Animal Feed Science and Technology 200 (2015) 66–75

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of dietary zeolite (clinoptilolite) levels on growth performance, feed utilization and waste excretions by gilthead sea bream juveniles (*Sparus aurata*)

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ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form
22 September 2014
Accepted 24 September 2014

Keywords:
Sea bream performance
Zeolite
Digestibility
Iron
Aluminum

ABSTRACT

Natural zeolites have been increasingly used in terrestrial animal and freshwater fish diets as a growth and health promoter, but few studies have considered marine fish. Therefore, this study was planned to investigate the effects of dietary zeolite (clinoptilolite) incorporation on growth, feed and nutrient utilizations, whole body composition, waste excretions, intestinal morphology, fillet iron (Fe) and aluminum (Al) accumulations in juvenile gilthead sea bream (*Sparus aurata*). In the experiment, zeolite was included at 0 (Z0, control), 10 (Z10), 20 (Z20), 30 (Z30) and 40 g/kg (Z40) in a commercial sea bream diet and fed to fish with an initial weight of about 9.1 g for 10 weeks. Dietary zeolite treatments had a significant increasing effect on final weight (linear, P=0.046; quadratic, P=0.002) and specific growth rate (SGR) (linear, P=0.057; quadratic, P=0.010). Feed conversion efficiencies (FCE) of fish were linearly improved (linear, P=0.013) whereas PER increased both linearly (linear, P=0.033) and quadratically (quadratic, P=0.005). Supplemental zeolite did not affect body moisture or lipid but there was a trend for an increase (linear, P<0.10) in ash and a quadratic trend in protein (P<0.10) were recorded. Increasing dietary zeolite levels increased liver Fe levels and led to a quadratic affect on fillet Al levels (P<0.05) but did not change liver Al or fillet Fe levels (P>0.10). No differences in ADCs for dry matter, protein and lipid were detected but ADCs for energy had a quadratic trend (P=0.068). A trend for a decrease in total and dissolved nitrogen (N) losses was detected as the level of zeolite increased in the diet (quadratic, P=0.071 and 0.089 respectively). Anterior intestinal folds and gut length did not change with increasing zeolite levels but posterior intestinal folds decreased (linear, P=0.013; quadratic, P=0.023) and gut index had a quadratic trend (P=0.081). Optimum dietary inclusion level of zeolite was estimated as 27.1 g/kg based on the maximization of SGR, PER and FCE values.

Abbreviations: ABW, average body weight; ADC, apparent digestibility coefficient; AIF, anterior intestinal fold; Al, aluminum; CF, condition factor; CI, carcass index; DFI, daily feed intake; DM, dry matter; DNW, dissolved nitrogen waste; FCE, feed conversion efficiency; Fe, iron; FW, final weight; GI, gut index; GL, gut length; HSI, hepatosomatic index; N, nitrogen; PER, protein efficiency rate; PIF, posterior intestinal fold; SEM, standard error of the means; SGR, specific growth rate; SNW, solid nitrogen waste; TNW, total nitrogen waste; TSW, total solid waste; VSI, viscerosomatic index; WG, weight gain; Z0, control; Z10, 10 mg/kg zeolite; Z20, 20 mg/kg zeolite; Z30, 30 mg/kg zeolite; Z40, 40 mg/kg zeolite.

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http://dx.doi.org/10.1016/j.anifeedsci.2014.09.023
0377-8401/

1. Introduction

Zeolites, a kind of clay, are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, and have infinite three-dimensional structures (Mumpton, 1999). They are used in livestock and fish feeds as an additive or filler due to their detoxifying and nutrient absorption and growth enhancing features (Eya et al., 2008; Mumpton, 1999). Furthermore, they are utilized for a wide range of purposes such as amelioration of effects of mycotoxins, treatments of diarrhea, milk fever, toxicities of ammonia organophosphate and heavy metals, absorption of radioactive substances, reduction of skeletal deformities and prevention of parasitic infections in terrestrial animal production (Papaioannou et al., 2005).

