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Tissue Mineral Element Content in Swine Fed Clinoptilolite

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Clinoptilolite is one of more than 30 naturally occurring zeolites present in extensive deposits of sedimentary rocks enriched with volcanic ash (Hawkins 1984). Clinoptilolite is of particular interest for application in agriculture and aquaculture because of its abundance, accessibility, and high cation exchange capacity. Considerable evidence is available suggesting a beneficial effect of the addition of clinoptilolite to the diet on growth of animals under some conditions (Mumpton and Fishman 1977; Pond and Mumpton 1984; Pond 1985; Pond et al. 1984; Pond and Yen 1987; Pond et al. 1985), in protecting against ammonia toxicity by binding ammonia (Pond 1984; Shurson et al. 1984; Varel et al. 1987) and in reducing cadmium-induced anemia (Pond and Yen 1983a). Although there has been no reported toxicity associated with clinoptilolite added to animal diets, its safety as a feed additive must be established firmly to warrant its widespread use as a growth promotant or protective agent against toxic elements in the environment. However, in a long term experiment with rats, Pond and Yen (1983b) observed no adverse effects at any stage of the life-cycle from feeding 5% clinoptilolite. Tissue concentrations of mineral elements were not measured in that report.

It is generally considered that clinoptilolite is stable at physiological pH and at the acidic pH (<2) of the stomach. Concentrations of liver and kidney ash, Cd, Zn, Fe, Mn, Cu, Ca, M, P, K, and N were unchanged in growing pigs fed a diet containing 3% when compared to zero percent clinoptilolite (Pond and Yen 1983b). The absence of a change in tissue Al concentration in animals fed clinoptilolite is of particular importance, because it provides evidence for high stability of clinoptilolite during its transit through the gastrointestinal tract. If a significant fraction of clinoptilolite were degraded during transit, some of the Al released from the aluminosilicate crystalline structure of the zeolite would be expected to appear in animal tissues after absorption of Al from the intestinal lumen. When one considers the cation exchange capacity of clinoptilolite and the complex physiological processes involved

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in digestion and absorption of ingested diet constituents, it is clear that more complete information is needed concerning the behavior of clinoptilolite in biological systems and its effect on total mineral element movement between the gastrointestinal lumen, body tissues, and excretory pathways.

The purposes of the experiment reported presently were 1) to test the hypothesis that tissue storage of major and trace mineral elements is altered by clinoptilolite addition to diets differing in concentrations of iron and calcium, and 2) to relate any observed shifts in mineral element concentrations to indices of edible tissue quality and composition.

MATERIALS AND METHODS

Thirty-two castrated male four-way cross (Chester White x Landrace x Large White x Yorkshire) growing pigs were assigned randomly at about 10 weeks of age (29 kg body wt) to eight dietary treatments arranged as a 2 x 2 x 2 factorial. The treatments were .5 or 1.1% Ca, 640 or 940 ppm Fe, and 0 or 2% clinoptilolite (Table 1). The diets were fed ad libitum for 12 wk. Pigs were kept in an environmentally controlled building (12 hr light, 12 hr dark cycle, 22 $\pm$ 2°C) in individual slotted-floor pens equipped with a wooden self feeder and an automatic watering nipple. Body weights were recorded at 0, 4, 8, and 12 wk. At d 84, all pigs were killed by electrical stunning and exsanguination and carcass measurements, including length (anterior edge of first rib to pubic bone), chilled carcass weight, weights of trimmed lean cuts (Boston butt, picnic, loin, ham), cross-sectional area of the longissimus muscle at the 10th-11th rib interface, and cross-sectional area of the subcutaneous fat covering the longissimus muscle at the 10th-11th rib interface. The color of the cut chilled surface of the longissimus muscle at the 10th rib was measured by the Hunterlab Spectrophotometer (Model D54 P-5; Hunter Associates Laboratory, Inc., Reston, VA). Liver and kidney from half the pigs in each diet group were weighed and a sample of the minced organ used for determination of S, P, K, Na, Ca, Mg, Fe, Mn, Cu, Zn, and Al content. Left radius-ulna from the same four pigs in each group was measured for weight, length and volume (by water displacement).

Data were subjected to general linear model least-squares means analysis of variance (SAS 1985). Body weight and blood hemoglobin and plasma mineral data were analyzed by split plot analysis with time, Ca level, Fe level, and clinoptilolite level as main effects. Daily weight gain, feed intake, and gain to feed ratio and all carcass and tissue mineral data were analyzed in a one-way analysis of variance with Ca level, Fe level and clinoptilolite as main effects. All interactions were tested.

