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Protection by Clinoptilolite or Zeolite NaA against Cadmium-Induced Anemia in Growing Swine (41652)

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Abstract. Weanling Landrace × Yorkshire swine were fed a basal diet or a diet containing 3% clinoptilolite (a natural zeolite) with or without 150 ppm $CdCl_2$ or 3% zeolite NaA (a synthetic zeolite) with or without 150 ppm $CdCl_2$ for 31 days. Hematocrit and hemoglobin were depressed significantly in animls fed Cd in the absence of zeolites, but not in their presence. Liver Cd concentration was increased dramatically by added dietary Cd but was significantly lower in animals fed clinoptilolite with Cd than in those fed Cd alone (11.4 vs 16.5 ppm). Liver Fe and Zn were decreased by dietary Cd; liver Fe was not affected significantly by clinoptilolite or zeolite NaA, but liver Zn was increased by zeolite NaA. Kidney dry matter, Zn, and Cd concentrations were increased by dietary Cd; neither clinoptilolite nor zeolite NaA affected kidney Cd concentration. Zeolite NaA increased kidney dry matter both in the presence and in the absence of dietary Cd. Plasma urea-N, K, Na, and Mg were unaffected by Cd or by either zeolite. The data illustrate the different effects of dietary clinoptilolite compared with zeolite NaA on blood plasma, liver, and kidney concentrations of minerals and provide evidence that both zeolites offer some protection against Cd-induced Fe-deficiency anemia; the magnitude of this protection and the effects of each zeolite on tissue concentrations of Cd and other materials need further quantification.

Natural and synthetic zeolites have cationic binding properties of potential value in protecting animals and humans from tissue accumulation of toxic minerals. One such element is cadmium (Cd), whose innate toxicity is well documented (1–3). Clinoptilolite, a naturally occurring zeolite, has the capacity to bind ammonia in the gastrointestinal tract (4); although its Cd-binding capacity has not been quantified, its selectivity for other divalent cations (Ca > Fe > Mg) (5) suggests the possibility of biologically important Cd selectivity. Zeolite NaA (a synthetic supplied by PQ Corporation, Lafayette Hill, Pa.), has a high ion-exchange capacity for Cd (6). The purpose of the present experiment was to determine whether the presence of a natural zeolite, clinoptilolite, or a synthetic zeolite, zeolite NaA, in the diet protects the animal against the effects of dietary Cd.

Materials and Methods. Weanling Landrace × Yorkshire pigs were assigned randomly at 4 to 5 weeks of age to six dietary treatments as follows: Basal (B), B + 3% clinoptilolite (C), B + 3% zeolite NaA (A), B + 150 ppm $CdCl_2$ (Cd), B + Cd + C, and B + Cd + A. The basal diet contained by analysis, 0.94 ppm Cd, 0.82% Ca, 0.80% P, and 17.2% protein supplied by the following ingredients: corn (68.3%), soybean meal (25%), ground limestone (0.5%), dicalcium phosphate (2.0%), iodized salt (0.4%), trace mineral premix (0.4%), complete vitamin premix (0.4%), and dextrose (3.0%). Clinoptilolite and zeolite NaA were added to diets 2 and 5 and to 3 and 6, respectively, at the expense of dextrose. Cadmium chloride was added to diets 4, 5, and 6, at 150 ppm to supply 92 ppm of Cd. The trace mineral premix supplied the following (milligrams per kilogram mixed diet): Cu (as CuO), 10; Fe (as $FeSO_4 \cdot 7H_2O$), 160; Mn (as MnO), 20; Zn (as ZnO), 100. Clinoptilolite was mined from Castle Creek, Idaho (supplied by R. B. Laudon, Double Eagle Petroleum and Mining Company, Casper, Wyoming) and had the following characteristics: particle size, minus-50 mesh; purity 66%, Ca ion-exchange capacity < 0.6 meq/g; NH_4 ion-exchange capacity 0.78 meq/g; water 15.4%, Al 4.5%, Si 26.5%, Na 1.44%, K 0.78%, Ca 2.33%, Mg 1.25%, Fe 1.37%, Cu 7 ppm, Ni 15 ppm, Mn 310 ppm. Zeolite NaA (supplied by H. S. Sherry, PQ Corporation, Lafayette Hill, Pa.) contained 10.65% Na, 15.35% Si, 15.30% Al, and 21.8% water (6). Pigs were penned in groups of three in two replicate

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pens per diet (2 pens × 3 pigs × 6 diets = 36 pigs) and given feed and water *ad libitum* for 31 days. Blood was drawn from the anterior vena cava of each pig at Days 14 and 28 for determination of hematocrit and hemoglobin and at Day 28 for plasma Ca, P, urea-N (clinical analyzer), K, Na, and Mg (atomic absorption spectrophotometry). On Day 31, all pigs were killed by bolt-gun stunning and exsanguination and liver and kidney were removed, weighed, and dried. The liver and kidney were ashed and analyzed by atomic absorption spectrophotometry for Cd, Zn, Ca, Mg, Fe, Mn, and Cu. Data were treated by analysis of variance and Duncan's Multiple Range Test.

