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Effects of Dietary Zeolites (Bentonite and Mordenite) on the Performance Juvenile Rainbow trout *Onchorhynchus myskis*

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Abstract: Zeolites are natural or synthetic crystalline aluminosilicates with ion exchanging properties. Zeolites have been increasingly used in various application areas such as industry, agriculture, environmental protection, and even medicine. Supplied as dietary additives to livestocks such as swine, ruminants and poultry, they improve weight gain, increase feed conversion ratios and promote animal health. The aim was to asses the effects of the natural zeolites, bentonite and mordenite on the performance and body composition of rainbow trout. During the feeding trial, quadruplicate groups of 15 rainbow trout (average initial weight ± SD, 104.2 ± 0.7) were grown in freshwater (salinity: 0; temperature: 14-16 ^{o}C) over 90 days. Fish were hand-fed, two times a day with diets containing 40% crude protein supplemented with 0 (control), 2.5, 5.0 and 10% bentonite or mordenite. Alpha cellulose replaced bentonite or mordenite in the control diet in order to keep the diet isonitrogenous and isoenergetic. There was a statistically significant differences ($P<0.05$) in percent weight gain, specific growth rate and feed efficiency for fish fed dietary bentonite at 5 and 10% and dietary mordenite at 2.5% compared to those fish on the control diet. Whole body composition of rainbow trout was not adversely affected by the addition of bentonite or mordenite to diets.

Key words: zeolites, bentonite, mordenite, rainbow trout, body composition

INTRODUCTION

Natural zeolites are a family of minerals of volcanic origin that are made of crystalline aluminosilicates with excellent ion exchanging properties. Zeolites have been increasingly used in various application areas such as industry, agriculture, environmental protection, and even medicine. Bentonite is a zeolitic material that is a mixture of minerals of the montmorillonite group (Bates, R.L. and Jackson, J.A., 1997). Mordenite is one of the rarer members of the zeolite group of minerals found in silica-rich rocks and altered volcanic ash beds (Tsxhernich, R.W., 1992). Of more than 40 naturally occurring zeolites, bentonite and mordenite were chosen because they are more abundant zeolites and the beneficial effects of natural zeolites have been reported to improve nutrient utilization, increased body weights and feed efficiencies (Kondo, N. and Wagai, 1968; Mumpton, F.A., 1999; Willis, W.L., *et al.,* 1982) in poultry, swine, and ruminants.

Bentonite and various bentonite clays have long been used in animal feeds as binding and lubricating agents in the pelleting process (Mumpton, F.A. and Fishman, P.H., 1977). In addition, bentonite has been used both as a dietary supplement in domestic animal feed to enhance feed utilization and improve growth (Fenn, P.D. and Leng, R.A., 1989; Fenn, P.D. and Leng, R.A., 1990; Ivan, M., *et al.,* 1992; Walz, L.S., *et al.,* 1998). Moreover, bentonite is used as a non-nutritive adsorbing material to bind a wide range of mycotoxins, thus reducing their toxic effects (Araba, M. and Wyatt, R.D. 1991; Kececi, T., *et al.,* 1998; Santurio, J.M., *et al.,* 1999; Scheideler, S.E., 1993; Winfree, R.A. and Allred, A., 1992). Zeomite mordenite ore reduced the toxicity of aflatoxin to growing chicks by 41%, as indicated by weight gains, liver weight, and serum biochemical measurements (Harvey, R.B., *et al.,* 1993). In aquaculture, there are contradictory results on the biological benefits of adding zeolites to fish feeds. Leornard (Leonard, D.W., 1979) added 2% clinoptilolite to trout diets and found a significant improvement in weight gain over a 64-day feeding period.

