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Effects of Dietary Zeolite and Perlite Supplementations on Growth and Nutrient Utilization Performance, and Some Serum Variables in Common Carp, (*Cyprinus carpio*)

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Abstract

Zeolite and perlite are natural volcanic rocks with ion exchanging properties. This experiment was carried out to determine the effects of different dietary levels of natural zeolite and perlite on growth performance, apparent digestibility coefficients of dry matter and protein, ash contents of bone and scales as well as blood parameters in common carp *Cyprinus carpio*. Five experimental diets tested were a control diet, and zeolite and perlite additions at levels 2.5 and 5%. Each treatment was tested in 3 replicates tanks using fish with an initial average weight of 30±0.5 g. Fish were hand-fed, twice a day for 8 weeks. The results showed that the dietary perlite and zeolite had no an influence on the fish condition factor (P>0.05). There were improving effects of the dietary additions, particularly at 5 %, on growth performance of fish compared to the control. The dry matter and protein apparent digestibility coefficients (ADC; means± S.D) for the experimental diets were, respectively, Z 2.5 (Zeolite 2.5): 83.0±0.89 and 91.99±0.28; Z 5 (Zeolite 5): 87.48±0.76 and 95.14±0.92; P 2.5 (Perlite 2.5): 84.87±0.71 and 93.42±0.64; P 5 (Perlite 5): 87.92±1.27 and 95.80±0.57. Significantly higher ash contents were found in bones and scales of fish fed with the experimental diets in comparison with the control diet (P<0.05). The increase of dietary zeolite resulted in a significantly higher concentration of Ca in serum. While the zeolite and perlite diets did not affect serum Mg, P and total protein contents of serum, a significantly enhanced glucose value was observed in fish fed Z 5. The Na and cholesterol content of serum decreased with adding perlite and zeolite, although K content increased (P<0.05). In conclusion, the results of this study suggest that dietary supplement of zeolite and perlite can be used as a new aquafeed ingredient for commom carp.

Keywords: *Cyprinus carpio*, dietary zeolite, dietary perlite, digestibility, growth performance, blood parameters.

Introduction

Zeolites are crystalline, hydrated aluminosilicates of alkali (e.g. Na^{+}, K^{+}) and alkaline (e.g. Mg^{+2}, Ca^{+2}) earth cations, consisting of three dimensional frameworks of SiO^{-4} and AlO^{-5} tetrahedra linked through the shared oxygen atoms (Papaioannou, *et al*., 2005). On the other hand, perlite is defined as a naturally occurring glassy volcanic siliceous rock with a marked concentric onion-like structure of fractures, caused by concentration during cooling (Mathialagan and Viraraghavan, 2002). There are various hypotheses concerning the function of zeolite in diet of fish: as dietary supplements in animal diets, data in the published literature provide evidence of a growth promoting effect when zeolites are used as additives in animal nutrition, provide oxygen-enriched air to aquacultural systems, have beneficial effects in fish hatchery systems, and allowing reductions in energy use and increasing production (Pond and Mumpton, 1984).

Studies in Japan using zeolite as a dietary supplement for animals showed that the test animals generally grew faster than control groups with a simultaneous decrease in the amount and cost of the feed (Mumpton and Fishman, 1977). Dietary inclusion of zeolites in fish has also been reported to improve growth and feed utilization (Mumpton and Fishman, 1977; Papaioannou *et al*., 2005). However, it is difficult to make a general conclusion about the performance improving effects of dietary zeolites in aquaculture. Research data reported in the published literature provide evidence of a growth promoting effect when zeolites are used as additives in animal nutrition. The effect of dietary zeolites on feed intake varies in different researches. For instance, Leonard (1979) added 2% natural zeolite (clinoptilolite, the most abundant zeolite in the nature) to trout diets, and found a significant improvement in weight gain over a 64-days feeding period. The use of clinoptilolite at 5

and 10% levels did not affect the growth of coho salmon (Edsall and Smith, 1989). Furthermore, Reinitz (1984) demonstrated that dietary inclusion of zeolite (sodium bentonite), at 5, 10 and 15% adversely affected weight gain in rainbow trout. Inclusion levels of zeolite into animal diets range from 1 to 10 %, but the recommended level is 1 %for synthetic zeolite, and 10 % for nature zeolite (Shariatmadari, 2008).

