July 23, 1962

To the Graduate Council:

I am submitting herewith a thesis written by Paul Thomas Chandler entitled "Responses of Holstein Calves to Dietary Calcium, Phosphorus and Vitamin D_3 Using Response Surface Techniques." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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RESPONSES OF HOLSTEIN CALVES TO DIETARY CALCIUM, PHOSPHORUS AND VITAMIN D_3 USING RESPONSE SURFACE TECHNIQUES

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Paul Thomas Chandler

August 1962

28.
33

ACKNOWLEDGEMENTS

The author expresses a sincere appreciation to his major professor, Dr. R. G. Cragle, for suggestions and supervision in conducting this study. Acknowledgement is given to Dr. N. S. Hall, Director of the UT-AEC Agricultural Research Laboratory for the graduate assistantship and for the facilities that were used to conduct the reported study.

Appreciation is expressed to Drs. D. F. Johnson, Jr. and F. W. Sigler for the performance of autopsies. The author is grateful to the members of his committee for reading this manuscript and for their suggestions in its preparation.

The author is grateful to Mrs. Viola L. Gibbons for technical assistance and to Mr. H. H. Davis and Mr. W. H. Wilson for animal care.

Acknowledgement is given the American Lecithin Company and Chas. Pfizer and Company, Inc., for their grateful donation of lecithin and terramycin. Appreciation is expressed to my wife, Patsy, for the typing of this manuscript.

522622

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CHAPTER I

INTRODUCTION

The metabolism of calcium, phosphorus, and vitamin D has been a classical topic of nutrition studies through the past fifty years. Many experiments have been conducted to determine the relationship of calcium, phosphorus, and vitamin D in the animal body. All results have led to the conclusion that vitamin D will affect the absorption and/or retention of calcium and phosphorus, and the amount of calcium will affect calcium and phosphorus metabolism. However, the effect of the dietary level of phosphorus has been questioned (1, 56, 65, 69). The chemistry and site of the majority of these actions is still to a great extent unknown.

The usual procedure for studying a problem of this type is to hold two of the factors constant and vary the third. Information obtained from studies of this type is limited in that it relates to only one set of conditions. A mathematical description of responses as related to any dietary combination within limits of calcium, phosphorus, and vitamin D would be desirable.

The primary objective of this study was to quantitatively relate calcium, phosphorus, and vitamin D over a comparatively wide range of each and to determine the dietary combinations for maximum absorption, retention, and growth in the dairy calf. A secondary objective was to check the validity of mathematical techniques in describing biological systems.

CHAPTER II

REVIEW OF LITERATURE

The Effects of Vitamin D Upon Absorption and Excretion of Calcium and Phosphorus

The effect of vitamin D upon calcium and phosphorus metabolism has been investigated extensively. It is known that dietary and endogenous vitamin D increases the absorption of calcium from the intestine and decreases the excretion of calcium into the gut. Duncan and Huffman in 1936 (22) and Smith in 1958 (62) observed that young calves would show a progressive downward trend in plasma calcium and inorganic phosphorus when solar ultraviolet radiation or some form of vitamin D was lacking in their dietary regime. Conrad and Hansard (15) reported that massive doses of vitamin D increased the true digestibility of calcium and phosphorus in 6-month-old Hereford calves. The apparent digestibility of calcium was 20.6 per cent for those calves receiving no vitamin D and 52.2 per cent for those receiving 5 million U.S.P. (United States Pharmacopoeia) units daily. Phosphorus had an apparent digestibility of 40.3 and 58.2 per cent, respectively. Increased absorption of calcium due to vitamin D feeding was accompanied by several related effects, including decreased endogenous fecal losses, a transitory increase in absorbed radiocalcium in blood, more rapid movement of radiocalcium through plasma, a threefold increase in net calcium retention, and increased deposition of radiocalcium in areas

of new bone growth.

It has been shown by Shelling (60) and Albright and Sulkowitch (1) that fecal phosphorus excretion depends upon the amount of fecal calcium. Vitamin D decreases fecal phosphorus excretion by decreasing the calcium excretion. This decrease in calcium excretion will cause an increase in the amount of calcium absorbed. It was suggested that the rise in inorganic serum phosphorus levels during additions of the vitamin is due to an accompanying decreased activity of the parathyroid glands because of the increased calcium absorption (1). In addition to increasing the absorption of calcium, vitamin D increases the urinary excretion of phosphorus (1, 60). It is probably because of this property of the vitamin that demineralization occurs with large doses.

The vitamin D and phosphorus relationships have not been studied to as great an extent as has the calcium relationship. Meintzer and Steenbock (51) showed, in four out of five experiments with rats, that there was an increase in phosphorus retention when vitamin D was given. This is probably secondary to an effect on calcium absorption. It has been shown that rats on a low calcium diet show a five-fold increase in phosphorus retention when treated with vitamin D (18). Growth hormone was also investigated in this study and it was found to show a three-fold increase in phosphorus retention. Vitamin D plus growth hormone did not show any additive effect. Vitamin D raised serum calcium and inorganic phosphorus but the hormone had no such effect. In hypophysectomized rats, on a low calcium diet, vitamin D

failed to induce growth or calcification although it did increase serum calcium. It seems that the presence of pituitary hormones are necessary for complete vitamin D response.

Vitamin D may increase total phosphorus absorption by causing re-excreted phosphorus to be re-absorbed at a lower point in the digestive tract. Bergein (5) stated that rats secrete considerable phosphorus into the upper tract. The phosphorus secreted into the upper tract was re-absorbed in the lower intestines to produce an ultimate positive balance of the element. In animals deficient in vitamin D, there was a failure for this re-absorption to take place and hence a loss of the phosphate from the body.