Clinoptilolite is a natural zeolite that has been used in diets of Coho salmon, *Oncorhynchus kisutch* (Edsall and Smith, 1989), rainbow trout, *Oncorhynchus mykiss* (Eya et al., 2008; Leonard, 1979; Obradović et al., 2006; Reinitz, 1984; Yiğit and Demir, 2011), European sea bass, *Dicentrarchus labrax* (Dias et al., 1998), common carp, *Cyprinus carpio* (Kanyılmaz and Tekelioğlu, 2009; 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). Previous studies with rainbow trout, common carp and shrimp suggested that dietary inclusion of zeolite promoted growth rate and feed utilization (Eya et al., 2008; Galindo et al., 2006; Khodanazary et al., 2013; Obradović et al., 2006). However, to the best of our knowledge, clinoptilolite has not been investigated in gilthead sea bream, an important species in the Mediterranean mariculture industry. Plus, past studies have generally dealt with the effects of zeolites on growth and feed utilization, but there is still a scarcity of information about its effects on nutrient digestibility coefficients, nutrient balances, intestinal morphology and heavy metal accumulations in fish. Therefore, the present study was planned to evaluate the effects of dietary clinoptilolite incorporations on growth performance, apparent nutrient digestibility, nutrient utilizations, waste excretions, intestinal morphology as well as iron (Fe) and aluminum (Al) accumulations in fillet and liver of juvenile gilthead sea bream, *Sparus aurata*.

2. Method

2.1. Zeolite and diet preparation

The zeolite material was procured from a commercial mining company (Gordes Zeolite, İzmir, 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 105 °C. 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, 10, 20, 30 and 40 g/kg (named as Z0, Z10, Z20, Z30 and Z40 respectively) in place of alpha cellulose (Table 2). Distilled water was added to the mixtures until a dough-like consistency, and then the material was pressed through a meat mincer with a 2 mm die. The pellets were dried at 65 °C and stored at +4 °C until use.

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

Component	g/kg
Moisture	124
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

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

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

	Z0	Z10	Z20	Z30	Z40
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

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite.

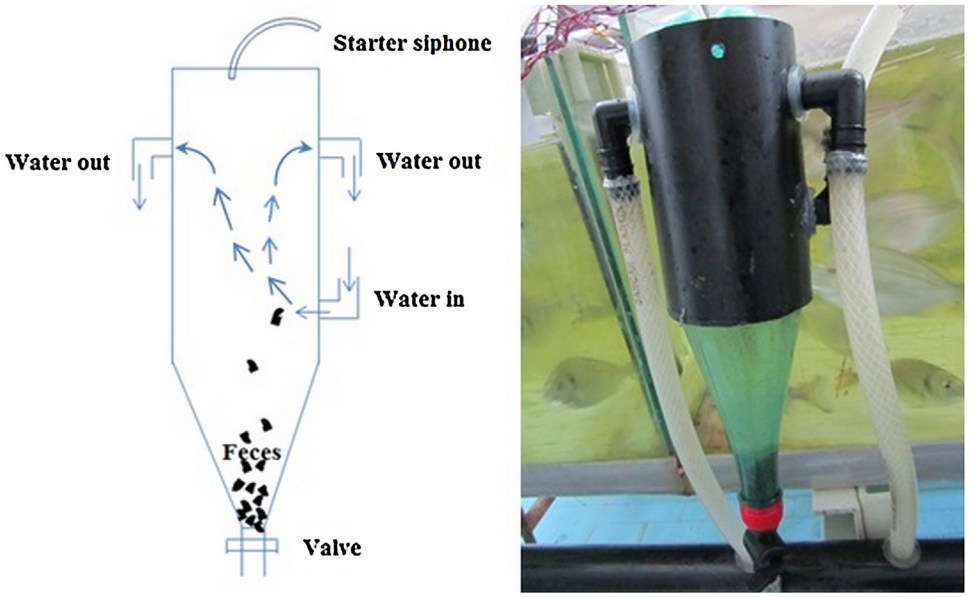

Fig. 1. A modified TUF feces collection system (Ke-TUF). Modified from Satoh et al. (1992).

2.2. 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 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%. Water turnover rate in the system was 1 h. The culture system was also provided with continuous aeration through an air compressor. Water temperature was maintained at about 25 °C with thermostatic heaters.

