PLASMA ALKALINE PHOSPHATASE VALUES IN WEANLING PIGS AS INFLUENCED BY DIETARY CALCIUM : PHOSPHORUS RATIO AND CESTRUM DIURNUM INGESTION

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SUMMARY

Twenty Yorkshire piglets, weaned at approximately 4 weeks of age were used in this study designed to study the variation in the plasma alkaline phosphatase levels as influenced by dietary calcium and phosphorus and 3% Cestrum diurnum ingestion. Plasma alkaline phosphatase values decreased significantly in the pigs fed either normal calcium: phosphorus or low calcium: high phosphorus diets supplemented with 3% C. diurnum. The decrease in the enzyme values was attributed to the observed histological lesion of retarded osteocytic osteolysis and chondrolysis due to the direct toxic action of C. diurnum factor(s) on resorbing osteocytes.

INTRODUCTION

The ingestion of leaves of the shrub, Cestrum diurnum, has been increminated as a cause of chronic debilitating diseases in horses and cattle (Krook et. al., 1975a, b). The disease is characterized by hypercalcemia, hypercalcitoninism, chronic wasting, lameness, dystrophic calcinosis of major vessels, tendons and ligaments, osteopetrosis and osteonecrosis (Krook et. al., 1975a, b).

Cestrum diurnum belongs to the Solanaceae family and contains factor(s) with action(s) similar to 1, 25 dihydroxycholecalciferol (Wasserman et al., 1975, 1976), the active metabolite of vitamin D_3 (Holick et al., 1971; Lawson et al., 1971 and Norman et al., 1971). However, C. diurnum factor(s) is (are) practically insoluble in water and in fat but soluble in a chloroform and methanol mixture (Wasserman et al., 1976). In C. diurnum poisoning in cattle and horses, Krook et al., (1975a, b) reported that the plasma alkaline phosphatase values remained within normal range in the horse, while it dropped very low in the cattle. The study reported here was, therefore, designed to investigate the variation in plasma alkaline phosphatase levels in young growing pigs as influenced by dietary calcium : phosphorus ratio and C. diurnum ingestion.

MATERIALS AND METHODS

Twenty Yorkshire piglets were used in this study. They were weaned at approximately 4 weeks of age and divided into 2 groups of 10 pigs per group based on litter sex and body weight.

Each group was fed a diet containing either normal calcium and phosphorus ratio (1.2% Ca: 1%P) or low calcium and high phosphorus ratio (0.8% Ca: 1.6%P) for 10 weeks (Table 1) after when 2 pigs from each diet group were necropsied. The remaining 8 pigs per diet were divided into 2 groups of 4 pigs and continued on their

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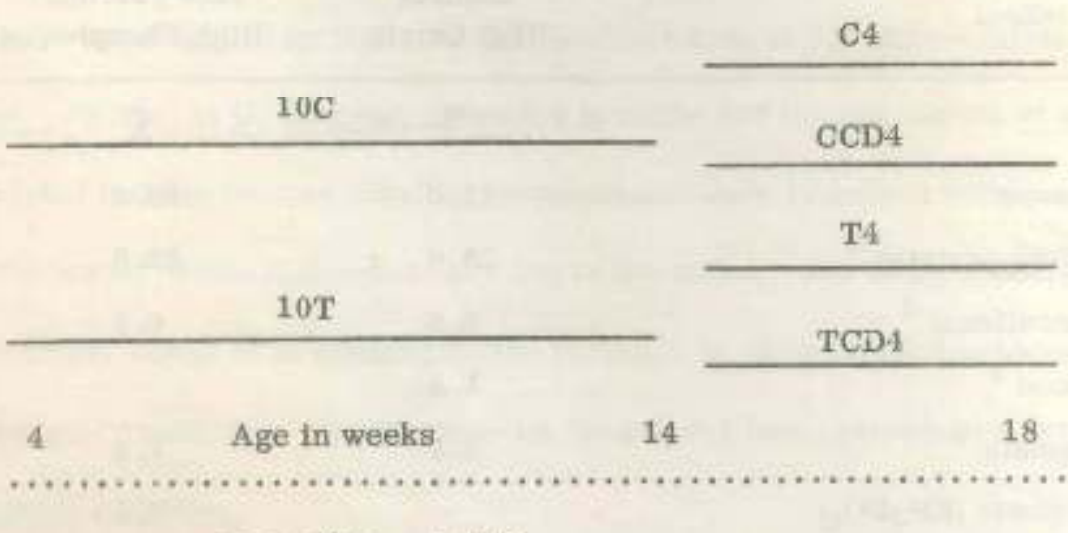
Table 1. Composition of Diets