The effects of vitamin D upon calcium and phosphorus are usually very rapid. Migicovsky (52) has shown that the absorption of calcium through the intestinal mucosa as measured by bone deposition comes under the influence of vitamin D within 2-4 hours after the vitamin is administered to the chick. It was concluded that vitamin D affects the excretion rate which in turn could bring about an increase in the absorption rate per bone. It could not be stated whether the action of the vitamin on the bone was direct or indirect. Harrison et al. (40) maintained rats for three weeks on a vitamin D free diet which contained an adequate amount of calcium. The calcium concentration in blood serum fell from 10 to 6 mg per cent during that period. Within 24 hours following the administration of a single dose of 100 I.U. (International Units) of vitamin D_2, this had risen to 9 mg per cent and 2 days later it had reached almost 11 mg per cent.

Schachter and Rosen (59) demonstrated a depression in Ca-45 transport within two days after rats had been given a rachitogenic diet. Other results pointing toward the rapid action of vitamin D is the protective effect of massive dosage in milk fever (16, 42).

Vitamin D shows its greatest effect when the calcium in the ration is low. Steenbock and Herting (63) showed with rats that when a diet contained adequate calcium (0.45 per cent) and increased amounts of phosphorus, the effect of vitamin D was not as great as when the calcium content was low. The difference seemed tc be due to the higher value of serum calcium maintained by the higher calcium intake. It was stated that the action of the vitamin is not limited to the absorption and deposition of bone salts, but that the vitamin has a more widespread effect on organic tissue metabolism of which increased growth is one manifestation.

Vitamin D has also been shown to stimulate appetite as well as growth. Hugot and Causenet (44) showed that the rats maintained on a low calcium diet ad libitum ate considerable more when given vitamin D. The increased food intake corresponded to the gain in weight of these animals. Duncan and Huffman (22) observed anorexia and a cessation of or decrease in the rate of growth in young calves when vitamin D was deficient.

The Biochemical Action of Vitamin D on Calcium and Phosphorus Absorption

A number of theories has been brought forward in the past to explain the action of vitamin D on calcium absorption. One of these is that the absorption effect is brought about by vitamin D causing

an increase in the acidity of the intestinal tract and thereby causing calcium salts to be more soluble (55). Harrison and Harrison in 1951 (37) suggested that the effect of vitamin D in increasing the efficiency of calcium absorption is observed only under conditions in which the calcium of the intestinal contents is poorly soluble. Another theory by Neuman and Neuman in 1958 (54) implicates local citrate production in the intestine as a possible explanation. The citrate formed within the absorbing cell of the mucusa could complex calcium and transfer the soluble, un-ionized calcium citrate parenterally. However, this theory has been challenged because of the findings that the vitamin D dependent transport system for calcium as characterized by Schachter and Rosen in 1959 (59) was unable to transport Sr-85 and stable strontium was unable to inhibit Ca-45 transport. Since the pK values for the citrate complexes of calcium and strontium are similar, it would be expected that strontium could compete with calcium if such a system existed. It was suggested that the transport system of Ca-45 is dependent on the production of high energy phosphate bonds.

Harrison and Harrison in 1960 (38) demonstrated another type of mechanism concerning calcium and phosphorus absorption. Using the in vitro everted intestinal sac technique, similar to the system of Schachter and Rosen (59), it was shown that another action of vitamin D is to increase the rate of diffusion of calcium through the intestinal wall. This increased diffusibility of calcium could be the result of an alteration of the permeability of the intestinal epithelial cells to calcium, possibly as the result of the incorporation of vitamin D

to cell surfaces. The vitamin D effect on calcium transfer was not a nonspecific alteration of the permeability of the intestinal wall to cations since no effect of vitamin D on Na-24 transfer was found. The effect of vitamin D on the active transport of Ca-45 is observed in the most proximal portion of the small intestine adjacent to the pylorus and is dependent upon the energy of oxidative metabolism (38, 59). The action of vitamin D upon the rate of diffusion of calcium is exerted along the entire length of the small intestine and is not affected by inhibition of oxidative mechanisms (38). Harrison and Harrison (39) observed in 1961 that the intestinal transport of phosphate is dependent upon the presence of calcium. It was stated that the primary action of vitamin D in phosphate transport is to increase the rate of penetration of calcium across the cell surface and this to increase the concentration of calcium at that site in the cell which the calcium-activated system for phosphate transport is located. The effect of vitamin D upon the cell permeability to calcium was confirmed.

The Sites of Calcium and Phosphorus Absorption and Secretion

The sites of calcium and phosphorus absorption and secretion have not been completely established. Bergein (5) in working with rats stated that there is a large secretion of phosphorus into the first part of the small intestine with a later absorption in the terminal portion of the small intestine. He has also stated that the secretion of phosphorus seems to be associated with calcium absorption. Harrison and Harrison (37) found that the major site of Ca-45

absorption in rats is from the proximal portions of the small intestine. At 4 hours after dosing, little radiocalcium remained in the proximal two-thirds of the small intestine and the major portion of the unabsorbed radiocalcium the small intestine and the major portion of the unabsorbed radiocalcium was located in the distal one-third of the small intestine. Fournier (27) and Fournier and Dupuis (28, 29) are of the opinion that the absorption of calcium and phosphorus from the stomach of the rat is of considerable importance.

The location of calcium and phosphorus absorption in sheep and camels was studied by Fomina (26). It was found that the contents of these elements in the various sections of the digestive tract differed with the stage of digestion. The content of phosphorus was lower commencing with the duodenum all the way down to the lower part of the large intestine. The content of calcium increased in the direction from the stomach to the large intestine. Smith et al. (61) observed a large endogenous secretion of phosphorus into the rumen and proximal small intestine of sheep. Lofgreen et al. (48) presented data which indicated that all segments of the gastrointestinal tract of the calf may play a role in the excretion of phosphorus. The jejunum in particular appears to be important in this respect.