Fish were selected from a large population produced in the institute's marine hatchery at the Beymelek Unit, Antalya, size-graded and then transferred to the experimental unit. Twenty five fish were randomly allocated to each tank and acclimatized to the experimental conditions for 2 weeks. During the adaptation period, fish were fed the control diet at a level of 4% body weight. The number of fish per tank was reduced to 20 at the beginning of the trial since some fish were used to measure initial body composition and some other weak fish were eliminated. Average initial weight of fish was 9.06 ± 0.04 g. Each of five treatments was tested in triplicated tanks for 10 weeks. Fish were fed by hand twice a day at 09:00 and 15:30 h at levels of 4, 3 and 2.5% of body weight during first 6, 7–8th and 9–10th weeks respectively. 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 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) and measured as 24.77 ± 0.18 °C, 5.10 ± 0.16 mg/L, 7.68 ± 0.04, 37.35 ± 0.1 ppt, respectively. Total ammonia nitrogen (TAN) and nitrite were monitored every 3 days (APHA, 1995) and detected as 0.01 ± 0.00 mg/L, 0.23 ± 0.01 mg/L and respectively.

2.3. Sample collection and analysis

Feces were collected during the last 10 days of the experiment using a modified Tokyo University of Fisheries (TUF) feces collector, called Kepez-TUF (Ke-TUF) (Fig. 1). Briefly, in the original TUF (Satoh et al., 1992) water is passed through a tube where feces accumulate and the tube must be detached from the rearing tank to collect them. We added a valve to form a feces collection column, making them easier to obtain. At the beginning of the experiment, twenty five fish were killed by an overdose of anesthetic (2-phenoxyethanol) for determination of initial body composition. At the termination of the experiment, 16 fish from each tank were killed by an overdose of anesthetization; four for determination of organ indices, four for whole body composition, four for intestinal morphology and the remaining four for fillet and liver Fe and Al contents. To determine anterior and posterior intestinal fold (AIF and PIF respectively) heights, the respective intestinal samples were fixed in 4% buffered formaline for 24 h. After dehydration, the samples were embedded in paraffin. The histological sections (6–7 μm; Leica RM 2125 R, Bensheim, Germany) were stained with haematoxylin and eosin (H&E), photographed with a camera (Olympus DP72, Tokyo, Japan) attacted to a microscope (Olympus BX53, Tokyo, Japan) (Takashima and Hibiya, 1995). Then mucosal fold heights were measured with ImageJ software (National Institute of Health, Bethesda, Maryland, USA) (Escaffre et al., 2007).

Whole body, liver and fillet samples were kept at −20 °C until analysis. For fillet and liver Fe and Al analysis the samples were digested in concentrated nitric acid using the Berghof Speedwave MWS-2 microwave digestion system (Berghof Products and Instruments, GmbH, Eningen, Germany). The digested materials were diluted with deionized water and passed through a membrane filter with a 0.45 μm pore size. Fe and Al concentrations were measured by an inductively coupled plasma emission optical emission spectroscopy (ICP-OES, Varian, Palo Alto, CA, USA) following the manufacturer recommendations. Proximate analyses of diet and fish were performed according to the methods of AOAC (1990): dry matter by drying in an oven at 104 °C until constant weight; ash content by incineration in a muffle furnace at 600 °C for 2 h; crude protein (N × 6.25) by the Kjeldhal method after acid digestion. Lipid was determined with an automatic extraction system (ANKOMXT15 Extractor, ANKOM Technology, Macedon, USA). All analyses were conducted in duplicate except lipid which was analyzed in triplicate. Chromic oxide concentrations of the diets and feces were determined according to Furukawa and Tsukahara (1966). Gross energy was calculated using conversion factors of 39.5, 23.7, and 17.2 MJ/kg for fat, protein, and carbohydrate, respectively.

2.4. Data calculation

Growth and feed utilization parameters were calculated according to formulas given below.

$$\text{Weight gain(WG g/fish)} = W_t - W_0,$$

$$\text{Specific growth rate (SGR\%/day)} = \frac{100(\ln W_t - \ln W_0)}{t},$$

$$\text{Feed conversion efficiency (FCE)} = \frac{\text{WG (g)}}{\text{dry feed intake (FI)(g)}}$$

$$\text{Protein efficiency rate (PER)} = \frac{\text{WG (g)}}{\text{protein fed (g)}}$$

$$\text{Condition factor (CF)} = \frac{W_t}{L^3}$$

$$\text{Viscerosomatic index (VSI\%)} = 100\left(\frac{\text{visceral weight (g)}}{\text{body weight (g)}}\right)$$

$$\text{Hepatosomatic index (HSI\%)} = 100\left(\frac{\text{liver weight (g)}}{\text{body weight (g)}}\right)$$

$$\text{Gut index (GI\%)} = 100\left(\frac{\text{digestive tract length (cm)}}{\text{fish length (cm)}}\right)$$