Diet Ingredient	Control High Calcium	Low Calcium: High Phosphorus
	%	%
Ground yellow corn	72.0	69.8
Soybean meal (50% protein) [1]	23.0	23.8
Salt (trace mineralized) [2]	0.5	0.5
Ground Limestone [3]	1.5	-
Dicalcium phosphate [4]	2.5	2.8
Potassiumdiphophate (KH_2PO_4)	-	2.6
Vitamin-Zn supplement (Hopro R)	0.5	0.5
	100.0	100.0
Total Ingredients %		
Calcium	1.2	0.8
Phosphorus	1.0	1.6
Protein	18.0	18.0

1. Beacon, The Beacon Milling Company, Inc. Cayuga, N.Y.
2. Blusalt, International Salt Company, Clarks Summit, Pa.; contained NaCl, 96%; Mg, 0.1%; Iron, 0.15%; Mn, 0.2%; Sulfate, 0.05%; Cu, 0.03%; Co, 0.01%; Zn, 0.008%; I, 0.007%.
3. United States Steel Corp., Buffalo, N.Y.; contained Ca, 38% and Mg, 1%.
4. International Minerals and Chemical Corp., Skakee, Ill.; Ca, 24% and P, 18.5%
5. Hopro-R, Smith Douglas, Borden Chemical, Borden, Inc., Norfolk, Va. contained vitamin A, 90,8000 U.S.P. units/kg; vitamin D_2, 54,480 U.S.P. units/kg; vitamin B12, .0454 mg/kg; niacin, 576.5 mg/kg; riboflavin, 181.6 mg/kg; d-pantothenic acid, 249.7 mg/kg; $ZnCO_3$ .132%; $FeSO_4$, .7H_2O, 0.66%; I, 0.022%.

respective diets with or without incorporation of 3% of dried leaves of C. diurnum (Table 2).

Table 2. Design of Experiment



C. 1.2% Ca: 1.0%P

CCD 1.2% Ca. + 3% C. diurnum leaf meal

T 0.8% Ca: 1.6% P.

TCD 0.8% Ca. + 3% C. diurnum leaf meal

Diets fed to 10 pigs on each diet for 10 weeks, then 2 pigs on each diet necropsied and remaining 8 pigs per diet split into 2 groups of 4 pigs and continued on respective diets with or without incorporation of dried leaves of Cestrum diurnum* at 3%.

* The leaves of Cestrum diurnum were air-dried, ground to a rather fine powder and thoroughly mixed with the feed at the rate of 3 kg per 100 kg feed. No feed ingredient was thus removed for the addition of C. diurnum leaves.

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The pigs-were separated into 2 groups of 10 pigs in each group and each group was housed in a partitioned concrete floor room. At 11 weeks of experiment, the remaining 8 pigs in each group were further divided into 2 subgroups of 4 pigs and each subgroup was housed in a partitioned concrete floor room.

Pigs were fed free choice throughout the experimental period. Water was also supplied free choice.

A 10ml. blood sample was taken from the anterior vena cava weekly for determination of alkaline phosphatase according to Sigma method (Bessey et al., 1946).

RESULTS

The average weekly plasma alkaline phosphatase values in Sigma units/100ml. on ordinate as function of age in weeks of abscissa are represented in Fig. 1.

Pooled regression analysis of plasma alkaline phosphatase values as function of age in weeks are presented in Table 3.

In the C pigs, plasma alkaline phosphatase value showed a significant regressive decrease (P 0.01) throughout the experimental period.

In the CCD pigs, a progressive significant (P 0.05) decrease in the plasma alkaline phosphatase was also recorded at 11 weeks of experiment with further significant (P 0.05) at the end of the experimental period.