Results. Daily body weight gain, gain/feed, and plasma urea-N, K, Na, and Mg concentrations were unaffected by diet (mean ± standard deviation for each trait was, respectively, 405 ± 104 g; 0.545 ± 0.143 g/g; 13.6 ± 2.8 mg/dl; 25.5 ± 27 mg/dl; 244 ± 8 mg/dl; 1.64 ± 0.11 mg/dl). Effects of diet on liver and kidney weight and dry matter content and on concentrations of minerals in tissue dry matter are summarized in Tables I and II, respectively.

Data on hematocrit and hemoglobin at 28 days are summarized in Fig. 1. Hematocrit and hemoglobin were not affected by diet at 14 days but at 28 days were reduced by dietary Cd ($P < 0.01$) (Fig. 1); the effect was partially ameliorated by clinoptilolite or zeolite NaA. Plasma Ca at 14 and 28 days was depressed ($P < 0.05$) by Cd and was elevated slightly by zeolite NaA in the presence or absence of added Cd ($P < 0.05$); plasma P was depressed by zeolite NaA ($P < 0.01$) but was not affected by clinoptilolite or by the addition of Cd to the diet. Plasma urea-N was not affected by diet nor were plasma K, Na, and Mg concentrations.

Liver Cd concentration was increased dramatically ($P < 0.01$) by dietary Cd; livers of pigs fed clinoptilolite and Cd had lower Cd concentrations than those of pigs not fed clinoptilolite with Cd ($P < 0.01$). Zeolite NaA had no protective effect against liver Cd uptake. Zn concentration was decreased by Cd ($P < 0.01$) and was increased by zeolite NaA ($P < 0.01$) but not by clinoptilolite. Concentrations of Ca, Mg, and Mn were not significantly affected by diet, although there was a trend for livers of pigs fed Cd to contain lower levels of each of these three minerals. Liver Fe was reduced ($P < 0.01$) by dietary Cd, but was unaffected by clinoptilolite or zeolite NaA, except for a tendency for zeolite NaA to depress liver Fe.

Kidney dry matter was increased by Cd ($P < 0.05$); expressed as a percentage of live body weight it was increased ($P < 0.01$) by zeolite NaA in the presence or absence of added Cd (0.120 for pigs fed zeolite NaA compared with 0.103 for those not fed zeolite NaA). Cd, Zn, Fe, Cu, and K concentrations of kidney dry matter were significantly affected by diet. Cd concentration was increased dramatically by

TABLE I. EFFECT OF DIETARY CLINOPTILOLITE (C) AND ZEOLITE NaA(A) ON CONCENTRATIONS OF Cd, Zn, Fe, Mn, Cu, Ca, AND Mg IN LIVER DRY MATTER OF GROWING SWINE[a]

Diet	Basal (B)	B + C	B + A	B + Cd	B + Cd + C	B + Cd + A	SD	Prob.
Liver wt, % of body wt	2.55	2.71	2.74	2.92	2.65	2.60	0.34	NS
Liver dry matter, %	28.8	29.8	28.3	28.7	28.2	29.6	1.76	NS
Ca, ppm	5.1	5.1	4.7	4.3	4.3	3.8	2.5	NS
Mg, ppm	637	654	645	610	650	615	44	NS
Cd, ppm	0.10[b]	0.05[b]	0.21[b]	16.5[c]	11.4[d]	15.5[c]	6.3	<0.01
Zn, ppm	163[b]	156[b]	270[c]	122[b]	122[b]	188[c]	48	<0.01
Fe, ppm	495[b]	418[b]	332[b]	46[c]	47[c]	40[c]	125	<0.01
Mn, ppm	5.1	6.1	6.2	5.1	4.9	4.3	3.1	NS
Cu, ppm	11.2	14.4	13.2	9.7	9.8	8.4	8.1	NS

[a] N = 6 animals/diet; animals killed at Day 31.

[b,c,d] Means in the same line with different superscripts differ significantly.