$$\text{Nutrient (N, lipid, energy) intake (g or kJ/kg ABW/day)} = \frac{\text{(nutrient intake (g or kJ)/kg ABW)}}{\text{days}}$$

$$\text{Nutrient (N, lipid, energy) gain (g or kJ/kg ABW/day)} = (\text{(final body nutrient content (g or kJ)} - \text{initial body nutrient content (g or kJ))/(kg ABW)/days}$$

$$\text{Nutrient (N, lipid, energy) retention} = \left(\frac{\text{nutrient gain (g or kJ)}}{\text{nutrient intake (g or kJ)}}\right),$$

where W_t(g) is fish body weight at day t and W0 at day 0, t (days) is the duration of experiment

$$\text{Apparent digestibility coefficient of dry matter (ADC)} = 1 - \left(\frac{(Cr_2O_3\ \text{Faeces})}{(Cr_2O_3\ \text{Food})}\right)$$

$$\text{ADC of nutrients} = 1 - \left(\frac{(Cr_2O_3\ \text{Food})}{(Cr_2O_3\ \text{Faeces})}\right) \times \left(\frac{(\text{Nutrient Feces})}{(\text{Nutrient Food})}\right)$$

$$\text{Total solid waste (TSW) (g/kg fish)} = \frac{\text{((ingested feed (DM))} \times (1 - \text{(ADC DM of feed))}}{\text{WG (kg)}}$$

Table 3
Growth performance and feed utilization efficiency and organ indices of juvenile gilthead sea bream fed the experimental diets. Values are means of three replicates.

	Z0	Z10	Z20	Z30	Z40	SEM	P values	
							Linear	Quadratic
IW (g/fish)	9.05	9.10	9.08	9.03	9.05	0.04	0.545	0.444
FW (g/fish)	50.7	52.6	53.8	54.5	52.0	0.78	0.046	0.002
SGR (%/day)	2.73	2.78	2.82	2.85	2.78	0.03	0.057	0.010
FI (g/fish)	50.2	50.1	53.0	51.9	49.7	0.76	0.770	0.011
DFI (g/kg ABW/day)	22.8	22.2	23.3	22.2	22.1	0.16	0.001	0.129
FCE	0.83	0.87	0.85	0.88	0.87	0.01	0.013	0.149
PER	1.72	1.85	1.80	1.84	1.80	0.02	0.033	0.005
VSI (%)	7.50	7.33	7.04	6.97	7.52	0.24	0.764	0.051
HSI (%)	1.45	1.30	1.48	1.41	1.33	0.09	0.600	0.707
CI (%)	90.1	92.0	92.2	91.0	91.8	0.78	0.262	0.171
CF	1.99	1.91	2.07	2.07	1.97	0.08	0.668	0.486

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means; IW, initial weight; FW, final weight; SGR, specific growth rate; FI, feed intake; DFI, daily feed intake; ABW, average body weight; FCE, feed conversion efficiency; PER, protein efficiency rate; VSI, viscerosomatic index; HSI, hepatosomatic index; CI, carcass index; CF, condition factor.

$$\text{Solid nitrogen waste (SNW) (g/kg fish)} = \frac{((\text{N in ingested feed (DM)}) \times ((1 - \text{ADC of N}) \times (\text{N content of feed}))}{\text{WG (kg)}}$$

$$\text{Dissolved nitrogen waste (DNW)(g/kg fish)} = \frac{((\text{N in feed} \times \text{ADC of N}) - (\text{N retention}))}{\text{WG (kg)}}$$

$$\text{Total nitrogen waste (TNW) (g/kg fish)} = \frac{((\text{SNW g/kg fish}) + (\text{DNW g/kg fish}))}{\text{WG (kg)}}$$

2.5. Statistical analysis

Polynomial contrasts were used to detect linear and quadratic effects of various dietary zeolite levels on the observed response variables. Significant treatment effects were considered at $P \leq 0.10$. Optimum dietary zeolite level that maximizes three selected main variables (SGR, PER and FCE) was estimated using second-degree polynomial regression analysis. A statistical package JMP v.8.0 for Windows was used for all statistical analyses.

