanari et al., 1996; Dias et al., 1998), and it had been reported to extend the passage time from the digestive tract. Moreover, it was stated by the researchers that the pH of the stomach might affect these properties and each fish species might react differently to such feed additives (Dias et al., 2010). Different results in the conducted studies suggested that many variables might have a direct effect on the results of the study. Considering that even the properties such as the type, geographic location, and particle size of the zeolite used (Mumpton and Fishman 1977; Willis et al., 1982) might affect the results of the studies, other variables like the age, weight, fish species, grow-out systems, the nutritional content of the rations, nutritional needs of the species and the ambient conditions (temperature, pH, etc.), it can easily be said that having diverse results was very predictable.

More research was required on biochemical blood values in fish to obtain normal values related to fish biology and clinical pathology (Manera and Britti, 2006; Fazio et al., 2013; 2016). Difficulties considering determining reference values of blood parameters in rainbow trouts were reported by many researchers (Roscoe Miller et al., 1983; Yilmaz and Ergun, 2018). Plasma lipoproteins were involved in transporting insoluble lipids from their locations (eg: muscle, liver) to the areas where they will be used (Henderson and Torcher, 1987). According to Hoar et al. (1992), lipoproteins in fish were defined as chylomicrons, VLDL, LDL, and HDL, similar to humans. It was reported that in the blood VLDL; in terms of triglycerides, LDL; in terms of cholesterol, and finally HDL; had been reported to be rich in cholesterol and phospholipids (Mckay et al., 1985). Plasma lipoprotein values differ very much in terms of fish species at feeding and breeding periods. HDL values dominate total lipoproteins in bony fish and cover more than 50% of total lipoproteins. These values vary both between separate species and between the same species (Hoar et al., 1992). HDL values were reported as 238 mg/dl in young red salmon and 3300 mg/dl in pink salmon before ovulation (Babin and Vernier, 1989). High HDL values were thought to be related to high cholesterol (Hoar et al., 1992). Moreover, while rainbow trout fasted for 8 weeks, VLDL and LDL values decreased by 67% - 47% respectively, while HDL values were not affected (Black and Skinner, 1986).

The reference blood values of fish were still unclear, there was a very limited number of trials regarding the effects of dietary zeolite inclusion to the fish diets. Khodanazary et al. (2012) reported that the addition of %2.5 zeolite had no effects on serum cholesterol levels in common carp which was parallel with this experiment. Kanyilmaz and Tekellioglu (2016) stated that different rates of dietary zeolite inclusion had a significant effect on serum cholesterol and triglycerides but no effect on BUN levels of gilthead seabream. The researchers (Kanyilmaz and Tekellioglu, 2016) also stressed that the reason for the alterations in serum cholesterol and triglycerides might be originated from absorption of short-chain fatty acids by zeolites (Ly et al., 2007). Jawahar et al. (2016) reported that different rates of dietary zeolite level elevated triglyceride and cholesterol compared to the control group in snakehead murrel. All the researchers strongly stressed that the mechanisms behind the possible effects of dietary zeolite to blood chemistry values were still unknown (Khodanazary et al., 2013; Jawahar et al., 2016; Kanyilmaz and Tekellioglu, 2016). The current experiment showed that triglyceride and VLDL levels were highest with the addition of 1% zeolite and the lowest with the addition of 2.5% compared to the control group. It was observed that there was a significant difference only between these two groups in terms of triglyceride and VLDL values and there was no difference among the other groups. In line with these results, it could be said that adding zeolite up to 2.5% did not change the triglyceride and VLDL levels in rainbow trouts. However, considering the lowest feed utilization of the Z25 group among the experimental groups and the lowest VLDL and triglyceride values could be accepted to be in parallel as an indication that some blood parameters will be negatively affected by the increasing rates of zeolite. Moreover, VLDL, one of the lipoproteins found in blood serum, was synthesized directly from triglyceride (Ando and Mori, 1993).

In the light of the final growth and feed utilization values of the study, considering that the worst growth and feed utilization rate was seen in the Z25 group, it was concluded that rainbow trouts

cannot fully benefit from the feed they received. It could be concluded that the high rate (2.5%) of the zeolite's negative effect on growth is also in line with the growth results when viewed in blood values.

CONCLUSION

The researches about natural zeolites, especially clinoptilolite, were focused on their use as animal feed additives. This research aimed to evaluate the effects of using different levels of natural zeolite (clinoptilolite) in rainbow diets. If growth and feed utilization values were compared with similar studies conducted by other researchers, some studies showed parallelism in some aspects and incompatibility with others. This could be originated from many factors such as; initial weights, species of the animals, different rations, the amount, and the origin of the natural zeolite used. Also due to the biological features of the fish, the presence of a wide range of reference blood values in the natural and controlled environment caused the study results related to the blood values to be limited. More in-depth studies should be carried out to investigate the real effects and mechanisms of dietary zeolites in fish species.

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