The Effect of Dietary Intake and Calcium:Phosphorus Ratio Upon Calcium and Phosphorus Metabolism

The levels of calcium and phosphorus in the diet have a direct influence upon the absorption and metabolism of these minerals. It was suggested by Albright and Sulkowitch (1) that calcium in the diet

has more influence on phosphate absorption than the phosphate in the diet has on calcium absorption. They found that an increase in the ingested calcium was followed by an increase in fecal phosphorus excretion. However, an increase in the phosphate ingested was not followed by an increase in fecal calcium excretion. Van Reen et al. (65) showed with rats that the amount of calcium in the diet has a definite effect on the absorption of phosphorus. It was stated that, with a normal level of phosphate in the diet, a high level of calcium will reduce its absorption. It is thought that this is through the formation of an insoluble calcium phosphate salt. The reverse relationship of excess phosphorus reducing calcium absorption has been questioned.

These results have been confirmed by work with large animals. Lueker and Lofgreen (50) studied the effects of intake and calcium to phosphorus ratio on absorption of these elements by sheep. An analysis of covariance was used so that actual intake and ratio would be separate. The results indicate that the amount of calcium absorbed is influenced only by calcium intake, and the amount of phosphorus absorbed is influenced only by phosphorus intake. The metabolic fecal calcium excretion was found to be fairly constant, unaffected by ratio and total intake. The excretion of fecal metabolic phosphorus was significantly influenced by the amount of both calcium and phosphorus absorbed. The amount of calcium absorbed exerted about half as much influence on metabolic fecal phosphorus excretion as the amount of phosphorus absorbed but the effect was in an opposite direction. An increase in phosphorus absorption causes an increase in metabolic

fecal phosphorus excretion which tends to be offset when more calcium is absorbed. Therefore, the Ca:P ratio exerts an influence upon the excretion of metabolic fecal phosphorus even though it does not affect absorption. The amount of calcium absorbed is important in governing the metabolic fecal excretion of phosphorus.

It has been shown by Axelsson and Eriksson (3), with two adult wethers, that the apparent absorption of calcium varies with the amount of calcium in the ration ($r = +0.490$), and that phosphorus is positively correlated with the amount of phosphorus in the ration ($r = + 0.599$). The amount of either element fed did not affect the absorption of the other which is in agreement with the previous reviewed work. Dowe et al. (20) investigated the effects of various Ca:P ratios in growing beef calves. The various ratios were obtained by adding calcium to a basal ration with a constant phosphorus level. It was found that body weight gains decreased as the ratio became larger or as calcium was added to the ration. Plasma inorganic phosphorus content was not depressed below a level considered adequate for proper nutrition. It was postulated that excess calcium depressed gains by interferring with the digestibility and/or absorption of nutrients and mineral elements other than phosphorus. Berry et al. (6) noted that increased dietary calcium lowered blood and plasma Ca-45 activity, increased stable calcium retention, depressed plasma P-32 levels, and depressed urinary P-32 excretion in swine. Buescher et al. (11) observed that high calcium additions to a normal phosphorus ration decreased growth rate, feed consumption, and feed efficiency

in swine. Lloyd et al. (46) observed similar results in early weaned pigs and found that the apparent digestibility of total carbohydrates was reduced as the calcium level was increased. The effects of high calcium intakes on the absorption of other nutrients have been reviewed by Davis in 1959 (19).

A study of Patton et al. (58) was conducted to find the effects of various ratios as to the utilization of calcium and phosphorus in humans. Eighteen young college women were used in this study and it was found that calcium retentions are related to the ratio of calcium to phosphorus and are greater when the ratio is more than one. In this study, with constant phosphorus intake, phosphorus balances were not affected by any increase in calcium intake. The indications were that the balance of both minerals was more closely related to intake than to the ratios of calcium to phosphorus. A study of urine was also made and it was found that a constant increase of urinary calcium was shown as the ratio increased with calcium being held constant. When these values were placed on a percentage basis it was found that the per cent of intake found in the urine decreased with increasing intake.

Factors That Affect the Efficiency of Calcium and Phosphorus Metabolism

It has been observed that the efficiency of calcium retention decreases as the amount in the diet increases to higher levels. As the amount of calcium in the diet increased, the per cent of calcium absorption decreased in the rat (12, 36). Gato et al. (30) found that rats on a high calcium diet (two times the control) excreted

three times as much calcium as the control group. The calcium content of bone was found to be less than that of the control group after a 12-day feeding period.

The results reviewed have indicated that the calcium status of the animal is a very important factor in the physiological behavior of calcium. Hansard and Plumlee in 1954 (36) found that the current calcium intake was of less influence upon the endogenous losses than was the calcium status of the animal at the time of measurement. The total absorption and retention of radiocalcium were increased in rats with low calcium body stores, whereas radiophosphorus was absorbed but subsequently re-excreted by way of the kidneys and less was retained. The behavior of the Ca-45 retained by the tissues was practically independent of the dietary calcium and phosphorus levels studied, except that the calcium depleted animals demonstrated greater Ca-45 concentration and rate of turnover in all tissues. It has also been shown in other work that a period of calcium deprivation is followed by an increased rate of absorption and an increase in the potential ability for new bone formation (56).

