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Reproduction and Progeny Growth in Rats Fed Clinoptilolite in the Presence or Absence of Dietary Cadmium

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Zeolites are three-dimensional crystalline, hydrated aluminosilicates of alkali and alkaline earth cations with distinctive cation exchange capabilities (MUMPTON & FISHMAN 1977). They are abundant in sedimentary rocks enriched with volcanic materials (HAWKINS 1983). Clinoptilolite, one of more than thirty naturally occurring zeolites, is particularly of interest for application in agriculture and aquaculture because of its abundance, accessibility and properties. Extensive deposits of clinoptilolite exist in the western United States, Japan, Bulgaria and the Soviet Union (HAWKINS 1983). Clinoptilolite reduces ammonia concentration in the portal blood of rats given a toxic oral dose of ammonium carbonate (POND et al. 1981). The effect is associated with the ion-exchange and cation binding capacity of clinoptilolite. Toxic cations such as cadmium (Cd) (NATIONAL RESEARCH COUNCIL 1980) may be bound by clinoptilolite to the extent that their adverse effects on animals are reduced (POND & YEN 1983). A body of evidence from widely scattered laboratories (POND & MUMPTON 1983) suggests beneficial effects of dietary clinoptilolite on animal performance under some conditions. No data are available on the effects of long-term feeding of clinoptilolite on animal reproduction and subsequent progeny growth and development or on the influence of clinoptilolite on the toxic effects of Cd ingestion over extended periods of time.

The purposes of the experiment reported here were to determine the effects of long-term ingestion of clinoptilolite on reproduction in female rats and on the postnatal development of their progeny and to ascertain whether or not clinoptilolite offers protection against the toxic effects of long-term Cd ingestion.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing an average of 45 g were caged individually in wire-bottom stainless steel cages and fed *ad libitum* the four diets shown in Table 1. The clinoptilolite used was taken from a tuff at Buckhorn, NM (kindly provided by Richard B. Laudon). Analytical and characterization data have been reported (SHEPPARD & GUDE 1982). Body weight of each rat was recorded weekly from 6 to 13 weeks of age. At about 13 weeks, a young adult male was placed in each cage until mating (assessed by presence of sperm in daily vaginal smear). Pregnant females were continued on the same diets to which they had been assigned during growth, and body weights were recorded after parturition and at 1, 2 and 3 weeks postpartum. Feed intake was not recorded.

Table 1. Composition of Experimental Diets

Diet No.	1	2	3	4
Diet Designation	Basal	Clin[a]	Cd	Clin[a] + Cd
	%	%	%	%
Corn, ground	72.5	72.5	72.5	72.5
Corn starch	5.0	-	5.0	-
Soybean meal, 44% protein	18.6	18.6	18.6	18.6
Dicalcium phosphate	2.28	2.28	2.28	2.28
Limestone, ground	.48	.48	.48	.48
Iodized salt	.38	.38	.38	.38
Vitamin premix[b]	.38	.38	.38	.38
Trace mineral premix[c]	.38	.38	.38	.38
Clinoptilolite[d]	-	5.0	-	5.0
Cadmium chloride	-	-	+ [e]	+ [e]
Total, %	100.0	100.0	100.0	100.0

[a] Clin = clinoptilolite.

[b] Supplies the following (units/kg of diet): vitamin A, 5016IU; vitamin D_3, 669IU; vitamin E, 67IU; vitamin K, 3.34 mg; vitamin B_{12}, 25 mcg; riboflavin, 5.0 mg; niacin, 26.75 mg; d-pantothenic acid, 20.0 mg; choline 1045 mg (as choline chloride); biotin, 83 mcg; thiamin, 2.1 mg.

[c] Supplies the following (mg/kg of diet): Cu (as cupric oxide), 9.5; Fe (as ferrous sulfate heptahydrate), 152; Mn (as manganous oxide), 19; Zn (as zinc oxide), 95. Ground limestone used as carrier, 3%.

[d] Provided by Dr. Richard B. Laudon, Double Eagle Petroleum and Mining Co., Casper, Wyoming; clinoptilolite mixed at Castle Creek, ID, -50 mesh, 72% purity, 15.4% moisture, 12.2% Al_2O_3, 63.4% SiO_2; NH_4^+-exchange capacity, 1.88 milliequivalents of NH_4^+ per gram (other characteristics of the tuff are reported by Sheppard and Gude, 1982).