Therefore, the use of perlite and zeolite in fish diets has not been studied extensively and the existing results are inconclusive. The present experiment was conducted to evaluate the effects of zeolite and perlite inclusions in common carp diets on growth and nutrient utilization performance, apparent digestibility coefficients of dry matter, protein as well as ash contents of bone, scales and blood serum biochemical properties.

Materials and Methods

Experimental Diets

A control diet was formulated to contain 29.35% crude protein and 7.68% crude fat using wheat (10%), corn (20%), soybean meal (60%), fish meal (26.5%), plant oil (6%) and other supplemental ingredients (7.5%) (Table 1). The control diet was either supplemented with 2.5 and 5 % of zeolite (named Z 2.5 and Z 5, respectively) or perlite (P 2.5 and P 5 respectively). Zeolite (clinoptilolite) used as raw material and expanded perlite added to experimental diet. Expanded perlite is an economical insulation for high and low temperature and cryogenic applications. Ingredients and composition of five diets are shown in Table 1. All ingredients were finely grinded, soybean oil, sunflower oil, molasses and deionized water (30%) was added and mixed in a homemade mixer (IKA® T25 digital ULTRA-TURRAX®) and then pelleted (3.5 mm diameter) using a meat chopper. Pellets were then air-dried and screened to remove fines before feeding. To all diets, 1% chromic oxide (Cr_2O_3) was added as an indigestible marker in order to measure dry matter and protein digestibility coefficients.

Experimental Fish and Feeding Trial

A total of 150 juvenile carp purchased from a private fish farm were transported to the fishery research center, Gorgan University of Agricultural Sciences and Natural Resources, Iran. The carp were acclimatized for one month in 8 tanks (300 L capacity) and then randomly distributed into 15

Table 1. Ingredients and nutrient composition of the experimental diets

Ingredients of control diet (g/kg)					
Fish meal (clupea meal)	265.0				
Soybean meal	300.0				
Wheat meal	100.0				
Corn meal	200.0				
Soy oil	30.0				
Sunflower oil	30.0				
Vitamin premix[1]	7.5				
Mineral premix[2]	7.5				
Lysine	10.0				
Methionine	14.0				
Lysetin	2.0				
CaCo3	4.0				
Molasses	10.0				
Salt	10.0				
Cr2O3	10.0				
Zeolite	0.00				
Perlite	0.00				
Experimental diets	Control	Z 2.5	Z 5	P 2.5	P 5
Control diet	100	97.5	95	97.5	95
Zeolite	-	2.5	5	-	-
Perlite	-	-	-	2.5	5
Calculated analysis (% dry matter)					
Ash	8.75	10.96	13.41	11.05	13.75
Crude protein (N× 6.25)	29.35	28.61	27.88	28.61	27.88
fat	7.68	7.48	7.29	7.48	7.29

[1]Each kg of vitamin premix containing: vitamin A, 120000 IU; vitamin D3, 80000 IU; vitamin E, 8000 mg; vitamin K3, 200 mg; vitamin B1, 600 mg; vitamin B2, 1000 mg; vitamin B6, 600 mg; vitamin B12, 1600 mg, vitamin C, 10400 mg; vitamin Nicotinic acid, 6000 mg; vitamin Calciumed pantothenate, 1800 mg; vitamin Folic acid, 320 mg; vitamin d- Biotin, 32 mg; vitamin Inositol, 4800 mg; vitamin Antioxidant, 1000 mg.