There are many indirect effects upon the absorption and metabolism of calcium and phosphorus. Seasonal changes have long been evident in calcium and phosphorus metabolism. These changes have been correlated with the intensity of solar ultraviolet rays. Duncan and Huffman (22) found a seasonal increase in calcium during the early summer months and a corresponding decline during the early winter months. The inorganic phosphorus values showed a stronger tendency

to increase to a maximum during July and August and then decrease to a minimum during January and February. It was also shown that the exposure of rachitic calves to early spring sunshine caused the concentrations of calcium and inorganic phosphorus in the plasma to increase markedly. There was a significant increase of these constituents in normal calves during the summer months and a corresponding decline during the winter months. Vasil'eva (66) has observed these changes in growing calves and related them to the vitamin content of the forage. All of these factors relate back to the action of vitamin D upon calcium and phosphorus metabolism.

Age has been shown to have a definite effect on calcium metabolism (33, 35). Hansard et al. in 1954 (35) observed that the absorption and/or true digestibility values were the greatest in young cattle. These values decreased rapidly to sexual maturity then more slowly to maturity and again decreased in the aged animals. This indicates that the rate of exchange of Ca^{++} between blood and other tissues decreased significantly with increased age (33). Daily endogenous fecal calcium increased with increasing age and body weight from 0.42 gram at 10 days to 3.3 grams at 6 months to 8 grams at maturity (35). The quantity of endogenous fecal calcium per kilogram of body weight showed little change as the animals grew from 1 month of age to maturity. This indicates that the animal's decreased retention of calcium comes about by an increased endogenous loss. The age effect is more of a hormonal controlling effect than any of the other factors. There are other factors which affect the absorption

of calcium and phosphorus but they will not be mentioned in this review. Some of these have been reviewed by Nicolaysen et al. in 1953 (56).

Some Factors Affecting the Dietary Requirements of Vitamin D

The dietary requirement for vitamin D varies with age, species, and the calcium:phosphorus ratio. In the rat when the ratio is about unity and the content of calcium and phosphorus in the diet is adequate, growth and calcification proceed in the absence of vitamin D (69). Species other than the rat are more dependent upon vitamin D for normal calcium metabolism. Gershoff (32) showed the vitamin D requirements to vary greatly with age in cats. Rickets were produced on vitamin D deficient diets in kittens but the cats which survived the acute rickets present during their rapid growing period later developed a spontaneous healing of their rickets. This indicated a lowering of the vitamin D requirements as the cats became older.

Patrick and Bacon (57) observed that the nutritional importance of vitamin D as an aid in calcium utilization is greater in the chick than in the rat. Walker (68) found in rats that unfavorable calcium: phosphorus ratios may actually increase the vitamin D requirement enormously. Gershoff (31), in his studies with chicks, found that the degree of radiocalcium absorption in the absence of vitamin D varied with the mineral content of the diet. It was observed that chicks given a diet containing 1.14 per cent calcium and 1.02 per cent phosphorus (Ca:P ratio one) absorbed more Ca-45 than birds given a diet containing 1.16 per cent calcium and 0.27 per cent phosphorus (Ca:P ratio four). However, in the presence of vitamin D the absorption of

Ca-45 under both situations was the same.

Dietary Requirements for Calcium, Phosphorus, and Vitamin D

By using a slaughter method, Ellenberger et al. (24) reported in 1931 that calves weighing 109 pounds at birth contain 673 grams of calcium. At 1 year of age, they contain 2630 grams of calcium. This gives an average daily retention of 5.4 grams daily. It has been reported that calves retain from 60 to 90 per cent of the ingested calcium (7, 8, 21, 43). Therefore, a wide requirement range for young calves would be from 6 to 9 grams intake of calcium per day. Theiler et al. in 1927 (64) obtained fair growth in heifers fed a ration containing 5 grams of calcium daily. Converse in 1954 (17) fed Holstein and Jersey heifers 7 and 6 grams of calcium daily, respectively, from 6 to 18 months of age. This level was sufficient for normal growth but inadequate for gestation. Henderson and Weakley (41) stated that growing dairy animals fed rations low in calcium (0.29 per cent or less) grow nearly as well as normal fed animals for some time but over a period of 2 years do not make as good a gain as measured by weight and wither height. The National Research Council (49) has set the daily requirement of calcium for the 100 pound calf at 7 grams or 0.77 per cent of the air dried ration.

Henderson and Weakley (41) reported that rations containing less than 0.20 per cent of phosphorus will decrease the amount of inorganic phosphorus in the blood and give rise to a bone which is low in ash. Archibald and Bennett in 1935 (2) observed a larger

phosphorus retention with dairy heifers on an intake of 3.25 grams than on 1.8 grams of the element per 100 pounds body weight per day. These same workers found that 6 to 12 grams of calcium daily from birth to 2 years of age are sufficient for the growth of calves. Eckles et al. (23) states that 8 grams of phosphorus per day were insufficient for the maintenance of normal plasma inorganic phosphorus values in heifers weighing 800 to 1100 pounds. The N.R.C. (49) has set the requirement for this element at 6 grams per day or 0.66 per cent of the air dried ration for the 100 pound dairy calf. Lofgreen et al. (47) found that the true digestibility of phosphorus was approximately 92 per cent and about 7.5 per cent of the daily requirement would go for metabolic loss. Wise et al. in 1958 (70) studied the phosphorus requirements of calves and reported that for maximum feed intakes, growth rates, and bone density, the requirement may be lower than 6 grams per day per 100 pound calf. However, when they allowed for a safety factor, their findings confirmed the N.R.C. requirements.

It was found by Bechtel et al. in 1936 (4) that a daily intake of approximately 100 I.U. of vitamin D per 100 pounds of body weight, furnished by corn silage, effectively cured rickets in yearling heifers. The N.R.C. (49) has set the requirement for vitamin D at 300 I.U. per 100 pounds body weight per day. It is probable that, under usual farm conditions, adequate amounts of vitamin D are supplied by sun-cured roughages or provided by the action of sunlight.