[e] Added in corn carrier to provide 100 ppm $CdCl_2$ (61.3 ppm Cd) in the diet.

Number of pups born per litter, live birth weight and average total litter birth weight were recorded for all litters. Number of live pups and total litter weight were recorded at 1, 2 and 3 weeks postnatally. At three weeks, pups were weaned and dams were euthanized with diethyl ether, and a blood sample was obtained from the abdominal aorta of the unconscious dam through a ventral incision for determination of hemoglobin (SANFORD & SHEARD 1929), hematocrit and plasma urea nitrogen (TALKE & SHUBERT 1965). Liver and kidneys from each dam were weighed fresh and after oven drying for 24 hr at 55 C.

Two sets of one male and one female pup from each litter were randomly selected at weaning (3 weeks old), caged two per cage and randomly assigned within set (cage) to either the basal diet or the diet fed to their dams during gestation and lactation. Individual body weights were recorded weekly for 4 weeks (7 weeks old) and feed consumption was recorded for each rat. Males were euthanized with diethyl ether four weeks postweaning. Blood was collected from the abdominal aorta for hematocrit, and liver, kidney and testes were removed and weighed. Females were continued on their respective diets from weaning (3 weeks old) to 16 weeks of age; individual body weight and feed consumption were recorded weekly. At 11 weeks, each group was reduced to 5 females. Females fed the basal diet and whose dams had been fed the basal diet supplemented with clinoptilolite were discarded, leaving 6 treatment groups.

Young adult fertile males were introduced into each remaining cage until mating had occurred. Pregnant females were continued on their respective diets during gestation and were weighed immediately after parturition and one day postpartum. Numbers of total and live pups per litter were recorded, and total weight of each litter at birth and at one day of age was determined. Dams were euthanized as described previously for dams of the first generation, blood hemoglobin and hematocrit were determined and liver and kidneys were weighed. Pups were pooled by litter, euthanized at one day old and pup dry matter and ash percentages were determined by freeze-drying and ashing for 16 hr at 500 C. Cd, Zn and Fe concentration of ash of pups from 3 or 4 litters per treatment group were determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Postweaning body weight gain and body weights at parturition and during 3 weeks of lactation, reproductive performance and organ weights of dams after 3 weeks of lactation and pup preweaning weight gains are summarized in Table 2. There was no effect of dietary cadmium level (0 or 61.3 ppm) on body weight gain during growth, gestation or lactation. A supplemental level of 5 percent clinoptilolite resulted in a reduced body weight during gestation (12 and 13 weeks old) compared to body weights of rats fed diets not containing clinoptilolite. However, body weight at parturition and at weeks 1, 2 and 3 postpartum was similar for rats in all diet groups.

Reproduction performance (number of pups per litter and litter birth weight) was unaffected by diet. After 3 weeks of lactation, dam hematocrit, hemoglobin, plasma urea N, liver and kidney weight (percent of body weight) and liver and kidney dry matter content were unaffected by diet (Table 2). Body weight was less ($P<.05$) at one week of age in offspring of dams fed clinoptilolite and Cd than in offspring of dams fed other diets; although the trend continued to weaning, the difference was not statistically significant at 2 and 3 weeks.

Table 2. Effect of Clinoptilolite on the Growth and Reproduction of Female Rats Fed an All-Plant Diet With or Without Cadmium - Generation 1