[2]Each kg of mineral premix containing: Manganese, 520 mg; Copper, 120 mg; Zinc, 1200 mg; iron, 800 mg; Selenium, 10 mg; I, 40 mg; Co, 10 mg; Colin chloride, 24000 mg.

experimental tanks (100 L capacity). The mean initial weight of fish was 30±0.5 g. Health status of the fish during the acclimation and experimental period was good, there was no mortality during the experiment. Each of the five experiment groups were tried in three replicate tanks. The water was aerated using air-stone diffusers and conducted recirculating system. Dissolved oxygen, temperature and pH were 6-7 mgL^{-1}, 25± 0.3 ºC and 7.5, respectively.

During the 8 weeks experimental period, fish were hand-fed twice daily for 5 days a week (9:30 and 14:30 h) at a rate of 3% body weight in the calculation of daily feeding amounts of each treatment the diet dilution rates, because these are inert materials (Dias *et al*., 1998). The fish were weighted collectively with bi-weekly intervals and feeding rates were corrected accordingly. No feeding was done on the sampling days. Before weighting, fish in each tank were anaesthetized cite clove oil (100 pm) (Anderson *et al*., 1997).

Fecal Collection

At the end of experiment, all fish in each tank were dissected and faeces were collected from intestine. During the dissection the intestine was clamped immediately proximal to the anus and at the level of pelvic fins using surgical clamps. This section of the intestine was removed and the contents were gently squeezed into a plastic bottle. Fecal samples were lyophilized (ALPHA 1-2 LD *plus*) and stored at -20 °C until further analysis (Percival *et al*., 2001).

Chemical Analysis

Before the termination of the experiment, blood samples collected after 24 h fasting. Four fish were randomly captured from each tank and anesthetized with clove oil (100 ppm). The blood samples were collected from caudal vein using the syringe, and then transferred to syringed into 1.5 ml tubes (Nwanna *et al*., 2007). Tubes were centrifuged (Eppendorf AG 22331 Hamburg, Centrifuge 4515D, Germany) at 10000 rpm for 10 min, and the serum was separated and stored at -20 ºC before analysis**.** The serum was analyzed for P, Ca, Fe, Mg, K, and Na using spectrophotometric (). cholesterol and glucose concentration (Roch Germany, 1489348). Scales and the central bones were also collected from the fish in each tank after blood sampling. The scales were sampled from the ventral muscle section. The bones were separated from soaked fish warm (40 ºC) deionized water for 5 min and then the bone and scales were dried in an oven (105 ºC) and ashed at 480 ºCfor 48 h.(Nwanna *et al*., 2007). Chromic oxide the diets and faeces samples were determined according to Williams *et al*. (1962) and chemical composition analysis of diets and faeces were made for dry matter, ash, crude protein and fat, using AOAC (1990) procedures.

Statistical Analysis

Data are presented as mean± standard deviation. To test differences between dietary treatments, all data were subjected to one-way analysis of variance (ANOVA) using SPSS software (version 11). Duncan's multiple range test was used to separate the means among treatments with a probability level of $P<0.05$ (Duncan, 1955).

Results and Discussion

Growth Performance

Growth performance of fish fed with different experimental diets is presented in Table 2. Dietary inclusions of zeolite and perlite had a significant positive effect on the growth performance of fish ($P<0.05$), being more marked in fish fed Z 5. When the inclusion levels were considered, it was seen that 5 % better than 2.5 %. The influence of the dietary incorporations was not clear in terms of FCR and FCE, but the best value was observed in fish diet Z 5 without differing from the control.

