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Effects of zeolite on growth and hematology of rainbow trout (*Oncorhynchus mykiss*) kept at low temperatures

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Abstract

It is known that zeolite, tested in animal feeds in recent years, has a positive effect on fish growth and many physiological parameters. For this purpose, rainbow trout (*Oncorhynchus mykiss*) was fed with feeds containing zeolite at different rates (1%, 3%, and 5%) for three months in the present study. Fish growth parameters (live weight gain, specific growth rate, feed conversion rate, condition factor, and mortality rate) and hematological indices (total erythrocytes count (RBC), total leucocytes count (WBC), hemoglobin (Hb), hematocrit (Hct), total plate count (PLT), erythrocyte sedimentation rate (ESR), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and mean cell volume (MCV) were examined monthly during the experiment.

At the end of the feeding period, the changes in growth parameters were determined, and only condition factor was found statistically significant (p<0.05). The highest condition factor calculated was 0.70 in group Z3 at the end of the study. In the hematological indexes, different ratios of zeolite added diets were found to cause a change in the blood indices, of which WBC, ESR and MCV values were statistically significant (p<0.05).

Keywords: *Oncorhynchus mykiss*, Zeolite, Growth parameter, Hematology

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Introduction

Zeolite is a generic name given to many natural mineral groups. The most important of these are clinoptilolite, chabazite, and analsicum. They are crystalline in structure with smooth pores and abundantly obtained aluminum silicate-like compounds containing alkali and alkaline earth elements such as Na, K, Ca, Mg (Danabaş and Altun, 2011).

Dias *et al.* (1998) studied the influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass juveniles. Effects of zeolite in preventing acute toxicity of ammonia was studied in common carp by Peyghan and Takamy (2002) and in rainbow trout by Erguen *et al.* (2008). Danabas (2011) studied fatty acid profiles of rainbow trout fed with zeolite. Danabas and Altun (2011) investigated the effects of zeolite on some water and growth parameters of rainbow trout.

Ghiasi *et al.* (2011) worked on the influence of Iranian natural zeolite on accumulation of cadmium in *Cyprinus carpio*. Ramirez-Duarte *et al.* (2011) tested sodium chloride and zeolite during shipment of *Ancistrus triradiatus* under high temperature.

Cogun and Sahin (2012) used zeolite against lead toxicity in Nile tilapia. Khodanazary *et al.* (2013) studied the effects of dietary zeolite and perlite supplementations on growth and nutrient utilization performance, and some serum variables in common carp, *C. carpio*. Hu *et al.* (2014) and Kanyilmaz *et al.* (2015) used dietary zeolite and determined growth and feed utilization of gilthead sea bream juveniles. Alak *et al.* (2016) determined changes in G6PD in liver tissue of rainbow trout in the presence of zeolite. Johari *et al.* (2016) used zeolite for the disinfection of rainbow trout. Mutlu *et al.* (2016) investigated the effect of zeolite and copper sulfate to determine changes in blood of common carp. Alinezhad *et al.* (2017) investigated growth performance, immune parameters and pathological conditions of rainbow trout fed zeolite. Sheikhzadeh *et al.* (2017) determined growth performance, digestive enzymes and serum biochemical parameters after administration of zeolite composite to rainbow trout. Hamidian *et al.* (2018) used zeolite composites to monitor histology and stereology in rainbow trout. In a review, Ghasemi *et al.* (2018) mentioned many usage probabilities of zeolite in the aquaculture industry, especially for better water quality in fish farms and for better fish growth

The analysis of hematological parameters provides important information on the metabolic processes and stress situation of fish, which has an important role in ecosystems (Gaber and El-Kasheif, 2013). The analysis of hematological and biochemical parameters in the fish contributes to the assessment of animal health and ecological conditions (Pimpão *et al*., 2007).

The aim this study is to evaluate the effect of zeolite as a feed additive on the growth and hematological parameters of rainbow trout kept at low temperatures.

Materials and Methods

Trial conditions and materials

The research was carried out at Atatürk University Fisheries Faculty Inland Water Fish Application - Research Center and Fisheries Faculty Laboratories.