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There has been no apparent toxic effect reported in fish fed zeolite. Smith (Smith, R.R., 1980) reported that the addition of 10% sodium bentonite to a commercial trout feed increased the growth rate of rainbow trout *(Salmo gairdneri*) by 14% and reduced the amount of feed required per unit of weight gain by 20%. The use of natural zeolite (clinoptilolite) at 5 and 10% levels did not affect the growth of coho salmon (Edsall, D.A. and Smith, C.E., 1989). However, Reinitz (Reinitz, G., 1984) demonstrated that dietary inclusion of sodium bentonite, at 5, 10 and 15% adversely affected weight gain in rainbow trout. Our interest in bentonite grew out of previous preliminary study in this laboratory that showed a possible growth enhancing activity in rainbow trout fed diets containing low levels of supplemental bentonite (J. Eya, unpublished data).

The main objective of the presented study was to investigate the effects of zeolites (bentonite and mordenite) as feed supplement on the performance and body composition of juvenile rainbow trout.

MATERIALS AND METHODS

Fish and Facilities:

A feeding experiment was conducted in a laboratory at West Virginia State University. Juvenile rainbow trout (n=420, average initial weight ± SD, 104.2 ± 0.7 g) were obtained from a commercial source. The fish were randomly distributed to 28 tanks (15 fish per tank), and four tanks were randomly assigned to each treatment (four replicates/treatment). The glass tanks (152 L) were supplied with a continuous flow of dechlorinated city water at the rate of 3 L min $^{-1}$. Water temperature was thermostatically controlled at 14 – 16 oC and dissolved oxygen concentration ranged between 6.0 and 9.0 mg L $^{-1}$. Prior to the experiment, fish were acclimated to the experimental conditions for 14 days. During this period, the fish were hand-fed a commercial trout diet to satiation twice daily. A 12 hour light and 12 hour dark photoperiod was maintained in a temperature controlled room. Fish handling procedures and facilities met the guidelines of the Animal Care and Use Committee of West Virginia State University.

Diet and Feeding:

Seven semi-purified diets were formulated to be isonitrogenous (40% crude protein) and isoenergetic (17 MJ kg^{-1} Gross energy) and contained different levels of zeolite (0 (control), 2.5, 5.0 and 10% sodium bentonite or mordenite) (Table 1). Diets were adjusted by adding bentonite or mordenite at the expense of α -cellulose. The basal composition of the experimental diets was based on National Research Council NRS (1993). Mineral and vitamin mixes were formulated to equal or exceed the nutrient allowances recommended for rainbow trout by the National Research Council (National Research Council, 1993). All ingredients were thoroughly mixed and subsequently pelleted as semi-moist, 3 mm diameter pellets with a Hobart pellet mill and stored at –20 ^{o}C. The diets were thawed in a refrigerator (4 ^{o}C) six hours before feeding. Fish were hand-fed to satiation with the test diets twice daily for 90 days. Satiation feeding was achieved by allowing fish to eat until feeding activity stopped, with no feed remaining in the tank. Daily feed consumption was recorded. Fish in each aquarium were collectively weighed and counted biweekly. Before weighing, fish in each aquarium were anaesthetized with 3-aminobenzoic acid ethyl ester by transferring the fish to water containing the anesthetic (8 mg L^{-1}). When fish were removed for weighing, the aquaria were cleaned thoroughly and drained. No feeding was done on sampling days. On cleaning days, fish were fed only once in the afternoon. Fish were observed daily for unusual behavior, morphological changes and mortality. At the end of the 90 day feeding trial, fish in each aquarium were individually weighed and counted. The following indices were determined using the following formulas: Percent weight gain, PWG = ((final weight - initial weight)/initial weight) x100; Feed efficiency, FE = wet weight gain/dry feed fed; Average feed consumption, AFC = total feed consumed/number of fish; Specific growth rate, SGR = ((ln final weight - ln initial weight)/number of days) x 100). Five fish were randomly sampled from each tank for the determination of hepatosomatic index (HSI) and viscerosomatic index (VSI). The HSI and VSI were calculated by the following formulas: HSI = (liver weight/body weight) x 100 and VSI = ((liver + empty gastrointestinal tract + mesenteric fat)/body weight) x 100, respectively.