These results are partly in agreement with the

Table 2. The effects of perlite and zeolite on growth performance of common carp fish grown over 56 days

	Control	Z 2.5	Z 5	P 2.5	P 5
Initial weight (g)	30.25±2.76	29.62±2.56	30.35±3.02	28.95±2.49	30.14±2.48
Final weight (g)	$58.01±4.16^{c}$	$61.62±8.16^{abc}$	$66.41±10.89^{a}$	$59.48±10.08^{bc}$	$64.51±9.54^{ab}$
WG	$27.52±2.10^{b}$	$32.00±4.60^{ab}$	$36.06±4.29^{a}$	$34.53±3.83^{ab}$	$34.37±4.24^{ab}$
WG (%)	$90.42±9.74^{b}$	$108.51±18.87^{ab}$	$118.74±12.59^{a}$	$105.71±15.70^{ab}$	$113.83±6.70^{ab}$
FCR[1]	$1.84±0.13^{b}$	$2.03±0.33^{ab}$	$1.77±0.19^{b}$	$2.52±0.31^{a}$	$2.24±0.27^{ab}$
FCE[2]	$54.16±3.81^{ab}$	$50.00±8.00^{abc}$	$56.66±6.42^{a}$	$40.00±5.00^{c}$	$44.99±5.77^{bc}$
SGR (%/day)[3]	$1.14±0.09^{b}$	$1.30±0.15^{ab}$	$1.40±0.09^{a}$	$1.28±0.13^{ab}$	$1.35±0.05^{ab}$
CF[4]	$1.60±0.05^{a}$	$1.64±0.09^{a}$	$1.66±0.03^{a}$	$1.69±0.09^{a}$	$1.66±0.04^{a}$

The initial mean body weight: 30 ±0.5 g. Values are means ±S.D. of triplicate and values within the same row with different superscript letters are significantly different ($P<0.05$).

[1] FCR = Feed conversion ratio (g dry feed consumed/g body weight gained) (Bailey and Aianärä, 2006).

[2] FCE= Feed conversion efficiency (g wet weight gain/g dry diet fed) (De silva and Anderson, 1995).

[3] SGR = Specific growth ratio (100[ln(final weight)×ln(initial weight)/day]) (López et la. 2006).

[4] CF= Condition factor ((weight×10^5)/length^3) (Tacon and Rodrigues, 1984).

findings reported by Lanari *et al* (1996), who found that additions of zeolite at 2.5 and 5% to rainbow trout diets improved weight gain and feed efficiency. Likewise, Yildirim *et al* (2009) indicated that fish fed on diets supplemented with zeolite at 1 and 2 % had higher WG, SGR, protein efficiency rate and FCR than those fed diet without zeolite.

However, Dias *et al* (1998) reported that feeding European sea bass with diets containing 10 and 20% natural zeolite as a bulk agent did not result in a remarkable effect on growth and feed utilization. Absence of any significant effect of dietary zeolite on growth performance has also been reported for coho salmon and by Edsall and Smith (1989), red tilapia by Rafiee and Saad (2005) and *Astacus leptodactylus* by Zamani Kyasajmahaleh *et al*. (2007).

Mumpton and Fishman (1977) noted that the growth enhancing effect of dietary zeolite may be related to the type, properties and their incorporation levels. The improved performance is likely associated with an improved nutrient utilization (Olver, 1989) and detoxifying effects of zeolite (Harvey *et al*. 1993). The better nutrient utilization can be explained by a slower passage of pre-digested food through the intestine, leading to the improved nutrients absorption, particularly nitrogen (Dias *et al*., 1998; Mumpton and Fishman, 1977). Ammonia is also considered to act as a cell toxicant in higher animals, and the prevention of its accumulation to toxic levels in the intestinal tract could lead to a reduction of epithelial turnover, a sparing of energy and a better nutrient utilization (Papaioannou *et al*., 2005). Ergün *et al*. (2008) reported that the addition of clinoptilolite (2.5 %) to the diet of young rainbow trout significantly improved ammonia excretion rate.

The results of the present study reveal that supplementing perlite to fish diet could bring some beneficial effects on growth. These results are in consistent with Glodek (1980), who stated that perlite used as a carrier in animal feed led to faster growth. Another possible explanation for the effect of perlite on body weight gain is due to its effects on nutrient absorption and as a carrier for aluminum silicate (Scheideler, 1993).