TABLE II. EFFECT OF DIETARY CLINOPTILOLITE(C) AND ZEOLITE NaA(A) ON CONCENTRATIONS OF ASH, Cd, Zn, Fe, Mn, Cu, Ca, Mg, P, K, AND Na IN KIDNEY DRY MATTER OF GROWING SWINE[a]

Diet	Basal (B)	B + C	B + A	B + Cd	B + Cd + C	B + Cd + A	SD	Prob.
Kidney wt, % of body wt	0.54	0.52	0.61	0.53	0.52	0.57	0.06	NS
Kidney dry matter, %	18.2[b]	19.0[b]	19.9[b]	20.5[c]	20.1[c]	20.7[c]	1.32	<0.05
Kidney dry matter, % body wt	0.098[b]	0.101[b]	0.121[c]	0.108[b]	0.104[b]	0.118[c]	0.008	<0.01
Kidney ash, %	6.76	6.80	6.63	6.70	6.60	6.70	0.17	NS
Cd, ppm[d]	N.D.[b]	N.D.[b]	N.D.[b]	195[c]	166[c]	228[c]	111	<0.01
Zn, ppm	106[b]	99[b]	93[b]	134[c]	140[c]	164[c]	31	<0.01
Fe, ppm	34[b]	30[b]	39[b]	24[c]	31[b]	25[c]	8	<0.01
Mn, ppm	1.7	1.5	1.4	1.5	1.5	1.4	0.2	NS
Cu, ppm	11.7[b]	11.5[b]	7.6[b]	18.8[c]	17.8[c]	16.3[c]	5.6	<0.01
Ca, ppm	4.7	4.0	4.7	3.3	4.3	4.3	1.1	NS
Mg, ppm	545	549	512	584	503	540	90	NS
P, %	13.5	13.5	13.1	13.5	13.1	13.3	0.6	NS
K, %	1.74[b]	1.82[b]	1.78[b]	1.46[c]	1.75[b]	1.69[b]	0.19	<0.05
Na, %	0.69	0.66	0.70	0.60	0.65	0.62	0.11	NS

[a] $N = 6$ animals/diet; animals killed at Day 31.
[b,c] Means in the same line with different superscripts differ significantly.
[d] Not detected (less than 1 ppm).

the addition of Cd to the diet as expected, but neither clinoptilolite nor zeolite A had an ameliorating effect on tissue uptake. Kidney Zn concentration was increased ($P < 0.01$) by dietary Cd, in contrast to the decrease observed in liver as a result of Cd feeding; there was no effect of either clinoptilolite or zeolite NaA on kidney Zn. Kidney Fe was reduced ($P < 0.01$) and Cu increased ($P < 0.01$) by dietary Cd; neither zeolite source affected Cu concentration. Although kidney Ca, P, Mg, Mn, and Na concentrations were not affected by diet, K concentration was less ($P < 0.05$) in kidneys of pigs fed the diet containing added Cd; no biological explanation is apparent.

Discussion. The depressed hematocrit and hemoglobin of pigs fed Cd was expected, as an early sign of Cd toxicity is Fe-deficiency anemia (7–10) induced by interference with Fe absorption from the gastrointestinal tract (12). Clinoptilolite and zeolite NaA each protected the animal from Fe-deficiency anemia after 28 days of feeding diets containing Cd, indicating that some of the dietary Cd may have been adsorbed to the zeolite matrix in accord with the known ion-binding capacity of clinoptilolite and the specific affinity of zeolite Na for Cd (7). The extent of Cd-binding apparently was greater for clinoptilolite than for zeolite NaA, since the dramatic increase in liver concentration of Cd in animals fed Cd was partially ameliorated by clinoptilolite but not by zeolite NaA (16.5, 11.4, and 15.5 ppm Cd in liver dry matter in pigs fed Cd, Cd + clinoptilolite, and Cd + zeolite NaA, respectively). Mumpton (13) pointed out the greater stability at pH < 2 of clinoptilolite than of zeolite NaA. The degree of loss of the integrity of the crystalline structure of zeolite NaA during transit through the acid (pH < 2) environment of the pig stomach (14) has not been measured, but its protection from Cd-induced anemia implies maintenance of appreciable integrity of the crystal structure, and/or Cd binding capacity, into the duodenum where most Fe absorption occurs (15, 16). Absorption of Fe by pigs fed clinoptilolite or zeolite NaA in the absence of added dietary Cd was not improved as measured by liver Fe concentration.

The decreased liver Zn in the presence of added dietary Cd is not in agreement with the observation of increased Zn concentrations in newborn rat pups from dams fed Cd compared with those of pups from control dams (17), but is in agreement with the response of

