Plasma Calcium and Phosphorus In Weanling Pigs as influenced by Dietary Calcium: Posphorus 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 to investigate the variations in plasma calcium and phosphorus as influenced by dietary calcium and phosphorus and 3 *Cestrum diurnum.*

In the CCD and TCD pigs, hypercalcemia developed rapidly and persisted following the feeding of 3% *C. diurnum.* **While hypophosphatemia occurred in the CCD pigs, there was an initial and transient hyperphosphatemia with subsequent and terminal hyposphosphatemia in the TCD pigs.**

The hypercalcemia induced by the ingestion of 3% *C. diurnum* **in the CCD and TCD pigs is attributed to increased intestinal absorption of calcium and not to bore resorption. The initial hyperphosphatemic response in TCD pigs is also believed to be due to increase absorption of phosphorus from the high phosphorus diet.**

INTRODUCTION

The ingestion of leaves of the shrub, *Cestrum diurnum,* has been incriminated as a cause of a chronic debilitating disease 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 an dosteonecrosis (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 (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* toxicosis, Krook *et. al.* (1975a,b) reported that plasma calcium was elevated to moderate or severe degree in horses and cattle but later decreased toward low normal values in the cattle.

The object of this study therefore is to investigate the variation in plasma calcium and phosphorus in weanling 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 respective diets with or without incorporation of 3% of dried leaves of *C. diurnum* (Table 2).

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 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 10 ml. blood sample was taken from the anterior vena cava weekly for deter-

TABLE 1

Composition of Diets

Diet Ingredient	*CONTROL High Calcium*	*Lo Ca.*—Hip
	%	%
Ground yellow corn	72·0	69·8
Soyabean 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
Potassium diphosphate (KH_2Po_4)	—	2·6
Vitamin-Zn supplement (Hopro R) [5]	0·5	0·5
TOTAL	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.V.
2. Blussat, International Salt Company, Clarks Summit, Pa.; contained NaCl, 96%; Mg, O. 1%; Iron, O. 15%; Mn, O. 2%; Sulphate, O. 05; Cu, O .03%; Co, O. o1%; Zn, O. oo8%; I, O. oo7%.
3. United States Steel Corp., Buffalo, N.Y.; contained Ca, 38% and Mg, 1%
4. International Minerals and Chemicals Corp., Skakee, III., Ca, 24% and P, 18.5%.
5. Hopro-R, Smith Douglas, Borden Chemical, Borden, Inc., Norfolk, Va. contained vitamin A, 90,800 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; $ZnCo3.1.32\overline{N}$; FSO_4. 7H20, 0.66%; $CuSO_4$. 5H2O, O.66%; I, O.022%.

mination of plasma calcium and phosphorus in a Technicon Autoanalyzer.

RESULTS

1. Plasma Calcium

The average weekly plasma calcium values in mg/100ml on ordinate as function of age in weeks of abscissa are represented graphically in Fig. 1

Pooled regression analysis of plasma calcium as function of age in weeks are presented in Table 3. In the C pigs, a progressive significant ($P < 0.01$) increase plasma calcium value was recorded.