	Basal	Clin[a]	Cd	Clin[a] + Cd	SD	Prob
	N=7	N=9	N=9	N=8		
Weight at 6 wks, g	139	137	144	136	10.0	NS
9 wks, g	190	179	192	184	11.9	NS
12 wks, g	211	194	210	201	12.3	<.05
13 wks, g	230	211	226	215	11.8	<.01
Wt at parturition, g	269	247	255	242	20.4	NS
Wt at 3 wk postpartum, g	239	227	235	230	27.9	NS
No. pups/litter at birth	11.3	10.2	12.0	10.3	2.53	NS
Live pups/litter at birth	11.1	10.2	11.6	10.3	2.53	NS
Live pups/litter 3 wks	10.4	10.2	10.9	9.9	2.13	NS
Total litter birth wt, g	72.4	66.8	72.6	63.8	14.0	NS
Live litter birth wt, g	71.6	66.8	69.8	67.5	15.2	NS
Live litter wt 3 wks, g	373.0	361.0	349.0	316.0	47.9	NS
Dam at 3 wk postpartum						
Hematocrit	45.5	46.1	44.1	44.1	3.69	NS
Hemoglobin, g/dl	14.1	14.3	13.2	14.1	1.40	NS
Plasma urea nitrogen, ng/dl	22.8	22.1	23.7	27.7	6.19	NS
Liver wt, g	9.36	9.01	9.24	8.50	1.58	NS
Dry liver wt, g	2.70	2.58	2.50	2.38	.41	NS
% DM	28.85	28.57	28.15	28.09	.94	NS
Liver wt, % of B.W.	4.12	3.95	3.90	3.70	.37	NS
Kidney wt, g	1.29	1.31	1.39	1.36	.13	NS
Dry kidney wt, g	.31	.31	.33	.32	.03	NS
% DM	24.06	23.69	23.81	23.49	.71	NS
Kidney wt, % of B.W.	.57	.58	.59	.60	.04	NS

[a] Clin = clinoptilolite.

Body weight of all female pups at 14 weeks was 223.6±24.2 g, that of pups fed diets 3 and 4 was 230.8 and 220.6 g, respectively. Male pups weighed 139.3±19.0 g at 7 weeks; males fed diets 3 and 4 weighed 125.3 and 128.5 g, respectively. The difference among means was significant (P<.05). Daily feed consumption of females from 3 to 14 weeks old was unaffected by diet (11.6±1.5 g). Kidney and testes weights (percent of body wt.) were unaffected by diet (.68±.04 and 1.20±.10%, respectively). Males fed diet 3 or from dams fed diet 3 (Cd) during gestation had larger livers (percent of body wt.) than those fed other diets (4.65 vs 4.50±.25%, P<.05). Hematocrit at 7 weeks of age was less in males fed Cd (diets 3 and 4) than in other males (39.4 vs 40.9±2.0, P<.05). There appeared t be no important carryover effect of dam gestation diet on body weight gain, feed consumption, organ weights or hematocrit of pups postweaning.

Body weight at parturition was less ($P<.05$) in dams fed diet 3 or 4 than in dams fed other diets (240.8, 245.3 and 252.3±16.2 g for dams fed diets 3, 4 and all other diets, respectively). Body weight one day postpartum, liver and kidney weights and pup birth weight, dry matter and ash content of female offspring of the F_2 generation of rats fed the basal diet or Cd and clinoptilolite separately and in combination were unaffected by diet. Mean ± standard deviation for each of these traits was: body wt one day postpartum 246.7±16.8 g; liver wt 3.88±0.33 percent of body wt; kidney wt 0.50±.03 percent of body wt; pup birth wt 6.4±0.6 g; pup dry matter 19.9±3.1 percent; pup ash content 2.21±0.32 percent. Although live pup birth weight was unaffected by maternal diet, mean one-day weight of pups from dams fed Cd (diets 3 and 4) was significantly less ($P<.05$) than that of pups from dams fed other diets, suggesting an adverse effect of Cd on early lactation (7.0, 6.1 and 7.3±0.8 g for pups from dams fed diets 3, 4 and all other diets, respectively). Clinoptilolite alone had no effect on pup weight and, when fed in combination with Cd, failed to improve body weight above that of pups from dams fed Cd alone. Body weight expressed in g dry matter was less in pups from dams fed Cd and clinoptilolite together (diet 4) than in pups from dams in all other groups (1.0 for pups from dams fed diet 4 compared with 1.43±.39 g for all other pups, $P<.05$). The biological significance of this difference is unclear. The ash content of the bodies of pups one day of age was unaffected by maternal diet. Hematocrit at one day postpartum was less ($P<.05$) in dams fed Cd (diets 3 and 4) than in other dams (32.1 compared with 34.2±2.8 in all other dams). The Cd content of pup body ash was below the detection limit (<1 ppm) in all diet groups and Fe and Zn concentrations were unaffected by dam gestation diets (Fe concentration was 500, 380, 530, 290, 390 and 300 ppm for pooled pups from 3, 4, 4, 3, 3 and 3 litters from treatment groups 1 through 6, respectively; Zn concentration was 679, 755, 812, 724, 728 and 846 ppm, respectively, for the same ash samples). It appears, therefore, that placental transfer of Fe and Zn was unaffected by maternal diet and that a level of 61 ppm Cd in the gestation diet was too low to result in measurable Cd transfer to the fetus. There was no effect of diet on dam liver or kidney weight (g or percent of body weight), but dam hematocrit one day postpartum was reduced ($P<.06$) by dietary addition of Cd, both in the presence and absence of clinoptilolite (diets 3 and 4). Dams whose dams were fed Cd throughout life but who themselves were not fed Cd did not differ significantly, in any of the traits measured, from counterparts whose dams were not fed Cd.