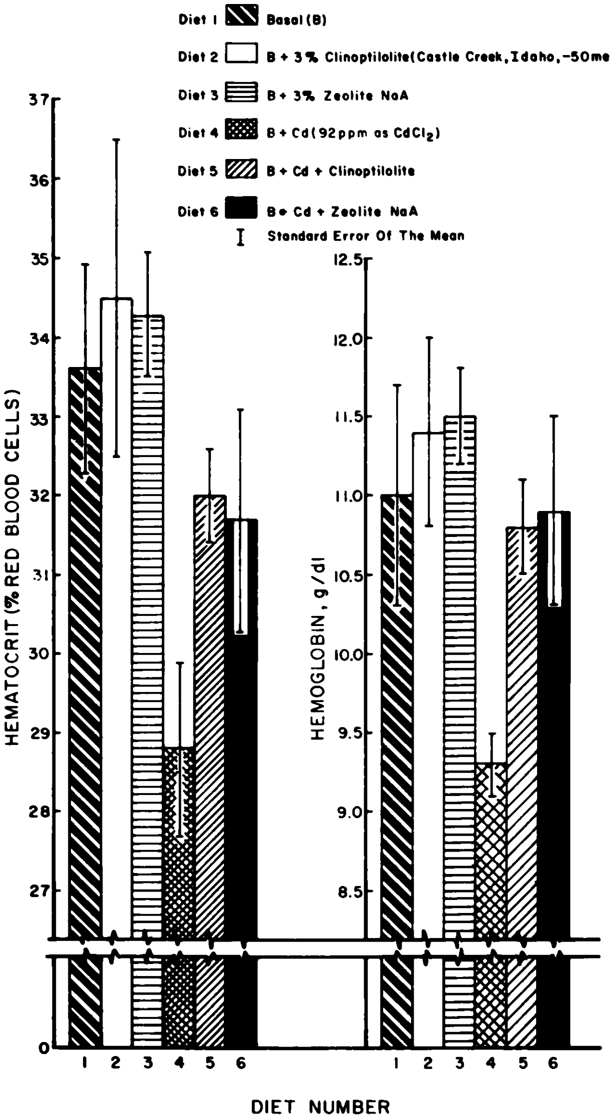

FIG. 1. Effect of dietary clinoptilolite and zeolite NaA in the presence or absence of dietary Cd on hematocrit and hemoglobin of weanling pigs after 28 days.

the liver of swine fed Cd (8); the increased Zn and Cu concentrations in kidneys of animals fed Cd may be related to increased excretion of Zn and Cu in Cd-fed animals. Urinary excretion of trace elements was not measured in this experiment. Cd and Zn compete for the protein-binding sites of metallothionein (1). Thus, mutual antagonism, together with important interactions between Cd, Fe, and Cu (18, 19), complicate the interpretation of the observed effect of zeolite NaA on liver and kidney Zn and of clinoptilolite on liver Cd concentration. The increased liver Zn concentrations in pigs fed zeolite NaA either in the presence or absence of added Cd indicates an improvement in bioavailability of dietary Zn induced by zeolite NaA. The mechanism of such an action is unknown, but is possibly due to selective Zn binding and subsequent release by zeolite NaA at the intestinal site most conducive to absorption, presumably the duodenum (1). The tendency for reduced liver Cu concentration of Cd-fed animals was expected (18).

The failure of clinoptilolite or of zeolite NaA to affect plasma concentrations of urea-N, K, Na, or Mg suggests no major shift in ion-exchange capacity due to zeolites. The increased plasma Ca and decreased plasma inorganic P in pigs fed zeolite NaA in the presence or absence of added Cd indicates an important biological effect unrelated to Cd binding. The reduced plasma P may have been a result of reduced P absorption due to formation of an insoluble aluminum–phosphorus complex, since partial destruction of the crystalline matrix of zeolite NaA during passage through the acid environment of the stomach would release Al. Levels of 500 ppm of Al in chick diets depressed weight gain and 355 ppm in mouse diets depressed P retention (20); release of 7 to 11% of the Al in zeolite NaA in the gastrointestinal tract of animals fed 3% zeolite A in the diet could result in an amount of free Al ion similar to that shown to affect animal performance adversely. The greater stability of clinoptilolite than of zeolite NaA at $pH < 2$ should yield less free Al ions for binding with P; the observed difference between clinoptilolite and zeolite NaA in plasma P response fits such a relationship. The rise in plasma Ca associated with the decline in plasma P may have resulted from bone resorption in response to hypophosphatemia. Bone tissue was not saved at slaughter for histopathology or chemical analysis. The higher dry matter content of kidneys from pigs fed zeolite NaA than that of kidneys from other pigs suggests the possibility of tissue damage.

The biological effects of feeding clinoptilolite and zeolite NaA to animals must be considered in terms of a complex and dynamic ion-exchange system operating in an environment of variable pH and cation concentrations and with differing orders of ion exchange preferences among cations present. A more complete knowledge of the effects of specific natural and synthetic zeolites is needed to interpret adequately the observed effects on plasma and tissue concentrations of Cd, Zn, Fe, Cu, Ca, and P. Our data illustrate the different effects of 3% of clinoptilolite compared with zeolite NaA in the diet on blood plasma and liver concentrations of the above elements. It is concluded that both zeolites offer some protection against Cd-induced Fe-deficiency anemia; the amount of each zeolite required for this protection and the amounts of each zeolite needed to minimize tissue concentrations of Cd are unclear.

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