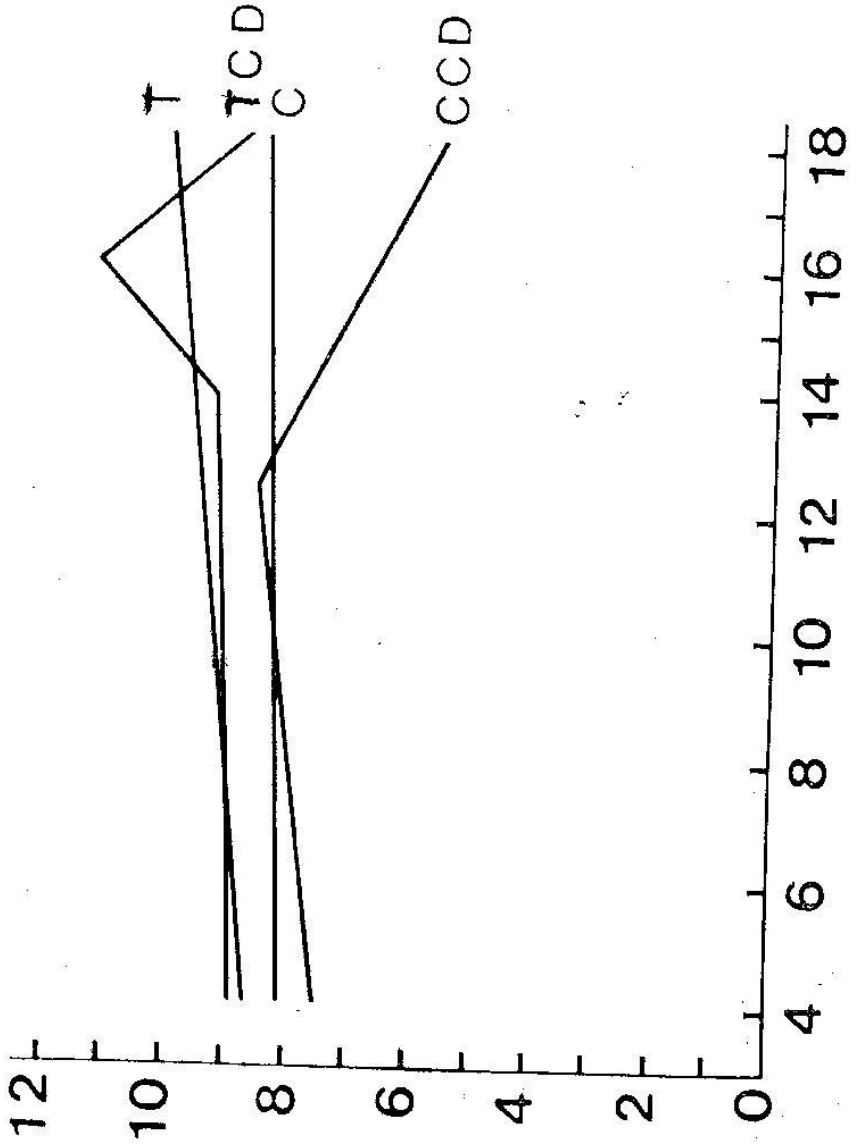
In the CCD pigs, the plasma calcium value rose slightly after 9 weeks of experiment although this increase was not significant ($P > 0.05$). However, a marked significant ($P < 0.01$) hypercalcemia was recorded at 12 weeks of experiment. The value reached a peak at 13 weeks of experiment before it dropped the 14th week of experiment. The decrease was not statistically significant ($P < 0.05$).

In the T-pigs, a significant ($P < 0.01$) hypocalcemia developed during the first 3 weeks of experiment but thereafter, the plasma calcium value increased linearly until later, a significant ($P < 0.01$) hypercalcemia developed.

In the TCD pigs, there was also an early significant ($P < 0.01$) decrease in plasma calcium value after 1 week of experiment. This was followed by a progressive insignificant ($P > 0.05$) increase with subsequent restoration of isocalcemia at 11 weeks of experiment. A marked significant ($P < 0.01$) hypercalcemia was recorded at 12 weeks of experiment, reached a peak at 14 weeks of experiment before slightly decreasing significantly ($P < 0.01$) at the end of the experiment.

2. Plasma Phosphorus

The average weekly plasma phosphorus values in mg/100ml on ordinate as function


T
TCD
C
CCD
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10
8
6
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2
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4
6
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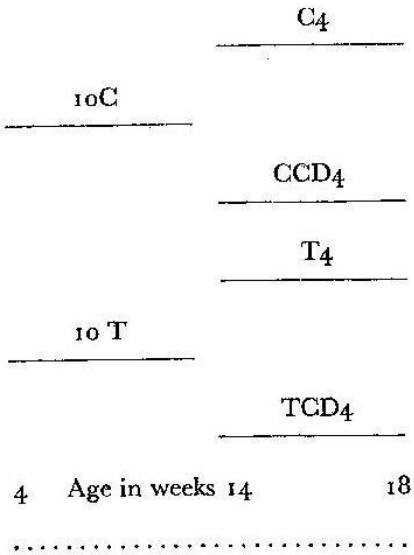
12
14
16
18

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 harvested in late October, 1974, on a horse farm in Miami Florida. They were air-dried; then 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.

of age in weeks of abscissa are represented graphically in Fig. 2.