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A total of 240 rainbow trout (not exposed to any infection or toxicity) weighing 100±25 g were used. 30 fish weighing 3000±150 g approximately, were placed in each tank (210 cm length, 60 cm width, and 30 cm depth). The fish were acclimatized to laboratory conditions for a period of 14 days. The zeolite was mixed to commercial trout feed in three different rates for the experimental groups. Water with a temperature of 10.8 ^{o}C, pH of 8.40, and dissolved oxygen concentration of 8.70 mg L^{-1} was used during the experiment.

Mixing zeolite to feed

The commercial trout feed (Aquamaks, Extruded) is lightly moistened with pure water and milled thoroughly with the aid of a stirrer.

The zeolite, obtained in the commercial form was mixed to the trout feed in three different rates as 1% for the first experimental group, 3% for the second experimental group and 5% for the third experimental group. Then zeolite + feed mixtures were compounded equally in the dough machine. After the proportional distribution of the feed, the feeds were passed through the pellet machine and brought to the level of the feed size that could be taken by the fish. In order to prevent the food from spoiling and deteriorating, it was dried and the humidity was reduced.

Calculating the growth parameters

In the study, four of the eight trial tanks were set up only for live weight control and fish with a total weight of approximately 3000 g fish / tank were fed twice daily. The amount of feed to be given to fish was calculated according to live weight gain every 15 days. When the feed ratio was calculated the formula (sum of live weight (g) + live weight gain (g) x 0.009) was used. Fish were weighed in groups of 1 g with a precision scale in containers filled with water every 15 days. During the weighing, the groups were counted and records were kept and the amount of feed to be given was readjusted for every period.

The specific daily growth rate (SGR) of the control and treatment groups was calculated with the following formula=100 × [{ln (final weight or length) −ln (initial weight or length)}/time]. Condition factor (CF)=(weight/(length)3) ×100 (Kim and Kang, 2004). The daily growth rate (DGR), feed evaluation rates (FCR), growth by weight (WG) and survival rate were calculated according to Danabaş and Altun, (2011).

Hematologic analyzes

The amount of hemoglobin was determined using cyanmethemoglobin method. The obtained hemoglobin value corresponding to the transmittance (% T) at 540 nm was read from the standard table and recorded as g $100cm^{-3}$. In the hematocrit assay, the micro hematocrit method was applied and recorded as % of total blood (Blaxhall and Daisley, 1973). The blood samples with anticoagulant were taken in hematocrit tubes (1.2 mm diameter and 7 cm length). After standing for 1 hour in the upright position (90^{o}), the separated serum part was measured with a ruler. The results were recorded in mm/h (Blaxhall and Daisley, 1973; Alak *et al*., 2012; Alak *et al*., 2018a; Alak *et al*., 2018b). Using Dacie's solution, Thoma slides were counted on a microscope 1/5 mm^2 and the resulting value was calculated as $10^6/mm^3$. After the same

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method of detecting the number of erythrocytes was applied, 4 mm^2 was counted for leukocytes and 9 mm^2 for cases where count was insufficient. The result is calculated as 10^3 mm^{-3} (Blaxhall and Daisley, 1973). The same method was applied with erythrocyte count determination. All squares were counted and calculated as 10^3 mm^{-3}. The other hematological indices such as mean volume of red blood cells (MCV), mean hemoglobin in red blood cells (MCH) and mean hemoglobin concentration per erythrocyte (MCHC) were also calculated (Alak *et al*., 2012; Alak *et al*., 2018a; Alak *et al*., 2018b).

Statistical analyses

The data obtained are given as mean±standard deviation. Data were analyzed by variance analysis (ANOVA) and averages were compared using Duncan's multiple comparison test. The significance level is taken as 0.05.

Results

Growth parameters

The results of growth parameters are given in Table 1. The highest average weight gain was in the Z3 group (6110 g) at the end of the trial period. When the daily live weight gain was analyzed, the highest value was determined between the 60^{th} and 75^{th} days in Z5 group as 73.3 g. Feed conversion rates were lower in the Z1 and Z3 groups than in the control group in the first 15^{th} days, but higher in the Z5 group. Between 15^{th} and 30^{th} days, the control group showed the highest average of all groups. Between days 45 and 60 only the Z3 group was higher than the control group. On the 60^{th} and 75^{th} days, the lowest average was found in the control group. In the last 15^{th} days of the study, the control group was higher than all other groups. When the condition factor averages were examined, the highest value was measured as 0.70 in group Z3 at the end of the study. Specific growth rates measured in the Z1 group were lower than that in the control group between days 60 and 75, however specific growth rates in the other two treatment groups were higher than in the control. No fish deaths were observed during the trial period.