Whole Body Composition:

Body composition of the fish was measured at the conclusion of the experiment. Five fish were randomly removed from each tank, homogenized for the determination of the whole body proximate composition

Table 1: Ingredient composition of experimental diets (on as-fed basis)

	Zeolites (%)						
	Bentonite				Mordenite		
	0	2.5	5.0	10.0	2.5	5.0	10.0
Ingredient				(%)			
Egg white (81% CP)	49.46	49.46	49.46	49.46	49.46	49.46	49.46
Dextrin	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cod liver oil	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Vitamin premix[1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
P-free mineral premix[2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Ethoxyquin	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy methyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zeolite	0.00	2.50	5.00	10.00	2.50	5.00	10.00
Sodium Phosphate	4.27	4.27	4.27	4.27	4.27	4.27	4.27
α - cellulose	11.77	9.77	6.77	1.77	9.77	6.77	1.77
Crude Protein	40.06	40.20	40.15	40.13	40.18	40.45	40.25
Gross Energy (MJ kg^{-1})[3]	17.09	17.13	17.12	17.11	17.18	17.24	17.15

- [1]Contains g/kg of premix: Biotin, 1.00; Calcium panthothenate, 1.30; Choline chloride; 368; Folic acid, 0.70; Inositol, 17.00; Niacin, 10.00; Pyridoxine hydrochloride, 0.30; Riboflavin, 0.30; Ascorbic acid polyphosphate (15% ascorbic acid); 200.00; Thiamine hydrochloride, 1.30; Vitamin B12, 0.007; Vitamin K, 1.70; Vitamin D3 (400,000 IU/g), 0.25; Vitamin A acetate (500,000 IU/g), 0.44; Vitam in E,6.70;and á -cellulose, 391.003.
- [2]Contains g/kg of premix: $AlCl_3.6H_2O$, 0.41; $CaCO_3$, 37.50; $CaCl_2$. $2H_2O$, 239.58; $CoCl_3.6H_2O$, 0.33; $CuSO_4.5H_2O$, 2.00; $FeSO_4$. $7H_2O$, 88.00; KCl, 212.30; KI, 0.20; $MgSO_4.7H_2O$, 253.04; $MnSO_4$, 3.48; NaCl, 151.83; Na_2SeO_3, 0.04; $ZnSO_4$. $7H_2O$, 11.29
- [3]Gross Energy value was estimated by ascribing 39.5 KJ kg^{-1} fat, 23.6 KJ kg^{-1} protein, and 17.2 KJ kg^{-1} carbohydrate (Higgs, D.A., *et al.,* 1995).

following AOAC (Association of Official Analytical Chemist, 1990). Percent moisture was determined by drying to a constant weight in an oven at 80 ^oC. Ash content was determined using muffle furnace at 600 ^oC for 3 h. Total nitrogen was measured using a Leco TruSpec N Nitrogen Determinator (Leco Corporation, St. Joseph, Michigan), and protein calculated as N x 6.25. Fat was determined by Chloroform-Methanol extraction procedure

Statistical Analyses:

All experimental data were subjected to one-way analysis of variance using General Linear Model of Statistical Analysis Institute Inc. (SAS., 1990). Means were separated by Fisher 's protected LSD procedure. Differences were considered significant at the 0.05 probability.

RESULTS AND DISCUSSION

Results:

The administration of bentonite (5 and 10%) and mordenite (2.5%) in the diet of rainbow trout was positively reflected in the weight gain and feed efficiency. Rainbow trout receiving a diet containing 5.0 and 10% bentonite and 2.5% mordenite had statistically significant differences ($P<0.05$) in weight gain and feed efficiency compared to those fed 0% level (Table 2). In comparison to fish fed the control diet, bentonite incorporation significantly ($P<0.05$) affected SGR while mordenite at 2.5, 5 and 10% levels did not affect SGR, which ranged between 0.89 and1.12 g day^{-1}. No significant differences were found between the diet supplemented with bentonite or mordenite and the control when AFC and survival of either the zeolite supplemented or control fish were compared (Table 2). In terms of the whole body composition (Table 3), crude protein content was higher for the 2.5 and 5% bentonite treatment but differed only from the control, and mordenite treatment of 2.5 and 10%. The lipid content was the least for the 10% mordenite treatment but differed only from all the levels of mordenite treatment. The 10% bentonite treatment produced the highest whole body moisture content than the 2.5.and 5% mordenite treatment. The ash content of the 10% mordenite

Table 2: Performance of juvenile rainbow trout fed diets supplemented with different levels of bentonite and mordenite[1].