RESULTS AND DISCUSSION

Dietary concentrations of Ca, Fe, and clinoptilolite had no effect on daily gain, daily feed intake or gain to feed ratio of

Table 1. Composition of Diets

Ingredients	High Calcium[a]				Low Calcium[b]			
	Basal[c] (B)	B+300 ppm Fe[d]	B+2% Clin	B+ Fe+Clin	Basal[c] (B)	B+300 ppm Fe[d]	B+2% Clin	B+ Fe+Clin
Corn, No. 2 yellow	76.3	76.3	74.3	74.3	78.0	78.0	76.0	76.0
Soybean meal	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6
Dicalcium phosphate	2.4	2.4	2.4	2.4	1.2	1.2	1.2	1.2
Ground limestone	0.5	0.5	0.5	0.5				
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin premix	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Trace mineral mix	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Ferrous sulfate·$7H_2O$[f]		+		+		+		+
Clinoptilolite (C)[e]			2.0	2.0			2.0	2.0
Total, %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Contained 1.16% Ca (mean of two samples of each high Ca diet) by analysis.
[b] Contained .53% Ca (mean of two samples of each low Ca diet) by analysis.
[c] Contained 640 ppm Fe (mean of two samples of each low Fe diet without clinoptilolite) by analysis.
[d] Contained 845 ppm Fe (mean of two samples of each high Fe diet without clinoptilolite) by analysis.
[e] Clinoptilolite from California deposit.
[f] Ferrous sulfate ·$7H_2O$ provides 300 ppm Fe added to the complete diet.

growing pigs (initial body wt 29.4, SD = 4.8 kg). Daily gain was 827 vs 819 g for pigs fed normal and high Ca; 824 and 823 g for pigs fed normal and high Fe and 829 and 817 g for pigs fed 0 and 2% clinoptilolite (overall mean 823 $\pm$ 44 g). Carcass and organ measurements were unaffected by diet. Carcass traits were: slaughter weight 103.0 $\pm$ 6 kg; chilled carcass weight 76.5 $\pm$ 4.7 kg; carcass length 79.8 $\pm$ 2.4 cm; longissimus muscle area at the 10th-11th rib interface 30.4 $\pm$ 2.6 sq cm; area of subcutaneous fat at the 10th-11th rib interface 60.3 $\pm$ 6.1 sq cm and sum of weights of trimmed Boston butt, picnic, loin, and ham 19.44 $\pm$ 1.61 kg. Liver and kidney weights were 1.37 $\pm$ .11 kg and .258 $\pm$ .024 kg, respectively, and, when expressed as a percentage of live body weight, were 1.33 $\pm$ .08 kg and .251 $\pm$ .021 kg, respectively.

The main effects of dietary Ca, Fe and clinoptilolite levels on kidney and liver concentrations of major and trace elements are summarized in Table 2. There were no effects of diet on concentration of P, Fe, Zn, or Al in either kidney or liver tissue. Level of dietary Ca had no effect on any element concentration, except for a decreased liver Cu level in pigs fed high Ca ($P<.05$). High dietary Fe had no effect on mineral element concentration, except for a decrease in kidney K ($P<.03$) and Mg ($P<.05$). The physiological significance of these isolated effects of dietary Ca and Fe level on tissue minerals and the importance of the observed Ca x Fe interactions for kidney Mg ($P<.01$) and liver S and Na ($P<.05$) are unknown. Liver S was increased by high Fe in pigs fed low Ca and decreased in those fed high Ca (.226 and .238% S for pigs fed high Fe or low Fe with low Ca diets vs .230 and .220%, respectively, with high Ca diets). The relatively high level of Fe present in the basal diet (about six times the level recommended by NRC 1988) may have precluded the demonstration of an effect of supplemental Fe on liver or kidney Fe concentration. In view of the absence of an effect of either dietary Ca or Fe level on animal performance or carcass measurements, it must be assumed that the relatively small observed shifts in concentrations of specific mineral elements (Table 2) were of no consequence to overall animal health or tissue or cellular integrity, since no pathological lesions or clinical signs of abberations in mineral element metabolism were evident. Concentrations of all mineral elements were well within the normal range in both tissues (NRC 1980). There was no effect of clinoptilolite on liver concentration of any mineral element measured.

The observed changes in kidney mineral element concentrations in response to the addition of clinoptilolite to the diet suggest that dietary levels of K, Mg and Cu need special attention when clinoptilolite is included at 2% of the diet and that the level of dietary iron may affect the copper status of pigs fed clinoptilolite. The presence of approximately .8% Fe in clinoptilolite provides a significant supplemental level of dietary Fe when added at 2% to the diet. The bioavailability of the Fe present in clinoptilolite is unknown, and may depend on

Table 2. Effect of Dietary Ca, Fe and Clinoptilolite Levels on Kidney and Liver Mineral Element Concentration