Lowercase superscripts (a, b) indicate significant differences among groups within each experimental time whereas superscripts in uppercase show significant differences among time. Each value is the mean ± SEM. of five individual observations.

Hematologic indexes

In this study, the effects of the diets supplemented with zeolite at different rates on the hematological parameters of trout are given below (Table 2). In the study, the highest amount of hemoglobin was measured as 11.880±1.628 g $100mL^{-1}$ on the 60^{th} day in the group fed with 5% zeolite feed, and the lowest was 5.040±1.383 g $100mL^{-1}$ on the 90^{th} day in the group fed the 3% zeolite feed. The highest amount of hematocrit was measured in the group fed with 5% zeolite supplemented feed at 60.200±8.136 g $100ml^{-1}$ on the 60^{th} day of the study and the lowest was 42.800±9.257 g $100mL^{-1}$ in Z3 on the 90^{th} day of the study. The highest erythrocyte sedimentation rate value was 0.064±0.005 mm h^{-1} in the Z3 group (3% zeolite additive feed) on the 30th day and the lowest was 0.018±0.083 mm h^{-1} in the Z1 group (1% zeolite additive feed) on the 60^{th} day. The effect of zeolite on the erythrocytes count was measured as 1.162±0.294 10^6 mm^{-3} (the

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highest) at the end of the 60th day in the group. The lowest value was determined as 1.038±0.136 10^6 mm^{-3} in the control group on the last day of research. When the effect of zeolite on the leucocytes count was examined, the highest value was measured as 9.960±1.796 in the group fed with 5% zeolite feed on the 60th day of the study. The lowest value was obtained in the group fed 3% zeolite (4.120±1.044 10^3 mm^{-3}). Thrombocyte count was given the highest level as 1.360±0.384 10^3 mm^{-3} in the Z1 group at the end of the study. The MCV value was compared with the all treatment groups, and group Z5 showed the highest value of 804.012±122.971 10^6 mm^{-3} at the end of the study.

At the end of the study, MCH value of Z3 group (58.264±4.226 µg $cell^{-1}$) was lower than in the control group. MCH values in the other two groups also were higher than in the control group.

The MCHC value was higher in the control group than in all the treatment groups at the end of the feeding time.

Table 1: Comparison of growth parameters between experimental fish fed different concentrations of zeolite in *Oncorhynchus mykiss.*