Experimental Approaches to the Problem

It is obvious that much work has been conducted to determine

the relationships of calcium, phosphorus, and vitamin D in animal nutrition and physiology. Most of the experimental designs have been of the type in which two of the factors are fixed and a third is varied. The information obtained from designs such as these are limited in that they describe only one set of conditions in one experiment.

Methods whereby the entire experimental area can be described have been introduced by Box and Wilson in 1951 (10) and Box in 1954 (9). There are methods where a minimum number of experimental points are used to derive regression equations which are used to describe the entire system. Mraz in 1961 (53) studied dietary calcium, phosphorus, and vitamin D_3 effects upon the metabolism of Ca-45, P-32, and Sr-85 in chicks. A central composite experimental design (9, 10) was used in which these three dietary ingredients were varied over a wide range. However, the statistical and mathematical possibilities inherent in the design were not fully exploited. Chandler and Cragle in 1962 (13) conducted a similar study in rats. Response equations were derived and used to plot responses over different dietary combinations. The errors associated with certain measurements were large and a quantitative description of all responses was not possible.

CHAPTER II

EXPERIMENTAL PROCEDURE

Design

A three dimensional central composite block design as described by Box and Wilson (10) and Box (9) was used to study the effects of calcium, phosphorus, and vitamin D_3 upon growth and related processes in Holstein male calves. Calcium and phosphorus levels were varied from 1.25 to 20 grams and 1 to 16 grams per day per 100 pounds body weight, respectively. Vitamin D_3 was varied from 3 to 30,000 I.U. per day per 100 pounds body weight (Table I). These variables were combined and added to the basal ration (Table II) to give the dietary combinations given in Table III. The calves (two per treatment) were fed these rations for a 5-week period or until death.

Basal Ration

A basal ration (Table II) consisting of casein, cerelose, lactose, lard, coconut fat, lecithin, minerals, and vitamins was formulated and mixed in a dry form. The minerals mixture was described by Hansard *et al.* (34) and was calcium and phosphorus free. The vitamins consisted of 73.4 per cent Vitamin Diet Fortification Mixture with vitamin D deleted (Nutritional Biochemical Corporation, Cleveland 28, Ohio) and 26.6 per cent of a mixture consisting of 6.25 per cent inositol, 55.00 per cent choline, 0.125 per cent folic acid and 38.625 per cent starch and lactose filler. Butylated hydroxy toluene

TABLE I

LEVELS OF VARIABLES AND CODED VALUES

Dietary variable	Level				
	-2[a]	-1	0	1	2
Calcium; gms/day/ 100 lb. calf (X_1)	1.25	2.5	5.0	10.0	20.0
Phosphorus; gms/ day/100 lb. calf (X_2)	1.00	2.0	4.0	8.0	16.0
Vitamin D_3; I.U./ day/100 lb. calf (X_3)	3	30	300	3,000	30,000

[a]Code value.

TABLE II

BASAL RATION

Material	Per cent by weight
Casein	25
Cerelose	30
Lactose	15
Lard	10
Coconut fat	5
Lecithin	2
Mineral mixture[a]	2
Vitamin mixture[a]	1
Additive[a]	10

[a]Described in text.

TABLE III

DIETARY COMBINATIONS AND ADDITIVES
TO THE BASAL RATION

Treatment number	Calcium		Phosphorus		Vitamin D_3	
	(grams)[a]	(code)	(grams)[a]	(code)	(I.U.)[a]	(code)
1	2.5	-1	2.0	-1	30	-1
2	10.0	1	2.0	-1	30	-1
3	2.5	-1	8.0	1	30	-1
3a	2.5	-1	5.66	0.5	30	-1
4	10.0	1	8.0	1	30	-1
5	2.5	-1	2.0	-1	3,000	1
6	10.0	1	2.0	-1	3,000	1
7	2.5	-1	8.0	1	3,000	1
7a	2.5	-1	5.66	0.5	3,000	1
8	10.0	1	8.0	1	3,000	1
9	5.0	0	4.0	0	300	0
10	1.25	-2	4.0	0	300	0
11	20.00	2	4.0	0	300	0
12	5.0	0	1.0	-2	300	0
13	5.0	0	16.0	2	300	0
13a	5.0	0	8.0	1	300	0
14	5.0	0	4.0	0	3	-2
15	5.0	0	4.0	0	30,000	2

[a]Per day per 100 lb. calf.

additions at a rate of 1 gram per pound of fat served as the anti-oxidate. The additive contained the desired amount of calcium, phosphorus, and vitamin D_3 (Table III) plus corn starch. Potassium chloride was added to certain diets so that the potassium level of all diets was constant. Calcium and phosphorus were added in the form of $CaCO_3$, KH_2PO_4 and $CaH_4(PO_4)_2 \cdot H_2O$. Crystalline vitamin D_3 was dissolved in corn oil and mixed with the additive. Terramycin was supplemented to the ration to obtain a feeding rate of 25 mg per 100 pound calf per day.

The calves were fed at a rate of 1.95 pounds per 100 pounds body weight per day. This rate is based upon 15 pounds of 13 per cent total solids of whole milk per 100 pound calf per day. The amount fed daily was divided into two equal portions and fed at 7:30 A.M. and 4:30 P.M. Each individual calf's feeding was suspended in 1.4 liters of warm water and fed through a nipple bucket. This basal ration with no calcium or phosphorus additions supplied 1.25 grams of calcium and 1.40 grams of phosphorus daily to the calf. The total intake of calcium and phosphorus (basal plus additive) and the ratio are given in Table IV.