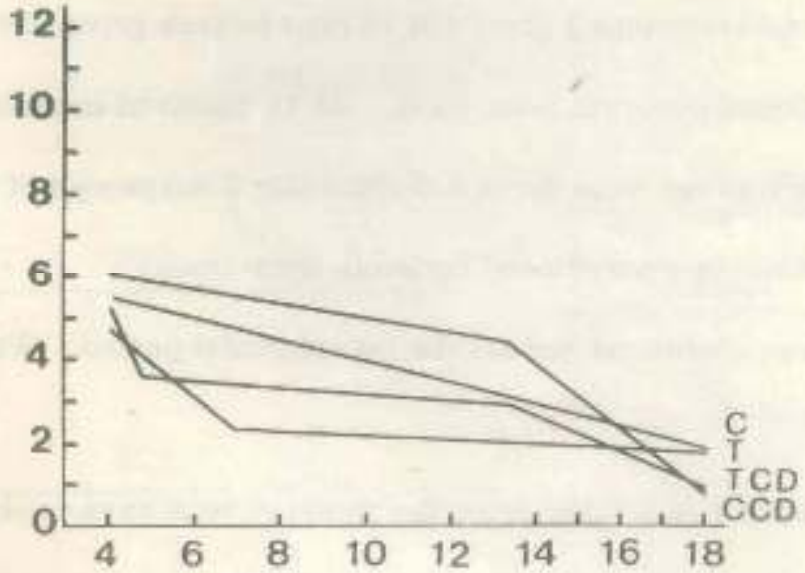

Fig. 1. The average weekly plasma alkaline phosphatase values in Sigma units/100ml. on ordinate as function of age in weeks of abscissa.

In the T pigs, an early significant (P 0.01) decrease of plasma alkaline phosphatase was recorded after 3 weeks of experiment with further significant (P 0.05) progressive decrease towards the end of the experiment.

In the TCD pigs, there was also an early significant (P 0.01) decrease in the plasma alkaline phosphatase value after one week of experiment with a further gradual but insignificant (P 0.05) decrease at 10 weeks of experiment. Thereafter the values decreased significantly (P 0.01) until the end of the experimental period.

Table 3. Regression Analysis of Plasma Alkaline Phosphatase (Y) as Function of age in Weeks (X).

Diet Group	Weeks	Equations	r Values	Probability	$\bar{X} \pm$ sem	Conclusion
C	4-18	Y = 6.53 - 0.255 X	-0.8725	0.01	3.73 " 0.337	Significant
CCD	4-14	Y = 6.75 - 0.177 X	-0.6264	0.05	5.16 " 0.283	"
	14-18	Y =14.81 - 0.781 X	-0.8689	0.05	2.31 " 0.637	"
T	4-7	Y = 7.88 - 0.800 X	-0.9644	0.01	3.50 " 0.535	"
	7-18	Y = 2.59 - 0.042 X	-0.6481	0.05	2.07 " 0.067	"
TCD	4-5	Y =14.17 - 2.230 X	-1	0.01	4.14 " 1.115	"
	5-14	Y = 3.89 - 0.073 X	-0.0726	0.05	3.20 " 0.215	not significant
	14-18	Y = 8.53 - 0.423 X	-0.9218	0.01	1.76 " 0.324	significant

DISCUSSION

Plasma alkaline phosphatase values decreased significantly in the CCD and TCD pigs after treatment with 3% C. diurnum. Although the enzyme is not specifically related to bone metabolism, its implication in bone apposition has long been recognized (Robison, 1923). Gomori (1943) showed calcification to take place in the centre of phosphatase - positive areas in embryonic cartilage while no calcification occurred in phosphatase-negative areas. Rutishauser and Mãjno (1951) and Mãjno and Rouiller (1951) showed that this enzyme occurs both in osteocytes in bone resorption and in the cytoplasm of actively eroding osteoclasts. Krook and Lowe (1964) and Brown et al., (1966) further showed an inverse relationship between serum calcium and serum alkaline phosphatase in experimental studies on nutritional hyperparathyroidism in horses and pigs respectively. These findings denote the enzyme as one of apposition as well as resorption. In the present study, the decrease in plasma alkaline phosphatase in pigs fed 3% C. diurnum is attributable to the observe histological lesion of retarded osteocytic osteolysis and chondrolysis due to the dire toxic action of C. diurnum factor(s) on the resorbing osteocytes.

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