Dry Matter and Protein Digestibility

The effects of zeolite and perlite addition on dry matter and protein digestibility coefficients are shown in Table 3. Supplementing zeolite and perlite increased digestibility coefficients of dry matter and protein compared with the control. The highest apparent digestibility coefficients of dietary dry matter and protein were achieved in the fish fed diets containing 5 % zeolite and perlite which are not different from each other (Table 3). Dias *et al* (1998) observed that the dietary use of zeolite at 10 and 20% had no significant effect on apparent digestibility coefficient of dietary protein. Also, Lanari *et al* (1996) investigated the effect of dietary zeolite in rainbow trout, and found that dry matter and protein digestibility were not influenced by the incorporations at 2.5 and 5%.

Bones and Scales Ash Content

For carp, significant ($P<0.05$) changes in ash content of bones and scale were observed at the levels of 2.5 and 5% of either perlite or zeolite, compared to the control diet (Table 4). Similarly, Leach *et al*. (1990) reported that using zeolite in the diet, had beneficial effects on bone ash and strength. Zolite and perlite have some ions which can be absorb by scales and bones.

Blood Serum Properties

The mineral contents of serum from carps are shown in Table 5. Mg and P did not differ significantly between the dietary treatments ($P>0.05$).

Table 3. Effect of zeolite and perlite on the apparent digestibility coefficients of dry matter and protein

	Control	Z 2.5	Z 5	P 2.5	P 5
ADC of dry matter (%)[1]	80.58 ± 0.55^{d}	83.00 ± 0.89^{c}	87.48 ± 0.76^{a}	84.87 ± 0.71^{b}	87.92 ± 1.27^{a}
ADC of protein (%)[2]	90.59 ± 0.49^{d}	91.99 ± 0.28^{c}	95.14 ± 0.92^{a}	93.42 ± 0.64^{b}	95.80 ± 0.57^{a}

Values are means ±S.D. of three replicates and values within the same row with different superscript letters are significantly different ($P<0.05$).

[1]ADC of dry matter (apparent digestibility coefficient of dry matter) = 100- 100 (%Cr in feed/%Cr in faeces) (Tibbetts *et al*., 2006).

[2]ADC of protein (apparent digestibility coefficient of nutrient) = 100- 100 (%Cr in feed/%Cr in feces) (%nutrient in faeces/%nutrient in feed) (Tibbetts *et al*., 2006).

Table 4. The effect of zeolite and perlite on ash contents of bones and scales

	Control	Z 2.5	Z 5	P 2.5	P 5
Ash of bones (%)	12.30 ± 0.26^{e}	13.73 ± 0.22^{a}	13.48 ± 0.16^{b}	12.95 ± 0.58^{c}	12.36 ± 0.56^{d}
Ash of scales (%)	4.05 ± 0.46^{b}	4.25 ± 0.54^{a}	3.72 ± 0.18^{d}	3.60 ± 0.26^{e}	3.97 ± 0.58^{c}

Values are means ±S.D. of three replicates and values within the same row with different superscript letters are significantly different ($P<0.05$).

Table 5. Serum mineral content of fish fed on perlite and zeolite

	Control	Z 2.5	Z 5	P 2.5	P 5
Na (mg/l)	159.33±2.88^{a}	151.66±2.30^{a}	140.33±9.07^{b}	153.33±4.04^{a}	156.00±8.88^{a}
K (mg/l)	4.76±0.66^{bc}	4.61±0.16^{c}	5.70±0.41^{a}	5.79±0.3^{a}	5.39±0.3^{ab}
Fe (mg/l)	109.66±12.66^{ab}	131.66±10.78^{a}	127.00±11.53^{a}	80.33±13.01^{c}	101.33±11.93^{bc}
P (mg/l)	4.53±1.02^{a}	4.00±0.10^{a}	4.20±0.50^{a}	4.00±0.98^{a}	4.00±1.32^{a}
Ca (mg/l)	9.10±0.10^{d}	9.26±0.25^{d}	10.90±0.00^{a}	10.47±0.03^{b}	10.03±0.05^{c}
Mg (mg/l)	3.13±0.37^{a}	2.93±0.45^{a}	2.90±0.75^{a}	2.26±0.30^{a}	2.80±0.65^{a}

Values are means ±S.D. of three replicates and values within the same row with different superscript letters are significantly different (P<0.05).