Animal Schedule

The calves were purchased from local farmers when they were from 3- to 7- days of age. They were transported to a heated, darkened calf barn and placed in individual 4 X 8 feet pens with wood shavings as the bedding. A commercial milk replacer (Peebles 10-28 Super Calf Kit, Western Condensing Company, Appleton, Wisconsin) was fed until

TABLE IV

TOTAL CALCIUM AND PHOSPHORUS INTAKE AND CA:P RATIO

Treatment number	Calcium	Phosphorus	Ca + P	Ratio Ca:P
	(grams)[a]			(ratio)
1	3.75	3.40	7.15	1.10
2	11.25	3.40	14.65	3.31
3	3.75	9.40	13.15	0.40
3a	3.75	7.06	10.81	0.53
4	11.25	9.40	20.65	1.20
5	3.75	3.40	7.15	1.10
6	11.25	3.40	14.65	3.31
7	3.75	9.40	13.15	0.40
7a	3.75	7.06	10.81	0.53
8	11.25	9.40	20.65	1.20
9	6.25	5.40	11.65	1.16
10	2.50	5.40	7.90	0.46
11	21.25	5.40	26.65	3.94
12	6.25	2.40	8.65	2.60
13	6.25	17.40	23.65	0.36
13a	6.25	9.40	15.65	0.66
14	6.25	5.40	11.65	1.16
15	6.25	5.40	11.65	1.16

[a]Per day per 100 lb. calf.

the animals were 21 days of age.

At 21-days of age, they were changed to the basal ration shown in Table II. There were no additions of calcium, phosphorus, and vitamin D_3 to the ration. The calves were kept on this depletion diet for one week. At this time, one of the additives as shown in Table III was made to the ration. The calves were randomly assigned to these treatments as they became available until two animals had been designated to each ration. The animals continued on the assigned ration for 5 weeks or until death. At the end of the 5-week feeding period, a 26-hour metabolism period was conducted.

Metabolism Period

Twelve hours before dosing, the calves were placed in metabolism stalls so that urine and feces could be collected. At the morning feeding, on the day of dosing, the calves were given an oral dose of 1.0 mc of Ca-45 and 0.5 mc of P-32. This material was pipetted (volume 2.5 ml or larger) into one-third of the morning feed and fed with the nipple bucket. The bucket was washed with a second one-third of feed and then with the third one-third of feed. This was followed by a water wash which the calf also ingested. At the end of feeding, no significant amount of activity could be detected in the nipple bucket.

After dosing, samples of urine, feces (if available), and blood were taken at 1, 3, 6, 9, 14, and 26 hours. After the last sampling period, the animals were removed from the stalls and taken to the slaughter room where they were stunned with a blow on the head and

bled by a heart-puncture. They were then sampled for bone, liver, kidney, and muscle.

Measurements

Weekly body weights were taken on all calves during the 5-week feeding period. Blood samples were taken at weekly intervals during the feeding period and analyzed for plasma calcium and phosphorus. Radioactive calcium and phosphorus were determined in plasma, urine, and fecal samples taken during the metabolism period. Total calcium and phosphorus were measured in urine and fecal samples. At slaughter, bone samples consisting of the fifth rib, transverse process of the lumbar vertebra, and femur were taken. These were analyzed for bone ash, total calcium and phosphorus and radioactive calcium and phosphorus. Radioactive calcium and phosphorus were also measured in liver, kidney, and sartorius muscle samples.

Analytical

Total calcium was determined by the complexometric titration as revised by Kamal (45) with certain modifications. Plasma samples were analyzed as suggested. The plasma procedure was used for urine. However, sample preparation was modified in that urine samples were dried in an air blast oven, ashed at 650° C. overnight, and then brought into solution in dilute HCl. An aliquot was chosen so that the calcium level would approximate that of plasma and then titrated similar to plasma samples. The milk procedure was used for the determination of calcium in fecal and bone samples. Dried fecal and fat free bone

samples were ashed overnight at 650° C. The sample ash was then brought into solution in dilute HCl. Aliquots were chosen to approximate that of milk samples and titrated by the procedure.

Total phosphorus was determined by the procedure of Fiske and Subbarow (25). Urine, fecal, and bone samples were ashed and brought into solution as described above. Plasma samples were wet ashed by heating 0.5 ml plasma, 1 ml concentrated H_2SO_4, and 1 ml 70 per cent $HClO_4$ over an open flame until a clear solution was obtained. Aliquots were chosen to read within the range of standards and then read on a Beckman Model B Spectrophotometer.

The per cent of ash in bone was determined by taking a dried fat free bone and ashing at 650° C. overnight. The fatty material was removed from fresh bone samples by extracting in a Soxhlet or 20 hours with 95 per cent anhydrous ethanol and then for 20 hours with diethyl ether. The samples were dried for 10 hours at 100° C. before ashing.

Calcium-45 was separated from P-32 by the procedure of Comar et al. (14). The supernatant containing the P-32 was dried in petri dishes. The Ca-45 and P-32 were counted with an end window, Geiger-Mueller tube.

Statistical and Mathematical

As can be noted in Table I, the levels of dietary variables (coded as -2, -1, 0, 1, 2) of calcium and phosphorus were doubled from one level to the next. Each successive level of vitamin D_3 was 10 times as much as the previous level. The equations for interpolation

are as follows:

Dietary Calcium: $\text{Log } Y = 0.301X + 0.699$

Dietary Phosphorus: $\text{Log } Y = 0.301X + 0.602$

Dietary Vitamin D_3: $\text{Log } Y = X + 2.477$

where Y = the dietary level in grams or I.U. per 100 pound calf per day and X = the code value. One dietary level to the next was assumed to result in an equal response as described by linear and quadratic terms. Using the coded values, a multiple regression model was constructed to estimate linear, quadratic, and interaction effects. The model (9, 10) was as follows:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3,$$

where Y is the measured response, X_1, X_2, and X_3 the dietary variables and the b's the regression coefficients.