3. Results

3.1. Growth, body composition and Fe and Al accumulations

Dietary zeolite treatments had a significant increasing effect on final weight (FW) (linear, P=0.046; quadratic, P=0.002) and SGR (linear, P=0.057; quadratic, P=0.010) (Table 3) with the highest values in fish on Z30. The FCE values of fish were linearly improved (linear, P=0.013) whereas PER increased both linearly (linear, P=0.033) and quadratically (quadratic, P=0.005) with increasing dietary zeolite level. Maximum SGR, PER and FCE values were estimated at 24.1, 24.3 and 33.0 g/kg zeolite levels, respectively, resulting in an optimum value of 27.1 g/kg. Although average FI was quadratically affected with increasing zeolite levels (quadratic, P=0.011), the DFI linearly decreased (linear, P=0.001). There were no discernible effects of zeolite supplementations on HSI, CI and CF of gilthead sea bream, whereas a quadratic trend was observed in VSI (quadratic, P=0.051).

Whole body ash concentrations of experimental fish tended to increase (linear, P=0.087; quadratic, P=0.076) and protein had a quadratic trend (quadratic, P=0.080) in response to increasing dietary zeolite levels, but moisture and lipid levels did not differ significantly regardless of the treatments (Table 4).

Liver Fe levels were increased (linear, P=0.001) from 22.65 in Z0 to 49.06 in Z40 mg/kg but there were no significant treatment effects on liver Al levels as well as fillet Fe (Table 4). There was a quadratic response in fillet Al levels of fish as the level of zeolite increased in the diet (quadratic, P=0.021).

3.2. Nutrient ADCs and utilizations

The ADCs of the experimental diets are shown in Table 5. No differences in ADCs for dry matter, protein and lipid were detected in response to dietary zeolite level but ADC of energy exhibited a quadratic trend (quadratic, P=0.068).

Nitrogen (N), lipid and energy utilizations of gilthead sea bream fed the experimental diets are presented in Table 6. Increasing dietary zeolite contents did not affect N balances but linearly reduced lipid intake of fish (linear, P=0.004).

Table 4
Proximate composition, liver and fillet Fe and Al levels of juvenile gilthead sea bream fed the different experimental diets (g/kg). Values are means of three replicates.

	Initial	Z0	Z10	Z20	Z30	Z40	SEM	P values	
								Linear	Quadratic
Whole body									
Moisture	707	669	700	658	665	661	4.18	0.138	0.698
Ash	43	32	30	31	34	34	1.52	0.087	0.076
Protein	161	164	162	159	165	166	2.22	0.293	0.080
Lipid	90	123	125	135	124	129	3.82	0.339	0.335
Liver									
Fe		22.7	24.9	29.5	46.1	49.1	4.20	0.001	0.356
Al		1.41	1.44	1.23	0.44	2.83	0.85	0.446	0.157
Fillet									
Fe		3.13	3.10	3.83	3.65	2.66	0.76	0.845	0.244
Al		0.25	0.95	1.12	1.95	0.22	0.47	0.497	0.021

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means; Fe, iron; Al, aluminum.

Table 5
Apparent digestibility coefficients of the experimental diets. Values are means of three replicates.

	Z0	Z10	Z20	Z30	Z40	SEM	P values	
							Linear	Quadratic
Dry matter	0.689	0.682	0.716	0.673	0.678	0.009	0.36	0.201
Protein	0.902	0.903	0.918	0.891	0.900	0.007	0.508	0.352
Lipid	0.967	0.969	0.971	0.964	0.969	0.002	0.419	0.804
Energy	0.829	0.841	0.863	0.837	0.848	0.006	0.139	0.068

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means.

Significant linear increases in lipid (P=0.049) and energy retentions (P=0.048) were detected as the level of zeolite increased in the diet.

3.3. Waste excretions

Estimated TSW, TNW, SNW and DNW values are presented in Table 7. The TSW varied between 325.2 and 355.9 g/kg fish among the treatments with a quadratic trend (quadratic, P=0.087). The SNW ranged between 7.11 and 9.01 g/kg fish without a significant effect of the treatments. The TNW and DNW tended to decrease linearly with increasing dietary zeolite levels (P=0.071 and 0.089, respectively).

3.4. Intestinal morphology

There appeared to be no effect of dietary zeolite on AIF morphology and GL of experimental fish (Table 8). However, PIF values decreased with zeolite levels (linear, P=0.013; quadratic, P=0.023) and GI exhibited a quadratic trend (quadratic, P=0.081). Histologic appearances of the anterior and posterior intestine of gilthead sea bream showed a normal villus structure in all treatments.