Pooled regression analysis of plasma phosphorus values as function of age in weeks are presented in Table 4.

There was no significant variation ($P > 0.05$) in the plasma phosphorus values of C pigs throughout the period of the experiment.

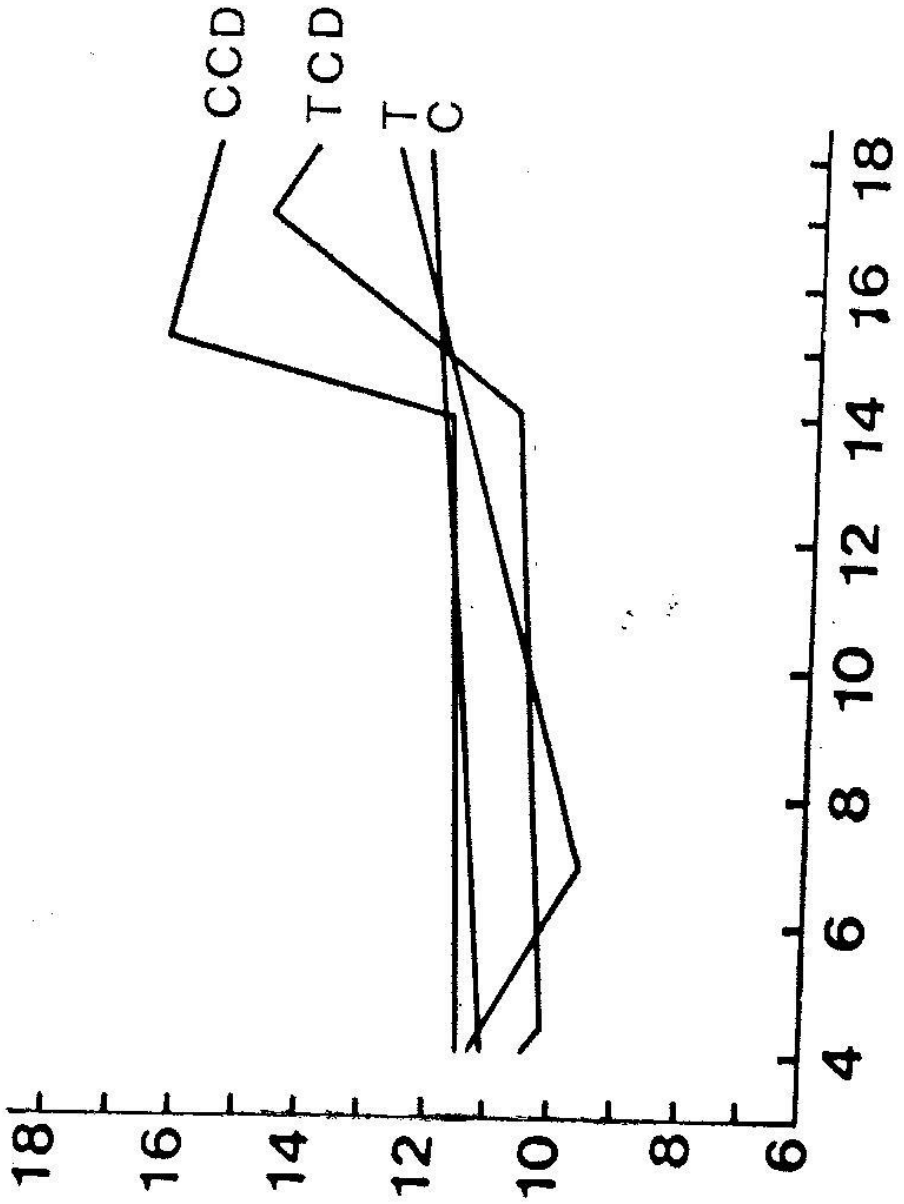
DISCUSSION

In the CCD pigs on the other hand, although there was a significant increase ($P < 0.05$) in plasma phosphorus value at 11 weeks of experiment a progressive hypophasphatemia developed at 12 weeks and persisted throughout the experiment with the value decreasing significantly ($P < 0.01$) at the end of the experiment.

In the T pigs, a significant ($P < 0.05$) linear increase in the plasma phophorus value was recorded throughout the period of the experiments.