Period (Days)	Groups	WGR (g $fish^{-1}$)	DGR	FCR	CF*	SGR (% day^{-1})
0-15^E	Control	3465.00±38.3^a	-4.72±8.03 ^{a}	0.39±5.33^a	1.95±0.022^a	-014±0.23 ^{a}
	Z1	3427.5±52.0 ^{a}	-13.17±1.64 ^{a}	-4.38±0.01^a	2.03±0.31 ^{b}	-0.41±0.05 ^{a}
	Z3	3442.5±19.17^a	-14.17±3.83 ^{a}	-2.73±0.47^a	2.10±0.12 ^{b}	-0.42±0.08 ^{a}
	Z5	3290.0±82.15^a	-5.17±9.65 ^{a}	1.44±5.05 ^{a}	1.39±0.03 ^{c}	-0.23±0.31 ^{a}
15-30^E	Control	3450±98.5^a	24.33±22.35 ^{a}	1.58±1.53^a	1.60±0.46a	0.66±0.56 ^{a}
	Z1	3390±10.95^a	-9.56±4.16 ^{a}	1.54±0.05 ^{a}	1.76±0.006 ^{b}	-0.29±0.13 ^{a}
	Z3	3280±46.18^a	-3.33±2.30 ^{a}	1.22±0.30 ^{a}	1.31±0.018 ^{b}	-0.28±0.20 ^{a}
	Z5	3230±7668^a	39.00±21.65 ^{a}	0.97±3.08 ^{a}	1.43±0.034 ^{c}	1.10±0.55 ^{a}
30-45^D	Control	4060±372.4^a	15.33±13.87 ^{a}	2.81±9.24^a	1.67±0.153^a	0.39±0.35 ^{a}
	Z1	3700±21.90 ^{a}	33.78±13.32 ^{a}	1.54±0.12 ^{a}	1.57±0.009 ^{b}	0.85±0.31 ^{a}
	Z3	3685±136.9 ^{a}	28.89±15.72 ^{a}	1.58±0.43 ^{a}	1.15±0.043 ^{b}	0.74±0.39 ^{a}
	Z5	3355±202.6 ^{a}	39.56±15.77 ^{a}	1.15±0.16 ^{a}	1.04±0.063 ^{c}	1.09±0.44 ^{a}
45-60^C	Control	4290±164.3^a	-14.56±30.16 ^{a}	1.65±0.13^a	1.76±0.067^a	0.38±0.71 ^{a}
	Z1	4040±0.001^a	17.22±29.31 ^{a}	1.38±0.16 ^{a}	1.18±0.00 ^{b}	0.38±0.70 ^{a}
	Z3	4025±71.20^a	30.56±14.31 ^{a}	1.80±0.03 ^{a}	1.24±0.022 ^{b}	0.71±0.31 ^{a}
	Z5	3780±175.2^a	50.11±35.30 ^{a}	1.06±0.93 ^{a}	1.06±0.049 ^{c}	1.19±0.79 ^{a}
60-75^B	Control	4655±202.6^a	13.11±43.59 ^{a}	2.16±0.27^a	1.42±0.062^a	0.25±0.92 ^{a}
	Z1	4460±43.81^a	21.78±26.19 ^{a}	3.24±0.73 ^{a}	1.12±0.011 ^{b}	0.45±0.54 ^{a}
	Z3	4335±82.15^a	35.67±28.78 ^{a}	2.22±0.27 ^{a}	1.25±0.024 ^{b}	0.75±0.56 ^{a}
	Z5	4800±591.5^a	6.78±24.08 ^{a}	2.65±0.32 ^{a}	1.06±0.049 ^{c}	0.14±0.48 ^{a}
75-90^A	Control	4955±246.4^a	13.56±50.97 ^{a}	2.42±1.83^a	1.39±0.069^a	0.23±1.01 ^{a}
	Z1	4650±10.95 ^{a}	6.89±29.40 ^{a}	0.81±0.49 ^{a}	1.15±0.003 ^{b}	0.12±0.58 ^{a}
	Z3	4605±115.0 ^{a}	54.22±44.89 ^{a}	0.74±0.50 ^{a}	1.21±0.30 ^{b}	1.04±0.82 ^{a}
	Z5	5050±646.3 ^{a}	-73.33±206.5 ^{a}	1.07±0.17 ^{a}	1.28±0.164 ^{c}	0.55±0.81 ^{a}

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Table 2: Comparison of hematological parameters between experimental fish groups fed with different concentrations of zeolite in *Oncorhynchus mykiss*.