Table 6
Nitrogen, lipid and energy utilization by juvenile gilthead sea bass fed the experimental diets. Values are means of three replicates.

	Z0	Z10	Z20	Z30	Z40	SEM	P values	
							Linear	Quadratic
Nitrogen								
Intake (g/kg ABW/day)	1.76	1.66	1.75	1.69	1.70	0.012	0.248	0.453
Gain (g/kg ABW/day)	0.53	0.53	0.52	0.55	0.54	0.009	0.119	0.349
Retention	0.302	0.319	0.299	0.326	0.318	0.007	0.11	0.984
Lipid								
Intake (g/kg ABW/day)	3.97	3.88	3.99	3.82	3.81	0.030	0.004	0.357
Gain (g/kg ABW/day)	2.59	2.67	2.93	2.67	2.77	0.010	0.247	0.205
Retention	0.653	0.688	0.734	0.700	0.727	0.028	0.049	0.301
Energy								
Intake (kj/kg ABW/day)	492.7	478.0	500.7	479.0	478.3	0.004	0.131	0.434
Gain (kj/kg ABW/day)	185.0	188.3	199.0	191.0	192.3	0.004	0.173	0.142
Retention	0.376	0.394	0.398	0.399	0.402	0.011	0.048	0.328

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means; ABW, average body weight.

Table 7
Estimated waste outputs (g/kg fish) of gilthead sea bream fed various levels of zeolite. Values are means of three replicates.

	Z0	Z10	Z20	Z30	Z40	SEM	P values	
							Linear	Quadratic
TSW	355.9	349.3	325.2	354.5	354.7	10.66	0.928	0.087
TNW	61.5	56.2	60.5	55.7	57.7	0.97	0.071	0.285
SNW	8.66	8.03	7.11	9.01	8.46	0.61	0.752	0.186
DNW	52.8	48.2	53.4	46.7	49.2	0.95	0.089	0.667

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means; TSW, total solid waste; TNW, total nitrogen waste; SNW, solid nitrogen waste; DNW, dissolved nitrogen waste.

Table 8
Anterior and posterior intestinal mucosal fold heights (mm), gut length (cm) and gut index of sea bream fed various level of zeolite. Values are means of three replicates.

	Z0	Z10	Z20	Z30	Z40	SEM	P values	
							Linear	Quadratic
AIF	0.58	0.52	0.68	0.55	0.52	0.066	0.631	0.543
PIF	0.97	1.01	0.89	1.01	0.66	0.060	0.013	0.023
GL	13.1	13.1	13.9	14.3	13.0	0.930	0.631	0.214
GI	0.89	0.88	1.00	1.05	0.83	0.079	0.839	0.081

Z0, control; Z10, 10 g/kg zeolite; Z20, 20 g/kg zeolite; Z30, 30 g/kg zeolite; Z40, 40 g/kg zeolite; SEM, standard error of the means; AIF, anterior intestine; PIF, posterior intestine; GL, gut length; GI, gut index.

4. Discussion

Fish grew from about 9.1 g to a range of 50.7 and 54.5 g at the end of the experiment with a comparable or better rate and feed utilization (SGR 2.73–2.85%/day and FCE 0.83–0.88) than the reported values (1.61–1.93% and 0.56–0.79 respectively) for this species (Pereira and Oliva-Teles, 2002; Santinha et al., 1999). Zeolite supplementation quadratically increased FW by 8%, 7.32%, 9.29% and 3.12% increments in Z10, Z20, Z30 and Z40 compared with Z0. Our results are consistent with those reporting a positive effect of dietary clays on growth performance such as Obradović et al. (2006), Eya et al. (2008) and Danabaş (2009) in rainbow trout, Galindo et al. (2006) in white shrimp (*L. schmitti*) and Khodanazary et al. (2013) in common carp. However there are some studies finding either no effect of dietary clay (Demir and Aybal, 2004; Dias et al., 1998; Edsall and Smith, 1989; Kanyılmaz and Tekelioğlu, 2009; Tekeşoğlu, 2010; Yiğit and Demir, 2011; Yıldırım et al., 2009) or negative results (Reinitz, 1984) on growth performance in fish. Dietary zeolite levels linearly decreased fish appetite when expressed in DFI per kg ABW, being in harmony with findings of Dias et al. (1998). Conversely, an improvement in FCE and PER values with zeolite levels was detected, implying that a better feed and protein utilization have a role in the improved growth performance. Although there is no general consensus in the literature about the effects of zeolite on FCE and PER in fish, our results are consistent with those of Galindo et al. (2006), Obradović et al. (2006), Eya et al. (2008), Danabaş (2009) and Khodanazary et al. (2013), who reported better feed utilization with the use of zeolite.