Table 6. Serum protein, cholesterol and glucose content of fish fed on perlite and zeolite

	Control	Z 2.5	Z 5	P 2.5	P 5
Total protein (g/l)	2.76±0.66^{a}	3.20±0.20^{a}	3.13±0.05^{a}	3.20±0.20^{a}	2.93±0.20^{a}
Cholesterol (mg/dl)	188.33±22.03^{a}	169.66±27.15^{ab}	156.66±10.11^{ab}	131.33±34.93^{b}	162.66±22.03^{ab}
Glucose (mg/dl)	82.00±10.81^{b}	101.00±26.21^{b}	164.33±21.45^{a}	100.66±18.87^{b}	116.00±20.29^{b}

Values are means ±S.D. of three replicates and values within the same row with different superscript letters are significantly different (P<0.05).

Nevertheless, it was reported that the aluminum content of zeolite may from indigestible complexes with P, which could eventually reduce the availability of P to laying hens (Öztürk *et al*, 1998). Feeding low levels of dietary P is thought to reduce serum P that this study which was found in the present study, where a decrease In P serum was noticed among fishes fed on diets containing supplemental materials, however, the differences was insignificant. According to Table 5, Ca concentrations were no significantly different between control diet and Z 2.5. Ca cations in zeolite are exchangeable with other cations such as $NH4^{+,}$ Mg^{++}, Na^{+}, potassium (K^{+}). Beneficial effects may also be related to Al, Si or Na contents of zeolite because these minerals were shown to influence Ca metabolism (Öztürk *et al*., 1998). However, Shriatmadari (2008) reported that the decrease of P availability may be due to the increase of dietary Ca utilization in zeolite. However, Enemark *et al*. (2003) recorded initial slight decline in Ca concentrations in serum which was likely caused by decreased availability of Ca from the zeolite containing diet. The decrease in animal serum content of P and Mg was partly caused by interference of zeolite with intestinal absorption and partly by a marginal dietary supplementation of these minerals (Trckova *et al*., 2004).

Fish fed the Z 5 diet had the lowest Na value compared with the rest (P<0.05). Fish fed the control, Z 2.5 and P 5 diet had significantly lower serum K than those fed Z 5 and P 2.5. The serum Fe levels differed significantly between dietary treatments and the highest value was recorded in fish fed the control diet followed by Z2.5 and Z5. Although there is a lack of information relating to the effects of zeolites on serum of fish in the literature, a study in swine showed that plasma K, Na and Mg were unaffected by dietary zeolite additions (Pond and Yen, 1983). Martin-Kleiner *et al*. (2001) also did not find any alterations in the serum of experimental animals fed zeolite, except 20% increase in K level.

Zeolite and perlite supplementation had not significant effect on the serum total protein concentrations after 56 days of rearing (Table 6). Cholesterol content of P 2.5 and glucose content of Z 5 were significantly different from other treatments (P<0.05). Wherease, Kanyilmaz, 2012 reported that blood glucose decreased with increasing of the zeolite level in giltheah sea bream (*Sparus aurata*). The exact mechanism of the effects of zeolite and perlite on the serum biochemical properties of fish is presently unknown and we have no explanation for this response.

Conclusion

The results of the present work revealed that the incorporations of zeolite or perlite into the diets of common carp had positive effects on dry matter, protein apparent digestibility coefficients, growth performance. The optimum inclusion level of zeolite and perlite appears to be 5 %. This research needs to more study for different levels of zeolite and perlite and digestive enzymes in experimental fih

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