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EFFECTS OF DIETARY ZEOLITE LEVELS ON SOME BLOOD PARAMETERS OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES

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Abstract:
In this study, it was aimed to investigate the effects of dietary zeolite supplementations on blood parameters of gilthead seabream (*Sparus aurata*). Zeolite was gradually included into the diets at 0%, 1%, 2%, 3% and 4% and fed to triplicated groups of fish for 10 weeks. Dietary zeolite levels did not affect red blood cell, white blood cell and hemoglobin levels of sea bream. On the other hand, serum glucose levels were linearly decreased whereas triglyceride quadratically increased with zeolite levels. There was a significant quadratic effect of dietary zeolite on serum cholesterol and alanine aminotransferase levels. Blood urea nitrogen, aspartate aminotransferase and alkaline phosphatase levels did not change in a particular trend with dietary zeolite levels, which was the case for sodium, potassium, calcium and magnesium. The results suggest that dietary zeolite inclusion up to 4% did not lead to any health impairment in gilthead sea bream when judged from blood parameters.

Keywords: *Sparus aurata*, Zeolite, Blood chemistry, Blood electrolytes, Hematology

Introduction

Zeolites, a kind of clay, are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, and have infinite three-dimensional structures (Mumpton, 1999). Former studies have showed that dietary zeolite supplementation in diets of livestock and rats was found to improve health and growth rate (Katsoulos et al., 2005; Demirel et al., 2011; Kyriakis et al., 2012; Pourliotis et al., 2012). The clays have been reported to protect the intestinal gut health and improve morphological characteristics of the mucosa (Albengres et al., 1985). As an unconventional feed additive, the clay minerals have an ability to absorb and detoxifying effect of noxious substances hence they are considered as protective against infections in warm blooded animals (Vondruskova et al., 2010).

Several clay mineral types including clinoptilolite, bentonite, modernite and sericite have been used in diets of Coho salmon, *Oncorhynchus kisutch* (Edsall and Smith, 1989), rainbow trout, *Oncorhynchus mykiss*, (Reinitz, 1984; Obradović et al., 2006; Eya et al., 2008; Ergün et al., 2008; Yiğit and Demir, 2011), European sea bass, *Dicentrarchus labrax* (Dias et al., 1998), common carp, *Cyprinus carpio* (Kanyılmaz, 2008; Khodanazary et al., 2013), tilapias, *Oreochromis niloticus and Tilapia zilli* (Hu et al., 2008; Yıldırım et al., 2009) and shrimp, *Litopenaeus schmitti* (Galindo et al., 2006). Of these aquatic species, particularly rainbow trout, shrimp and gilthead sea bream (*Sparus aurata*) (Kanyılmaz et al., 2015) have been reported to show higher growth performance when fed diets including zeolite (clinoptilolite). Earlier studies on different fish species mostly dealt with the effects of clays on growth and feed utilization. However, feeding gilthead sea bream with zeolite supplemented diets resulted in iron accumulations in the liver (Kanyılmaz et al., 2015), which could suggest that dietary zeolite can cause alterations in blood parameters. In addition, it has been reported that long term feeding with dietary zeolite in some terrestrial animals could cause physical irritations in the intestinal mucosa and subsequently affect some hematological variables as a result of their ion exchange features (Martin-Kleiner et al., 2001; Katsoulos et al., 2005; Mohri et al., 2008). Eventually, there is a serious scarcity of information about effects of dietary zeolite levels on blood parameters in fish. This study was planned to evaluate the effects of dietary clinoptilolite incorporations on blood chemical and hematological parameters of gilthead sea bream.

Materials and Methods

Zeolite and diet preparation

The zeolite material (Table 1) was procured from a commercial mining company (Gordes Zeolite, Manisa, Turkey). It was ground using a hammer mill, sieved to obtain particle size about 100 µm, washed with distilled water and then dried overnight at 105ºC. Composition of the zeolite used is presented in Table 1.

Experimental diets were prepared from a commercial sea bream diet (Çamlı Yem, İzmir, Turkey). First, the diet was ground with a hammer mill, and then zeolite was added at levels of 0, 1, 2, 3 and 4% (named as Z0, Z1, Z2, Z3 and Z4 respectively) in place of alpha cellulose (Table 2). Distilled water was added to the mixtures until a dough-like consistency, and then the resulting material was pressed through a meat mincer with a 2 mm die. The pellets were dried one night at 65°C and stored at +4°C until use.

Table 1. Chemical composition of clinoptilolite used in the experiment*

Component	g/kg
SiO_2	671
Al_2O_3	118
Fe_2O_3	14.7
MgO	11.5
CaO	21.8
Na_2O	3.8
K_2O	34.4
Moisture	124

*Statement of the supplier (Gordes Zeolite, İzmir, Turkey).