The data reported here are, to the authors' knowledge, the first to show that long-term ingestion of a natural zeolite, clinoptilolite, has no apparent deleterious effects on growth or reproduction of animals. There was no evidence of toxicity nor of teratogenicity of clinoptilolite addition to the diet at a level of 5.0% by weight, continuously during the growing period of female rats and throughout pregnancy and lactation. Furthermore, their progeny grew normally and had normal reproduction. The safety of clinoptilolite as a feed additive in animal production must be established, apart from the efficacy of its use as a growth promotant or as an agent to control diarrhea. The results of this experiment provide no evidence for problems in animal health associated with long-term use of clinoptilolite in normal diets of simple stomached animals.

Previous work (POND & YEN 1983) has shown that clinoptilolite offers some protection in growing animals against the Fe-deficiency anemia induced by dietary Cd (POND et al. 1973; RADI & POND 1979), a known toxic element for animals (WILSON et al. 1941; NRC, 1980). In adult rats in the present study a level of 61 ppm of Cd in the diet during growth, gestation and lactation was not associated with a significantly reduced hemoglobin after 3 weeks of lactation, although the mean for rats fed Cd in the absence of clinoptilolite was less than that for rats fed other diets. Male progeny of these dams had lower hematocrits at 7 weeks of age when continued on their dam's high Cd diet from weaning at 3 weeks of age to 7 weeks of age, but those transferred at weaning from their dam's high Cd to the basal diet containing no added Cd had normal hematocrit at 7 weeks of age. Female littermate progeny continued through growth and pregnancy on their dam's high Cd diets also showed reduced hematocrit one day after their young were born. Clinoptilolite in the diet in the presence of Cd appeared not to protect the progeny against Cd-induced anemia either in males killed at 7 weeks or in females killed at maturity, unlike the protection observed in pigs (POND & YEN 1983). The failure of clinoptilolite to protect against Cd-induced anemia in rats in the present experiment may be related to the longer relative portion of the life cycle during which Cd was ingested, allowing tissue Fe stores to be depleted more severely.

The smaller pup weight of progeny of second generation females fed Cd and clinoptilolite together than that of progeny of females fed other diets suggests the possibility of an adverse synergism betwee these two dietary additives. The absence of detectable Cd in pups from dams fed diets either containing or not containing added Cd an the failure of dam diet to affect pup ash Fe or Zn concentration suggests that none of these three elements were involved in the reduced dry body weight obtained in one-day-old progeny of females fed Cd and clinoptilolite together. The failure of a level of 61 ppm Cd in the gestation diet to increase measurably the Cd content or decrease the Zn and Fe content of the newborn progeny suggests that the placental barrier is effective in protecting the fetus fro Cd uptake. Cd does, however, cross the placenta of the rat fed higher levels of Cd (200 ppm) in the presence of dietary Ca insufficiency (POND & WALKER 1975). The higher ratio of dietary Ca to C used in the present experiment may have protected against placental transfer of Cd.

It is concluded that clinoptilolite at 5% in the diet of the rat throughout the postweaning portion of the life cycle and through on reproduction and lactation period is not associated with observable toxicologic or teratogenic effects. The protective effect of clino tilolite against the toxic effects of long-term continuous ingestio of Cd is inconsistent and needs further quantification.

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