All data were subjected to analysis of variance procedure. The sums of squares for treatments were subdivided into linear, quadratic, interaction, and deviation from regression components. The analysis of variance headings are shown in Table V. Response equations, standard errors of the regression coefficients, and coefficients of variation were calculated for all measured responses.

TABLE V

ANALYSIS OF VARIANCE HEADINGS WITH A BREAKOUT OF DEGREES OF FREEDOM

Source of variation	Degree of freedom	
Total	30	
Mean	1	
Group	1	
Treatments	14	
Linear		3
X_1		1
X_2		1
X_3		1
Quadratic		3
X_1^2		1
X_2^2		1
X_3^2		1
Interaction		3
X_1X_2		1
X_1X_3		1
X_2X_3		1
Deviation from regression		5
Error	14	

CHAPTER IV

RESULTS

The results are presented in the following sequence: (1) performance of calves on diets are given; (2) the average observed responses for certain measurements which were described with a non-significant deviation from regression (Table V) and were significantly affected by one or more dietary variables are presented in Table VI; (3) equations describing the various phenomena were derived from the observed data and the constants and standard errors of constants for these equations along with coefficients of variation are given in Table VII; (4) a correlation matrix showing correlation coefficients for certain responses are presented in Table VIII; (5) the treatment responses are predicted by use of the derived response equations and the values are compared to the observed values (Table VI); (6) three-dimensional figures which cover the entire experimental range are derived by use of the response equations (Figures 1-13); and (7) individual calf responses for all measurements are presented in the appendix.

Calves performed well on all treatments with the exceptions of treatments three, seven, and 13. Animals on these three rations displayed anorexia within 1 to 2 weeks after the beginning of treatment. Death followed anorexia within 1 to 3 days. Post mortem findings mainly revealed hyperemia and hemorrhage of the abomasum. Submucosal hemorrhages were also noted in the rumen. Strong fermented odor was

TABLE VI

OBSERVED AND PREDICTED VALUES FOR CERTAIN MEASUREMENTS

Response	Treatment number							
	1	2	3a	4	5	6	7a	8
Per cent gain (5-wk period)	54[a]	47	27	47	46	53	46	31
	45[b]	51	37	43	52	47	44	40
Plasma Ca mg % (5-wk period)	9.7	10.5	9.8	9.5	9.4	10.9	9.6	10.1
	9.8	10.5	9.9	9.5	9.6	11.0	9.8	10.1
Plasma P mg % (5-wk period)	18	15	20	18	16	12	18	18
	19	15	20	18	17	13	19	19
Urine Ca mg % (26-hr period)	25	31	17	20	14	67	19	15
	15	35	23	16	15	58	7	18
Urine Ca-45 (% dose 26-hrs)	0.07	0.69	0.14	0.03	0.03	1.35	0.21	0.04
	-0.08[c]	0.81	0.17	0.05	0.00	1.25	-0.04	0.12
Urine P mg % (26-hr period)	85	2	200	139	154	3	238	181
	109	-5	233	170	153	-2	273	167
Urine P-32 (% dose 26-hrs)	6.0	0.2	16.3	11.8	7.2	0.4	18.0	13.9
	6.2	-0.1	17.9	12.7	6.8	0.1	19.4	14.1
Feces Ca, % dry (26-hr period)	1.9	8.6	2.3	8.3	2.5	7.0	1.8	6.1
	1.4	7.9	2.5	8.2	2.3	7.0	2.8	6.6
Feces P, % dry (26-hr period)	1.3	2.6	1.3	4.9	1.3	1.2	1.2	3.1
	0.9	2.1	1.2	4.5	1.6	1.2	1.8	3.5
Femur ash %	43	47	47	46	46	48	46	49
	44	48	45	48	43	48	44	47
Liver Ca-45 % dose X 10^5/gm	5.2	3.3	8.4	4.1	4.9	5.8	5.4	4.0
	5.5	3.1	8.0	3.8	4.9	5.7	5.1	3.3
Liver P-32 % dose X 10^3/gm	3.1	4.3	3.0	2.6	2.9	5.6	2.5	3.7
	3.1	3.8	3.1	2.3	2.7	5.0	2.5	3.2
Muscle Ca-45 % dose X 10^5/gm	5.4	3.6	8.2	5.6	5.4	4.9	7.0	5.4
	5.6	3.7	7.6	5.3	5.3	4.8	6.2	4.9

TABLE VI (continued)

Response	Treatment number						
	9	10	11	12	13a	14	15
Per cent gain (5-wk period)	43[a]	48	51	51	51	37	43
	46[b]	46	50	53	41	37	40
Plasma Ca mg % (5-wk period)	10.1	10.1	10.8	10.3	9.6	9.9	10.5
	9.9	9.9	10.8	10.2	9.6	9.9	10.4
Plasma P mg % (5-wk period)	19	18	14	18	20	18	18
	18	18	13	17	20	18	17
Urine Ca mg % (26-hr period)	15	14	54	24	14	18	13
	24	19	55	28	12	17	19
Urine Ca-45 (% dose 26-hrs)	0.08	0.03	1.39	0.44	0.05	0.04	0.03
	0.25	0.20	1.30	0.49	-0.01[c]	0.00	0.15
Urine P mg % (26-hr period)	134	306	2	2	285	146	144
	139	280	9	8	236	115	156
Urine P-32 (% dose 26-hrs)	9.3	18.7	0.1	0.1	21.8	8.9	10.2
	9.8	17.7	0.3	0.5	19.2	8.1	10.2
Feces Ca, % dry (26-hr period)	4.2	1.9	11.5	4.0	6.8	2.9	3.4
	5.0	1.6	11.7	4.5	5.2	3.4	2.7
Feces P, % dry (26-hr period)	1.9	1.1	3.2	1.3	3.4	1.0	2.1
	2.1	0.8	3.4	1.5	3.0	1.7	1.4
Femur ash %	46	41	52	45	45	49	45
	46	43	50	45	46	47	46
Liver Ca-45 % dose X 10^5/gm	5.5	6.3	2.2	2.6	3.5	7.6	6.4
	5.1	6.3	2.7	2.6	4.6	7.7	6.7
Liver P-32 % dose X 10^3/gm	3.3	2.6	3.4	3.0	2.3	3.3	3.5
	3.4	2.4	4.1	3.3	2.6	3.4	3.9
Muscle Ca-45 % dose X 10^5/gm	6.9	6.3	4.0	2.2	4.9	6.3	5.2
	6.7	6.7	4.1	2.1	6.3	6.4	5.7

[a]Observed values (average of two calves).