Table 2. Nutrient compositions of the experimental diets (dry matter basis).

	Z0	Z1	Z2	Z3	Z4
Dry matter (g/kg)	950	954	956	952	953
Ash (g/kg)	115	122	132	141	151
Protein (g/kg)	481	469	470	476	480
Lipid (g/kg)	174	175	171	172	172
Carbohydrate (g/kg)	190	204	206	201	196
Energy (MJ/kg)	21.6	21.6	21.5	21.6	21.6
Iron (mg/kg)	388	441	474	527	681
Aluminum (mg/kg)	137	553	891	1205	1655

Experimental design and fish rearing

This study was conducted at the Kepez Unit of Mediterranean Fisheries Research Production and Training Institute, Antalya, Turkey. The experimental system was a closed recirculation system consisting of 15 rectangular tanks (65 L), sedimentation tanks, a protein skimmer, a biological filter and an ultraviolet filter. The system was subjected to an artificial photoperiod of 12 h light (350 lux) and 12 h darkness. Daily water renewal rate was 10% and water turnover rate in the system was one hour. The culture system was also provided with continuous aeration through an air compressor. Water temperature was maintained at about 25^{o}C with thermostatic heaters. Fish were selected from a large population produced in the institute's marine hatchery, Beymelek, Antalya, size-graded and then transferred to the experiment unit at the Kepez Unit. Twenty-five fish were randomly allocated to each acclimatized to the experimental conditions for 2 weeks. During this period fish were fed the control at a level of 4% body weight. The number of fish in each tank was reduced to 20 at the commencement of the study. Average initial weight of fish was 9.06 ±0.04 g. Each of five treatments was tested for 10 weeks. Fish were fed carefully twice a day at 09:00 and 15:30 h near the satiation (4% first 6 weeks, 3% 7-8th weeks and 2.5% 9-10th weeks). Even if rarely observed, uneaten pellets were siphoned. Experimental fish were collectively weighed every 2 weeks after a slight anesthetization with 2-phenoxyethanol at a dose of 0.3 mL/L (Velíšek and Svobodová, 2004). Water parameters such as temperature, dissolved oxygen, pH and salinity were monitored daily with YSI 55-12 FT DO and YSI 63-12 FT pH Meter (Yellow Springs Instrument, Yellow Springs, OH, USA). Total ammonia nitrogen (TAN) and nitrite were monitored every 3 days (APHA, 1995). Water temperature, dissolved oxygen pH, Salinity, TAN and nitrite levels of the water were 24.77 ± 0.18ºC, 5.10 ±0.16 mg/L, 7.68 ±0.04, 37.35 ±0.1 ppt, 0.01 ±0.00 mg/L, 0.23 ±0.01 mg/L respectively.

Sample collection and analysis

At the end of the feeding trial, fish were starved for 24 h and five fish were randomly sampled from each tank. Following anesthetization with 2-phenoxyethanol at a dose of 0.3 mL/L (Velíšek and Svobodová, 2004), their blood was taken from the caudal vein using heparinized disposable syringes. A part of the blood samples was separated into micro tubes (Miniplast 0.6 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/0.6 ml) as an anticoagulant agent and analyzed via a hematologic auto analyzer (MS4, Melet Scholoesing laboratories, Pontoise, Cedex–France). The remaining blood was transferred to biochemical tubes for serum analysis (BD Vacutainer SST II Advance 5 mL, Plymouth, UK) and centrifuged at 3500 rpm for 5 minutes (Elektromag M 4812 centrifuge, Istanbul, Turkey). Glucose, total cholesterol, triglyceride, blood urea nitrogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (Çelik et al.), aspartate aminotransferase (Ly et al.), calcium (Ca) in serum were determined using a VetTest chemistry analyzer (Model 8000, IDEXX Laboratories Inc., Westbrook, ME, USA) and magnesium (mg), sodium (Na) and potassium (K) were determined using a Roche/Hitachi chemistry analyzer (Model 911, Roche Diagnostics, Indianapolis, USA).

Data calculation and statistical analysis

Linear and quadratic effects were tested to reveal the trends resulting from the effects of various dietary zeolite levels on the observed response variables. Values were given as means ± standard errors. A statistical package JMP v.8.0 for Windows was used for the statistical analyses.

Results and Discussion

Growth and feed utilization data were given elsewhere (Kanyılmaz et al., 2015). In brief, mean final weights of Z0, Z1, Z2, Z3 and Z4 were 50.7, 52.6, 53.8, 54.5 and 52.0 g respectively. Feed conversion efficiencies of treatments with the same order were 0.83, 0.87, 0.85, 0.88 and 0.87. The study showed that nutrient ADC were generally unaffected by the diets. Overall, the zeolite supplementation made a positive contribution to the growth performance and feed utilization, and an inclusion level of 2.71% was estimated as optimum.