Component-%	PWG (%)	FE g gain g^{-1} dry feed	SGR g day^{-1}	AFC (g $fish^{-1}$)	Survival (%)
Cont-0	265[c]	0.78[cd]	1.06[cd]	408.3[a]	95.71[a]
Ben-2.5	302[bc]	0.82[bcd]	1.26[b]	441.5[a]	95.72[a]
Ben-5.0	369[a]	0.98[a]	1.36[a]	449.1[a]	93.55[a]
Ben-10.0	335[ab]	0.89[b]	1.27[b]	449.7[a]	92.14[a]
Mor-2.5	319[b]	0.87[b]	1.12[bc]	441.6[a]	90.75[a]
Mor-5.0	295[bc]	0.80[bcd]	1.03[cd]	438.7[a]	91.25[a]
Mor-10.0	261[c]	0.75[d]	0.89[d]	418.3[a]	89.89[a]
SEM[2]	14.08	0.03	0.06	24.35	2.94

[1]Values are means of four replicate tanks for each dietary treatment.
[a,b,c,d]Means in a column with different superscripts differs ($P<0.05$).
[2]Pooled standard error of mean values.

Table 3: Whole body composition (% wet weight basis) of juvenile rainbow trout fed diets containing different levels of dietary bentonite for 90 days[1].

Components- %	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Cont-0	72.49[ab]	15.43[b]	10.31[ab]	5.89[ab]
Ben-2.5	72.53[ab]	16.19[a]	10.16[ab]	6.10[ab]
Ben-5.0	72.76[ab]	16.19[a]	9.76[ab]	6.30[ab]
Ben-10.0	73.24[a]	16.03[ab]	8.68[b]	6.56[a]
Mor-2.5	71.06[b]	15.43[b]	11.07[a]	6.51[ab]
Mor-5.0	71.27[b]	15.78[ab]	11.08[a]	5.81[ab]
Mor-10.0	71.52[ab]	15.51[b]	11.13[a]	5.73[b]
SEM[2]	0.60	0.22	0.59	0.28

[1]Values are means of four replicate tanks for each dietary treatment. Proximate analyses were performed in triplicates for each treatment replicate.
[a,b,c]Means in a column with different superscripts differs ($P<0.05$).
[2]Pooled standard error of mean values.

Table 4: Visceral fat, hepatosomatic and viscerosomatic indices of juvenile rainbow trout fed diets containing different levels of bentonite for 90 days[1].

Component-%	Visceral fat (%)	Hepatosomatic index (%)	Viscerosomatic index (%)
Cont-0	3.72[bc]	1.60[a]	12.12[a]
Ben-2.5	4.69[a]	1.59[a]	11.78[a]
Ben-5.0	4.29[ab]	1.45[a]	11.17[a]
Ben-10.0	4.13[ab]	1.59[a]	11.62[a]
Mor-2.5	3.16[cd]	1.61[a]	11.51[a]
Mor-5.0	2.52[de]	1.64[a]	11.00[ab]
Mor-10.0	2.08[e]	1.10[b]	9.96[b]
SEM[2]	0.25	0.07	0.41

[1]Values are means of four replicate tanks for each dietary treatment.
[a,b,c]Means in a column with different superscripts differs ($P<0.05$).
[2]Pooled standard error of mean values.

treatment was less compared the 10 % bentonite treatment. The 10% mordenite treatment had the least visceral fat and hepatosomatic index (Table 4). The 10% mordenite treatment produced the least viscerosomatic index and significantly differed from all treatment groups except the 5% mordenite treatment. These data indicate that bentonite and mordenite can be fed to rainbow trout without adversely affecting performance and whole-body proximate composition.