Day	Group	Hb (g 100ml^{-1})	HCT (g 100ml^{-1})	ESR (mm h^{-1})	RBC (10^6 mm^{-3})	WBC (10^3 mm^{-3})	PLT (10^3 mm^{-3})	MCV (μm^3)	MCH (μg cell^{-1})	MCHC (μg cell^{-1})
0^C	Control	6.260±0.089^a	49.650±12.054^a	0.023±0.008^b	1.234±0.247^a	7.762±1.438^{ab}	1.260±0.357^a	458.002±158.235^b	52.691±10.672^a	12.549±3.730^a
30^B	Control	6.720±3.669^a	58.000±9.082^a	0.046±0.011^b	0.932±0.192^a	7.400±1.876^{ab}	1.160±0.622^a	638.810±141.466^b	69.707±25.344^a	11.863±6.308^a
	Z1	7.180±2.749^a	56.400±9.476^a	0.052±0.019^{ab}	1.080±1.134^a	4.840±0.698^b	1.120±0.328^a	533.328±126.463^b	65.849±22.926^a	13.153±5.697^a
	Z3	9.860±0.642^a	59.400±7.700^a	0.064±0.005^a	1.052±0.077^a	4.760±1.178^{ab}	0.920±0.460^a	560.203±62.708^a	94.253±10.676^a	16.918±2.409^a
	Z5	6.820±1.366^a	61.600±7.266^a	0.042±0.017^{ab}	0.848±0.104^a	5.240±1.161^a	1.040±0.497^a	741.391±88.484^b	82.042±21.840^a	10.945±2.059^a
60^A	Control	9.360±0.602^a	52.200±2.167^a	0.02±0.012^b	0.994±0.098^a	7.040±0.433^{ab}	1.160±0.167^a	542.449±71.530^b	94.741±9.177^a	17.569±1.799^a
	Z1	9.180±3.218^a	58.000±4.690^a	0.018±0.083^{ab}	1.162±0.294^a	9.360±2.718^b	1.120±0.109^a	509.845±130.577^b	84.501±34.109^a	16.062±5.055^a
	Z3	8.940±0.618^a	62.200±7.726^a	0.026±0.011^a	1.060±0.093^a	7.840±0.554^{ab}	1.080±0.109^a	557.771±53.697^a	84.996±10.561^a	15.293±2.480^a
	Z5	11.880±1.628^a	64.200±8.136^a	0.02±0.01^{ab}	1.070±0.234^a	9.960±1.796^a	1.280±0.641^a	626.350±183.298^b	117.417±36.989^a	18.682±2.755^a
90^C	Control	8.260±1.804^a	56.200±7.596^a	0.024±0.005^b	1.038±0.136^a	6.320±0.521^{ab}	0.920±0.228^a	543.896±97.597^b	82.317±28.991^a	15.002±3.880^a
	Z1	7.140±1.542^a	57.200±9.984^a	0.022±0.008^{ab}	0.834±0.110^a	4.920±0.901^b	1.360±0.384^a	692.021±163.500^b	88.276±28.726^a	13.082±4.593^a
	Z3	5.040±1.383^a	42.800±9.257^a	0.026±0.005^a	0.856±0.176^a	4.120±1.044^{ab}	1.200±0.748^a	482.508±52.044^a	58.264±4.226^a	12.128±1.375^a
	Z5	5.500±1.581^a	58.600±11.717^a	0.028±0.004^{ab}	0.746±0.136^a	4.800±1.483^a	0.960±0.497^a	804.012±122.971^b	75.530±14.982^a	9.306±2.477^a

Lowercase superscripts (a, b) indicate significant differences among groups within each experimental time whereas superscripts in uppercase show significant differences among time. Each value is the mean±SEM. of five individual observations. *p<0.05

Discussion

Yıldırım *et al*. (2009) reported that *Tilapia zilli* fed diets supplemented with 1% -2% of zeolite during 45 days resulted in higher weight gain, specific growth rate and better feed efficiency than the fish fed non-zeolite feeds.

Mostafa *et al.* (2010) reported that the addition of 20-40-80-120 mg L^{-1} zeolite in carps affected growth parameters and water quality criteria positively. Likewise, Yıldırım *et al.* (2009) reported that zeolite used as a feed additive improves growth parameters, feed evaluation ratios and water quality criteria of tilapia fish.

Stetca and Morea (2013) performed a research to determine the physiological effects of natural zeolites in fish feeding and found that 3% and 7% of zeolite addition to feeds did not alter the physiological status and 43% of growth rates in carp. Ghiasai and Jasour (2012) reported that feed conversion rate and specific growth rate significantly increased as a result of zeolite application in angel fish.

Danabas and Altun (2011) tried the effect of the addition of zeolite to pond water of rainbow trout and observed no statistical differences ingrowth parameters with zeolite concentrations of 1, 2, and 3 mg L^{-1}. The results of our study support these findings.

The level of low hemoglobin indicates that the mechanism of iron synthesis in fish is impaired. This is thought to be due to the anemic state of the fish, the hemoglobin level being caused by low-level hemolysis, and the restriction of aerobic glycolysis, which interrupts the synthesis of hemoglobin.