Dietary zeolite treatments had a significant negative linear effect on serum glucose (Linear=0.0001, Quadratic=0.798). There was a significant positive quadratic effect of dietary zeolite on serum triglyceride (Linear=0.006, Quadratic=0.018) levels. Serum ALT levels were quadratically responded to dietary zeolite levels (Linear=0.277, Quadratic=0.036). Serum AST concentrations were comparable among the treatments. There was no a remarkable trend in ALP levels in response to dietary zeolite. Cholesterol levels were quadratically effected (Linear=0.062, Quadratic=0.032), whereas there were no discernible effects of zeolite supplementations on BUN (Table 3) WBC, RBC and Hemoglobin (Hb) (Table 4), Na, K, Ca and Mg levels of gilthead sea bream (Table 5).

Table 3. Serum biochemical parameters in sea bream juveniles fed diets containing different level of zeolite (Glucose, BUN, Cholesterol, triglyceride (mg/dL), ALT, AST, ALP (IU/L).

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
Glucose	78.00	73.67	74.00	70.33	68.73	1.943	0.0001	0.798
BUN	22.43	24.00	22.40	23.70	23.53	1.505	0.639	0.905
Cholesterol	240.7	341.7	371.0	329.0	384.7	50.729	0.062	0.032
Triglyceride	238.3	232.0	202.7	275.0	289.5	14.069	0.006	0.018
ALT	11.00	7.67	16.00	10.00	7.00	0.699	0.277	0.036
AST	195.3	134.3	343.0	189.3	161.3	34.934	0.929	0.102
ALP	226.3	180.3	297.6	207.0	278.0	20.761	0.162	0.807

SEM = standard error of mean, BUN = blood urea nitrogen, ALT = alkaline phosphatase, AST = aspartate amino aminotransferase, ALP = alanine aminotransferase

Table 4. WBC (10^3 mm^3), RBC (billion/mm^3) and Hb (g/dL) levels in sea bream juveniles fed diets containing different level of zeolite.

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
WBC	224	259	187	224	228	42.412	0.939	0.991
RBC	2.42	2.73	2.45	2.41	2.61	0.277	0.955	0.758
Hb	9.07	9.43	9.10	8.70	9.17	0.786	0.797	0.956

SEM = standard error of mean, WBC = white blood cell, RBC = red blood cell, Hb = hemoglobin

Table 5. Na (mmol/dL), K (mmol/dL), Ca (mg/dL) and Mg (mg/dL) levels in sea bream juveniles fed diets containing different level of zeolite

	Z0	Z1	Z2	Z3	Z4	SEM	P values	
							Linear	Quadratic
Na	177.6	180.7	178.3	177.7	176.6	1.429	0.223	0.194
K	5.77	5.57	7.07	5.23	5.47	0.575	0.606	0.257
Ca	15.17	13.67	14.83	14.10	14.13	0.278	0.158	0.385
Mg	3.13	2.97	3.53	2.77	2.80	0.146	0.135	0.171

SEM = standard error of mean, Ca = calcium, mg = magnesium, Na = sodium, K = potassium

Blood parameters of fish are known to be affected by a number of factors such as season, water quality variables, age, sex, nutrition, health status, genetic characteristics, transportation, handling and other environmental conditions as well as those related to sampling and laboratory analysis methods (Bond, 1979; Rey Vázquez and Guerrero, 2007). In the present study, dietary zeolite supplementation significantly affected some blood parameters of gilthead sea bream. For instance, blood glucose levels varied between 68.73 and 78.00 mg/dL and were linearly reduced with increasing dietary zeolite levels. These glucose levels are within the range of reported values for gilthead sea bream (Roncarati et al., 2006; Peres et al., 2013) In agreement with the present findings, a previous study on common carp (Kanyılmaz, 2008) reported a significant decrease in blood glucose concentrations with increasing dietary zeolite contents. However, there are several other studies on various aquatic and terrestrial animals pointing out that dietary zeolite had no effect on blood glucose levels (Curtui, 2000; Yazdani and Hajilari, 2009; Ghaemnia et al., 2010; Demirel et al., 2011; Safaeikatouli et al., 2011; Peres et al., 2013). Çelik (2006) and Çelik et al. (2008) reported that when exposition of fish to heavy metals for a long time could have lowering effects on glucose levels. Although difficult to make direct connection with this, a decreasing trend in blood glucose levels could be due to increasing dietary iron and aluminum levels with zeolite in the present experiment (Table 2). Indeed, there were negative strong relationships between blood glucose and dietary iron (r^2 = -0.96) and aluminum (r^2 = -0.93) levels. Alternatively, dietary zinc, cobalt and chromium have been found to reduce blood glucose levels in fish by being involved in insulin activity (Watanabe et al., 1997; Vangen and Hemre, 2003). Compositions of these elements in the present zeolite material and their availabilities to the fish are unknown, and therefore further studies are required to clarify these points.