Mineral element	Calcium (Ca) L	Calcium (Ca) H	Iron (Fe) L	Iron (Fe) H	Clinoptilolite (Clin) L	Clinoptilolite (Clin) H	Mean	SD	Probability
No. of pigs	16	16	16	16	16	16			
			Kidney						
S, %	.181	.181	.186	.176	.186	.175	.181	.011	NS
P, %	.233	.236	.238	.231	.236	.233	.234	.011	NS
K, %	.243	.250	.254	.239	.254	.239	.247	.011	CaxClin,P<.04, Fe<.03; Clin<.04
Na, %	.134	.140	.137	.137	.138	.136	.137	.010	NS
Mg, %	.016	.015	.016	.014	.017	.014	.015	.001	Fe,CaxClin,P<.05; Clin,CaxFe,P<.01
Fe, ppm	65.6	57.4	62.6	60.4	62.4	60.7	61.5	8.3	NS
Al, ppm	1.13	1.63	1.38	1.38	1.38	1.38	1.38	.50	NS
Cu, ppm	9.50	7.88	7.50	9.88	10.12	7.25	8.69	2.26	Clin<.04; FexClin<.03
Zn, ppm	27.4	27.5	28.6	26.3	26.8	28.1	27.4	2.1	NS
			Liver						
S, %	.232	.225	.228	.229	.228	.229	.228	.006	CaxFe<.05
P, %	.348	.359	.353	.354	.356	.351	353	.024	NS
K, %	.305	.303	.306	.301	.306	.301	.304	.011	NS
Na, %	.073	.072	.071	.075	.072	.074	.073	.005	CaxFe<.05
Fe, ppm	255.3	218.6	233.1	240.8	228.1	245.8	236.9	44.5	NS
Al, ppm	2.6	3.1	2.9	2.8	3.1	2.6	2.8	1.2	NS
Mn, ppm	3.2	3.1	3.3	3.0	3.0	3.3	3.1	1.0	NS
Cu, ppm	10.8	8.3	8.7	10.4	8.4	10.6	9.5	2.0	Ca<.05
Zn, ppm	70.0	66.7	67.1	69.6	70.1	66.6	68.3	8.8	NS

its position within the crystalline structure of the clinoptilolite which would affect its accessibility for exchange with other cations over the variable pH range of the gastro-intestinal lumen during its transit.

The failure of liver or kidney Al to be affected by supplemental clinoptilolite in the diet suggests that the zeolite is stable in the milieu of the digestive tract contents and therefore that the absorption of Al or Si contained in the crystalline matrix of the molecule is nil or low.

The Ca requirement of the pig for maximum weight gain may be less than for maximum bone density (NRC 1988). The high Ca diet used in this experiment was expected to increase bone density, but the effects of dietary Fe and clinoptilolite level in modulating this response were unknown. Volume to weight ratio of the radius-ulna was decreased (.779 vs .753) and its weight relative to body weight was increased (1.82 vs 2.00%) in pigs fed high Ca; both of these responses indicate an increase in bone density due to high dietary Ca as predicted. There was no effect of either dietary Fe or clinoptilolite on bone traits and no interactions between or among dietary Ca, Fe and clinoptilolite. Radius-ulna (RU) weight was 199.1 $\pm$ 27.8 g; RU volume 152.5 $\pm$ 22.5 cm; RU length 19.2 $\pm$ .9 cm; RU dry matter 132.9 $\pm$ 18.4 g and 66.8 $\pm$ 1.8%; RU weight as a percentage of body weight 1.91 $\pm$ .15; RU weight to length ratio 10.4 $\pm$ .9. The absence of effects other than those due to dietary Ca level suggests little need for concern about reduced bone density in pigs fed clinoptilolite.

Previous work (Pond et al. 1978) had shown that Fe content of bone ash is reduced in pigs fed high Ca diets. Whether or not other criteria of body Fe status are affected by high Ca has not been clearly ascertained. There was no effect of diet on longissimus muscle color (Hunter 100-unit lightness L_L scale = 42.5 and 42.1 for normal and high Ca; 42.5 and 42.0 for normal and high Fe; 42.0 and 42.5 for 0 and 2% clinoptilolite, respectively; the overall color score mean was 42.3 $\pm$ 2.4). These readings correspond to normal (structure standard no. 3) color standards set forth by Agriculture Canada (1980). The meat color data corroborate the liver and kidney mineral element data (Table 2) which showed no effect of dietary Fe, Ca or clinoptilolite level on tissue Fe content.

It is concluded that the addition of 2% clinoptilolite to corn-soybean meal all-plant diets fed continuously to growing-finishing swine for 84 days is not associated with adverse effects on tissue mineral element concentrations even when dietary Ca and Fe concentrations are altered. The several observed changes in tissue mineral concentrations in response to dietary clinoptilolite do, however, suggest that dietary mineral element composition should receive special attention when clinoptilolite is added to the diet. The data indicate that the Al present in clinoptilolite is not available for absorption from the gastrointestinal tract in a sufficient amount to alter liver

and kidney Al concentration, implying that clinoptilolite is stable in the range of pH encountered in the gastrointestinal tract of the pig.

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