Changes in dehydration, nutrition, erythrocyte synthesis, and membrane permeability cause changes in hematocrit levels. Stress in fish causes a decrease in blood pH, increase in erythrocyte volume and a consequent increase in the percentage of hematocrit (Saravanan *et al.*, 2011). In the present study, the hematocrit findings obtained are higher than in the control and support this situation (Alak *et al.*, 2018a; Alak *et al.*, 2018b). The increase or decrease in the ESR value due to the number of erythrocytes indicates physiological dysfunction in fish (Jagtap and Mali, 2012). The decrease in the number of erythrocytes is considered to be a sign that erythropoietin tissues are

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affected, that the condition of the organism has deteriorated and that the anemia has developed. The sudden increase in erythrocytes can be explained by the stress-induced induction of catecholamine by contraction of the spleen and new erythrocytes participating in circulation. In hypoxia, hemoglobin has a lower oxygen binding capacity as well as decreased blood pH, since acid release occurs at a very high rate in the blood.

Leukocytes are defense cells of organisms. The species with a large number of leukocyte cells are able to fight with xenobiotics more effectively. The number of leukocyte cells is influenced by physiological and environmental factors. In fish, the platelet cells have a phagocytic ability and participate in the defense mechanism. These cells represent the link between natural and subsequently acquired immunity. Platelets are blood phagocytes that form the protective wall (Cuesta *et al*., 2003; Witeska, 2005; Alak *et al*., 2018a; Alak *et al*., 2018b).

High levels of glucocorticoids cause a decrease in the number of platelets and an increase in clotting time. Compared with the control group, the thrombocyte count was increased in the high dose zeolite treated groups. The most well-known physiological role of thrombocytes is to initiate blood coagulation in the hemostasis process (Engelmann and Massberg, 2012). The platelet cells represent the association between intracellular and extracellular molecules, including innate immunity and immune functions (Yeğin and Uçar, 2017). Under stress conditions, the blood coagulation system becomes more active and may cause an increase in the thrombocyte count (Casillas and Smith, 1977). It may be a negative effect of thrombocytopenia on fish because these cells are not only responsible for blood coagulation but also play a role in surface wounds and blood flow control (Campbell and Ellis, 2007).

In the present study, it was observed that the increase in MCV in high level zeolite groups was a macrositer result and marked anemia due to the swelling of erythrocytes. This increase is also due to a hypoxic increase or impaired water balance (osmotic stress) in stress-exposed fish; in this case, it increases the affinity of the blood oxygen (Harikrishnan *et al.*, 2009).
In this study, the MCHC value increased significantly in zeolite treated fish during the study period, which is thought that hemoglobin increasing might be influential in this change (Saravanan *et al.*, 2011; Alak *et al.*, 2018a; Alak *et al*., 2018b). The zeolite application has shown that it causes shrinkage of red blood cells (increased MCHC) and significantly increases MCH. High MCHC and MCH values have been reported to demonstrate the presence of large size RBC, which has less hemoglobin content; (Alwan *et al.*, 2009; Kumar and Banerjee, 2016). We think that the increase in MCV and MCH levels of fish with the effect of zeolite is an anemic condition effect. Similarly, in some studies performed on rainbow trout, it was noted that the high MCV level developed due to the macrositer type of anemia (Jayaprakash and Shettu, 2013; Kumar and Banerjee, 2016). The results of our study are similar to findings of the studies in which the effects of different substances on the hematological index of different fish species were investigated (Sinha *et al.*, 2000; Devi and Banerjee, 2007; Ramesh

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and Saravanan, 2008; Alwan *et al.*, 2009; Jahanbakhshi and Hedayati, 2015; Murussi *et al.*, 2015; Southamani *et al.*, 2015; Alak *et al.*, 2018a; Alak *et al.*, 2018b).

Twelve weeks feeding with different rates of zeolite included feeds in rainbow trout (*O. mykiss*) affected the hematological indices (Hemoglobin, hematocrit, erythrocyte sedimentation rate, erythrocyte, leukocyte, thrombocyte, mean erythrocyte volume, average hemoglobin per red blood cell and average hemoglobin per erythrocyte) and growth parameters. Based on the analyses done, it is considered that the determined concentrations of zeolite should not be suitable for the rainbow trout and that lower concentrations should be investigated.

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