Dietary zeolite did not affect BUN levels in the present study. Conversely, a former study on common carp (Kanyılmaz, 2008) found dietary zeolite led to a remarkable decrease in BUN. In terrestrial animals BUN is used as an indicator of the renal health but not in fish due to main nitrogenous excretion route being the gill not the kidney. Therefore, elevated BUN levels are suggested as an indicator of problem with nitrogen excretion by the gill (Glibert and Terlizzi, 1999). The comparable BUN levels among the treatments imply that dietary zeolite did not cause an adverse effect on nitrogen metabolism in sea bream.

In current study, cholesterol concentrations of gilthead sea bream were quadratically affected with dietary zeolite levels and varied between 240.70 and 384.70 mg/dL. This range is consistent with the findings for gilthead sea bream by Peres et al. (2013) but slightly lower than those of Roncarati et al. (2006). In contrast to our present findings, existing literature in fish point out that dietary supplemental zeolite had no effect on cholesterol levels (Kanyılmaz, 2008; Tekeşoğlu, 2010). Serum triglyceride levels showed a linear increase with dietary zeolite elevation in the present study. Previous studies report no effect of dietary zeolite on triglycerides levels of chick and fish (Curtui, 2000; Tekeşoğlu, 2010). The increase in cholesterol and triglycerides with zeolite supplementation could be partly resulted from a linear increase in lipid retention by fish fed zeolite added diets (Kanyılmaz et al., 2015). Dietary zeolite was found to have an ability of absorption of short chain fatty acids (SCFAs; butyrate, acetate and propionate) in large intestine of pigs (Ly et al., 2007). The SCFAs are fermentation products and known to reduce the synthesis of cholesterol and triacylglycerol in the liver (Ooi and Liong, 2010). Possible absorption of the SCFAs could be another reason of the cholesterol and triglyceride increasing effect of dietary zeolite, but these speculation remains to be studied.

The AST, ALT and ALP activities are associated with the tissues damages such as in the liver, gut and bile ducts (Roncarati et al., 2006; Maita, 2007; Peres et al., 2013). These variables in the present study, except ALT which had a significant quadratic contrast, were not significantly affected by the treatments, suggesting that dietary zeolite levels have no detrimental effect on fish health at least for gilthead sea bream. Similar findings were also reported by previous poultry and fish studies fed diets with varying zeolite levels (Curtui, 2000; Safaeikatouli et al., 2011; Vizcarra-Olvera et al., 2012).

Blood electrolytes are used as indictors for various physiological statuses in fish such as secondary stress response, growth and nutritional condition (Vangen and Hemre, 2003; Peres et al., 2013;). There was no significant clear trend in selected electrolytes in response to increasing dietary zeolite, being consistent with the reports from terrestrial animals (Alexopoulos et al., 2007; Demirel et al., 2011). However, Khodanazary et al. (2013) reported that dietary zeolite addition increased blood Ca and K levels, decreased Na concentrations and did not change Mg levels in common carp. Our results suggest that dietary zeolite did not alter mineral absorption and metabolism at a considerable level, but when used at levels higher than 4% there may be an adverse effect due to antagonistic relations between dietary ash levels and availabilities of certain minerals to fish such as Ca, Mg, Fe and P (Sugiura et al., 2000).

A reduction in RBC and Hb numbers below the normal range in fish is assumed to be an indicator of anemia (Houston, 1997; Maita, 2007). WBC number generally increases after deterioration of hemostasis due to an exposure to a stressful factor (Çelik, 2006). Oppositely a reduction in WBC number is also an indicator of impairment of immunity (Çelik, 2006). The Hb, WBC and RBC values obtained from the present study are within the range of literature data for gilthead sea bream (Molinero et al., 1997; Tort et al., 2002; Fazio et al., 2012). The hemogram values of sea bream were not altered by dietary zeolite levels in this study. These findings are inconsistent with those of Kanyılmaz (2008), who noted that dietary zeolite supplementation increased Hb values in common carp while similar to those of studies on fish (Eğrikılıç, 2009) and terrestrial animals (Katsoulos et al., 2005; Pourliotis et al., 2012; Yazdani and Hajilari, 2009).

Conclusion

The present findings show that dietary zeolite inclusion significantly decreased glucose whereas increased cholesterol and triglyceride levels. Other hematological and biochemical variables, except ALT levels, were not altered by the treatments. Overall the results suggest that dietary zeolite up to 4% did not affect health status of gilthead sea bream. Future studies should be focused on the effects of dietary zeolite on availabilities of minerals, gut health and microflora as well as immune parameters.

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