In the TCD pigs, the elevation of plasma phosphorus value reached a peak at 12 weeks of the experiments although this increase was not statistically significant ($P > 0.05$). The value later decreased significantly ($P < 0.005$) in the 13th week of the experiment.

In this study, the hypercalcemic response in the TCD and CCD pigs after the ingestion of 3% *C. diurnum* is considered the result of an increased intestinal absorption of calcium by *C. diurnum* factor (s). Normally, the amount of 1,25 $(OH)_2$ D_3 produced by the kidney is feedback regulated thereby controlling the degree to which calcium is absorbed from the digestive system (Omdahl and Deluca, 1973). Since *C. diurnum* contains factor(s) with action (s) similar to 1,25 $(OH)_2$ D_3, the primary point in vitamin D metabolism was by-passed and more calcium wa absorbed than could be physiologically


CCD
TCD
T
C
18
16
14
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18

TABLE 3

Regression analysis of plasma calcium (Y) as Function of age in Weeks (X)

Diet Group	*Weeks*	*Equations*	*r Values*	*Probability*	$\bar{} + = sem$	*Conclusion*
CC	4–18	$Y = 10.80 \quad 0.089 \; X$	0.7769	> 0.01	11.78 .. 0.132	significant
CCD	4–14	$Y = 11.36 \quad 0.039 \; X$	0.3897	< 0.05	11.71 ,, 0.101	non significant
	14–15	$Y = 47.30 \quad 4.230 \; X$	1	< 0.01	14.04 ,, 2.115	significant
	15–18	$Y = 20.40 \quad 0.263 \; X$	0.6547	< 0.05	16.06 ,, 0.259	non significant
T	4– 7	$Y = 13.55 \quad 0.573 \; X$	0.9250	< 0.05	10.40 ,, 0.389	significant
	7–18	$Y = 7.86 \quad 0.254 \; X$	0.9480	< 0.01	11.03 ,, 0.279	,,
TCD	4– 5	$Y = 13.46 \quad 0.740 \; X$	1	< 0.01	10.13 ,, 0.370	,,
	5–14	$Y = 10.08 \quad 0.050 \; X$	0.4044	< 0.05	10.56 ,, 0.119	non significant
	14–17	$Y = 8.27 \quad 1.369 \; X$	0.9814	< 0.01	12.95 ,, 0.900	significant
	17–18	$Y = 25.05 \quad 0.600 \; X$	1	< 0.01	14.55 ,, 0.300	,,

TABLE 4

Regression analysis of plasma phosphorus (Y) as function of age in Weeks (X)

Diet Group	*Weeks*	*Equations*	*Values*	*Probability*	*sem*	*Conclusion*
C	4–18	$Y = 8.09 + 0.022 \; X$	0.2334	< 0.05	8.33 + 0.107	not significant
CCD	4–14	$Y = 6.96 + 0.134 \; X$	0.6542	< 0.05	8.17 ,, 0.204	significant
	14–18	$Y = 14.86 - 0.514 \; X$	$=0.9418$	< 0.01	6.63 ,, 0.386	,,
T	4–18	$Y = 8.29 + 0.101 \; X$	0.5656	< 0.05	9.40 ,, 0.207	,,
TCD	4–14	$Y = 8.65 + 0.047 \; X$	0.1942	< 0.05	9.08 ,, 0.241	not significant
	14–16	$Y = = 3.22 + 0.910 \; X$	0.8102	< 0.05	10.43 ,, 0.648	,, ,,
	16–18	$Y = 33.47 - 1.395 \; X$	$=0.9771$	< 0.05	9.75 ,, 0.824	significant

accommodated and thus hypercalcemia resulted. A similar explanation had been proposed for the hypercalcemia in the natural and experimental *C. diurnum* toxicosis (Krook *et. al.*, 1975a, b, Wasserman *et. al.* 1975, 1976).

The hypophosphatemic response in the CCD pigs is in agreement with the findings in natural *C. diurnum* poisoining (Krook *et. al.*, 1975a, b) and in vitamin D toxicosis in rats (Belanger and Clark, 1967).

The transient but insignificant increase of plasma phosphorus at 12 weeks of experiments that is, after 1 week of feeding *C. diurnum* could only also be explained by a *C. diurnum* enhanced increased intestinal absorption of phosphorus from the high phosphorus diet.

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