ADC values obtained in the present experiment using Ke-TUF are within the range of those reported in the literature for gilthead sea bream (Pereira and Oliva-Teles, 2002; Santinha et al., 1999), except dry matter which was lower possibly due to inclusions of indigestible cellulose or zeolite (Ghaemnia et al., 2010) rather than an independent effect of Ke-TUF. In the present study, determination of the ADCs without an overestimation compared with the literature implies that a dry matter and nutrient leaching problem from feces after being voided or during the collection was within the acceptable range. Overall, this may suggest that application of this modified feces collector in future digestibility studies is possible. The present findings indicated that zeolite addition did not change ADC values, except energy which had a quadratic tendency. There is little information about effects of zeolite on ADC values of fish. Our results with low dietary zeolite levels are, to a large extent, compatible with those of Dias et al. (1998), who found high dietary zeolite levels (10 and 20%) were comparable in terms of ADC of diets. In contrast, Hu et al. (2008) in tilapia, Khodanazary et al. (2013) in carp and Li and Kim (2013) in growing pigs noted an improvement in dry mater and protein ADC values with clay incorporations. Hu et al. (2008) also noted a significant increase in intestinal protease and alkaline phosphatase activities in fish fed a diet with clay. We conclude that better energy ADCs could play a role on the improved growth and feed utilization in gilthead sea bream on zeolite added to diets. However, it appears that some other factor(s) also played a role, which will be discussed further.

All the experimental fish had a normal histological structure of AIF and PIF, containing the mucosa, lamina propria, muscularis and seroza, being compatible with results of our previous study on common carp (Kanyılmaz, 2008). Shortening or elongation of intestinal villus have been directly associated with low or high nutrient absorption capacities by some authors (Farhangi and Carter, 2001; Ma and Guo, 2008). While some researchers have reported a linear relation between growth and villus height (Stevens and Devlin, 2000; Yılmaz et al., 2007) others have been in favor of the fact that a change in villus height is independent from growth rate (Escaffre et al., 2007; Farhangi and Carter, 2001; Genç et al., 2006, 2007). In consistent with the latter group, a decreasing pattern in PIF values with increasing zeolite levels in the diet did not correlate

with growth rate and digestibility values in the present experiment. An inherent ability of zeolites to attach the mucus has been reported to reinforce the intestinal barrier, contribute to the regeneration of epithelium and preserve the mucosa from the toxic effects of drugs and toxins (Albengres et al., 1985; Hu et al., 2012, 2008; Li and Kim, 2013). Mucus is known to have a pivotal role in nutrition and gut health (Montagne et al., 2004). Considering that digestive tract of fish has a high protein turnover rate, an energy demanding process (Kaushik and Seiliez, 2010; Tacchi et al., 2012), a reduction in this cost through gut health improvement would contribute to the overall growth and nutrient utilization performance in animals (Kaushik and Seiliez, 2010; Parisini et al., 1999; Tacchi et al., 2012). We may infer that an enhancement of gut health through the use of dietary zeolite reduced protein turnover rate along the gut, enabling more energy and protein to be directed towards growth.

There was a significant linear decrease in lipid intakes with the increase in zeolite levels which appeared to depend on the amounts of feed consumed. Conversely, a positive significant linear effect of dietary zeolite supplementation was the case in lipid and energy retentions. There is no direct data about effects of dietary zeolite on the nutrient balances of fish in the literature. Yet, a recalculation of the literature data suggests that a growth enhancement with dietary clay supplementation was generally accompanied by higher nutrient retentions by fish (Danabaş, 2009; Eya et al., 2008; Obradović et al., 2006), being consistent with our findings. However, no effect of dietary clay on nutrient retentions was implied in rainbow trout (Reinitz, 1984), sea bass (Dias et al., 1998) and common carp (Kanyılmaz and Tekelioğlu, 2009). It is important to note that effectiveness of zeolites can change depending on its type, purity, structural feature, particle size, porosity and dietary levels (Papaioannou et al., 2005).