Discussion:

The results indicate that dietary bentonite supplementation improved weight gain, feed efficiency, specific growth rate, and body protein deposition by 14-39, 19-28, 5-26, and 4-5%, respectively. The mordenite treatment improved weight gain, feed efficiency, specific growth rate, and body protein deposition by 11-20, 3-12, -2-6, and 0-2%, respectively. The dietary incorporation of 5% bentonite and 2.5% mordenite were the optimum level for maximum percent weight gain (39% for 5% bentonite; 20% for 2.5%mordenite) and specific growth rate (28% for 5% bentonite; 6% for 2.5% mordenite), and highest feed efficiency (26% for 5% bentonite; 12% for 2.5% mordenite) in juvenile rainbow trout. The improved performance parameters are in agreement with the findings made by Lanari *et al.*, (1996) that addition of zeolites (2.5 and 5%) to rainbow trout diets improved weight gain and feed efficiency. Similarly, Dias *et al.,* (1979) also reported that European sea bass, fed with diets which had 10 and 20% incorporation of natural zeolite as a bulk agent had no adverse effect on protein digestibility, growth and feed utilization. Our findings and those of the aforementioned authors are in contrast with Reinitz, (1984) who found that the dietary addition of sodium bentonite, at 5, 10 and 15% levels, reduced weight gain in rainbow trout or the observations of Edsall and Smith, (1989) of no significant effect on the growth rate of coho salmon fed 5 and 10% clinoptilolite. Some of the observed differences between bentonite and mordenite may be due to differences in geographic source, particle size and pretreatment as indicated by Willis *et al.*, (1982). Similarly, Mumpton and Fishman, (1977) observed that the effectiveness of zeolites in promoting growth may be related to the type of zeolite, its properties and the supplemental level used in the diets. The improved performance is likely to be associated with the improved utilization of nutrients (Olver, M.D., 1989) and/or detoxifying effects of zeolites (Harvey, R.B., *et al.,* 1993; Ortatatli, M. and Oguz, H., 2001; Parlat, S.S., *et al.,* 1999; Rizzi, L., *et al.,* 2003), which has been documented repeatedly. The efficacy can be explained by slower passage of pre-digested food through the intestine which leads to the improved utilization of nutrients from the feeding dose, particularly nitrogen (Dias, J., *et al.,* 1998; Meisinger, J.J., *et al.,* 2001; Mumpton, F.A. and Fishman, P.H., 1977). Survival of rainbow trout was not significantly affected by zeolite treatment. The maximum mortality of fish fed any diet supplemented with dietary bentonite or mordenite was 10%. Although no significant change in mortality due to zeolites was observed in the studies of (Willis, W.L., *et al.,* 1982) or in the present study, other studies have reported an improvement in this parameter for laying hens and swine (Olver, M.D., 1989; Torrii, K., 1978).

In terms of the whole-body composition, results show that, compared to the control fish, bentonite treated fish had decreased fat content and an increased protein content. The higher moisture content of fish fed diet with 10% bentonite corresponded with low fat content. The significant increase in whole body crude protein content with diets containing bentonite correlated with improved FE and suggests that bentonite contributed towards a more efficient conversion of feedstuff nitrogen to animal protein. In the present study, the different dietary bentonite and mordenite treatment tended to reduce hepatosomatic and viscerosomatic indices, however, the variability of values was not clearly attributable to any dietary changes. Also, slightly higher visceral fat content in fish fed dietary bentonite and lower values for mordenite treated fish was observed in this investigation. The exact mechanism of the effects of bentonite or mordenite on the visceral fat deposition is presently not known and we have no explanation for this response.

In conclusion, these findings suggest that incorporation of bentonite or mordenite into the diets of juvenile rainbow trout had no adverse effect on performance and whole-body composition. The beneficial effect of bentonite or mordenite was better characterized by measuring weight gain, specific growth rate, feed efficiency and whole-body protein, than by measuring whole-body moisture, ash, lipid, visceral fat, hepatosomatic and viscerosomatic indices. The inclusion of bentonite at 5% and mordenite at 2.5% were the optimum levels for maximum percent weight gain, specific growth rate and feed efficiency. However, additional studies will be necessary to clearly determine the mechanism responsible for the beneficial effect of these zeolites.

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