Increasing dietary energy (lipid) to protein ratio and optimization of feeding and rearing conditions are suggested to decrease solid and dissolved N waste excretions (Cho and Bureau, 2001; Kaushik, 1998). In the present study, a quadratic tendency in TSW amounts in response to dietary zeolite inclusions was obtained with the lowest value in Z20. Some of calculated data (not shown) from the literature suggest a decrease in TSW fractions with dietary clay incorporations (Danabaş, 2009; Eya et al., 2008; Obradović et al., 2006) whereas others are in favor of an increase (Dias et al., 1998; Reinitz, 1984). Although there were no differences in SNW among the treatments, TNW and DNW values tended to decrease with increasing zeolite levels with the lowest figures in Z30. In consistent with this, an estimation of TNW from data of Danabaş (2009) revealed that a 37.7% reduction in TNW could be possible with 1% dietary zeolite in rainbow trout. The estimations regarding the effects dietary clay on N losses in other studies (Eya et al., 2008; Obradović et al., 2006) are also in parallel with this trend. The trend for decreasing N wastes in fish fed zeolite incorporated diets in the present experiment could be due to their better FCE and PER values, which further supports our observations of better fish performance with dietary zeolite.

Final proximate compositions of fish were not affected by the treatments with the exception of ash and protein, which had quadratic tendencies; this is partly consistent with some previous research (Dias et al., 1998; Kanyılmaz and Tekelioğlu, 2009). A critical consideration of dietary zeolite inclusion should be directed towards its effects on heavy metal concentrations in fish. Accordingly we examined Fe and Al accumulations in fillet and liver. Dietary Fe requirements of red sea bream, a closely related fish with gilthead sea bream, were estimated as 150 mg/kg diet (Sakamoto and Yone, 1978). Dietary toxic effects of Fe were seen at 1380 mg/kg in rainbow trout (Desjardins et al., 1987). In the present study, dietary Fe levels were higher than the requirement levels even in the control diet, and increased with the zeolite levels up to 681 mg/kg. Yet we did not observe an adverse effect of this level, such as deteriorated growth and feed utilization (Desjardins et al., 1987) possibly due to a form of Fe in the zeolite, Fe_2O_3, which is not available for fish (Maage and Sveier, 1998). However, a more than double increase in hepatic Fe concentrations of fish fed Z30 and Z40 compared to Z0 could suggest that zeolite used in the present study may contain an available Fe form other than Fe_2O_3. Yet, there were no differences in fillet Fe concentrations between the treatments and hence further studies are needed to clarify this conflicting point. Dietary requirements and bioaccumulations of Al through feed have not been documented in fish, but its dietary toxicity is negligible (Wilson, 2011). Indeed, a study on Atlantic salmon showed that administration of increasing dietary Al levels from 0 to 2000 mg/kg to fish brought neither a beneficial nor a clear adverse effect (Poston, 1991). Although Al levels of our experimental diets increased with the zeolite inclusions, the maximum Al level in Z40 (1655 mg/kg) was still within the safe range reported by Poston (1991). Although a significant quadratic trend in fillet, dietary zeolite levels appeared not to affect adversely Al levels of liver and fillet of gilthead sea bream.

With a 225 g of meal size a day (Dabeka et al., 2004), maximum total intakes of Fe and Al via gilthead sea bream fed Z20 and Z30 diets would be only 0.86 and 0.44 mg respectively. These amounts of Fe and Al from the fillets cannot impose a health risk considering the tolerable levels (100 and 7 respectively) (WHO, 1996, 2004).

5. Conclusion

The present findings show that dietary zeolite inclusion results in a significant improvement of growth rate, FCE and PER. The optimum zeolite dose in diets of juvenile gilthead sea bream is 27.1 g/kg without any threat to fish and human health in terms of fillet Fe and Al levels. Dietary zeolite also appears to be effective in terms of reductions in TNW and DNW. Future studies should be focused on the effects of dietary zeolite on amino acid, fatty acid and phosphorus utilizations as well as gut health.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

The General Directorate of Agricultural Research and Policy, Ministry of Food, Agricultural and Livestock, and Academic Research Projects Unit, Cukurova University, Turkey supported this research under grant no TAGEM/HAYSUD/2011/09/01/01 and SÜF 2010 D 04 respectively. The authors wish to express their gratitude to Yılmaz Emre, Aslıhan Polatoğlu and Talip Özgen for their supports during the study, and Gordes Zeolite, for generously providing the zeolite material.

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