[b]Predicted values.

[c]Negative values should be considered as zero.

TABLE VII

REGRESSION COEFFICIENTS, STANDARD ERRORS AND COEFFICIENTS OF VARIATION

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Response (Y)	b_o	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}	Coeff. Varia.
Av. wk. gain	46.01[a]	1.10	-4.64*	0.69	0.50	-0.61	-1.87	1.00	-2.62	-0.03	
	8.07[b]	2.61	4.17	2.51	2.80	4.46	2.80	4.30	3.57	3.98	19.65
Av. Plasma Ca	9.85	0.24*	-0.19	0.11	0.12	-0.02	0.07	-0.30	0.17	0.03	
(mg %)	0.18	0.06	0.09	0.06	0.06	0.10	0.06	0.10	0.08	0.09	5.74
Av. Plasma P	17.73	-1.42**	1.68**	-0.31	-0.55**	0.56*	-0.05	0.44	0.15	0.52	
(mg %)	1.17	0.38	0.61	0.37	0.41	0.65	0.41	0.63	0.52	0.58	7.70
Av. Urine Ca	23.10	9.03**	-8.00*	0.39	3.39*	-2.69	-1.27	-6.60	5.62	-5.29	
(mg %)	8.48	2.75	4.39	2.64	2.95	4.69	2.95	4.52	3.75	4.18	49.40
Urine Ca-45	0.25	0.27**	-0.21*	0.04	0.13*	-0.05	-0.04	-0.26*	0.09	-0.10	
(% dose)	0.16	0.05	0.08	0.05	0.06	0.09	0.06	0.09	0.07	0.08	37.51
Av. Urine P	139.21	-67.64**	86.29**	10.32	1.31	10.39	-1.00	-0.27	-10.28	-1.34	
(mg %)	34.10	11.00	17.60	10.60	11.80	18.90	11.80	18.20	15.10	16.80	38.18
Urine P-32	9.84[a]	-4.34**	7.80**	0.51	-0.21	1.57	-0.17	-1.10	-0.11	0.32	
(% dose)	1.38[b]	0.45	0.71	0.43	0.48	0.76	0.48	0.74	0.61	0.68	37.85
Feces Ca	4.96	2.52**	0.26	-0.17	0.42	0.02	-0.47	-0.27	-0.44	-0.18	
(%)	0.91	0.30	0.47	0.28	0.32	0.50	0.32	0.49	0.40	0.45	48.21

TABLE VII (continued)

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

Response (Y)	b_o	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}	Coeff. Varia.
Feces P	2.12	0.65**	0.71*	-0.08	0.00	0.20	-0.15	0.43	-0.40	-0.02	
(%)	0.58	0.19	0.30	0.18	0.20	0.32	0.20	0.31	0.25	0.28	58.25
Femur ash	46.00	1.86**	0.21	-0.21	0.07	-0.24	0.19	-0.43	0.16	-0.29	
(%)	2.04	0.66	1.06	0.64	0.71	1.13	0.71	1.09	0.90	1.01	4.80
Liver Ca-45	5.14	-0.91**	0.10	-0.26	-0.17	-0.60**	0.52	-0.52	0.80	-0.75	
(% dose X 10^5)	0.59	0.19	0.31	0.18	0.21	0.33	0.21	0.32	0.26	0.29	26.60
Liver P-32	3.38	0.42**	-0.51*	0.13	-0.03	-0.27*	0.06	-0.33	0.40*	-0.07	
(% dose X 10^3)	0.49	0.16	0.26	0.15	0.17	0.27	0.17	0.26	0.22	0.24	21.41
Muscle Ca-45	6.70	-0.65*	0.46**	-0.17	-0.32	-0.91	-0.17	-0.02	0.34	-0.39	
(% dose X 10^5)	0.71	0.23	0.37	0.22	0.25	0.39	0.25	0.38	0.31	0.35	28.68

[a]Regression coefficient.

[b]Standard error.

*$P < 0.05$.

**$P < 0.01$.

TABLE VIII

CORRELATION MATRIX

	Plasma Ca	Plasma P	Urine Ca	Urine Ca-45	Urine P	Urine P-32	Feces Ca	Feces P	Femur ash	Liver Ca-45	Liver P-32	Muscle Ca-45
% gain		1								$\bar{0}$.548**		$\bar{0}$.547**
Plasma Ca	1.000	$\bar{0}$.396*	0.570**	0.677**	$\bar{0}$.465**	$\bar{0}$.410*					0.393*	
Plasma P		1.000	$\bar{0}$.623**	$\bar{0}$.649**	0.617**	0.485**					$\bar{0}$.620**	
Urine Ca			1.000	0.943**	$\bar{0}$.604**	$\bar{0}$.567**					0.607**	
Urine Ca-45				1.000	$\bar{0}$.575**	$\bar{0}$.517**			0.364*		0.588**	
Urine P					1.000	0.890**	$\bar{0}$.460*				$\bar{0}$.526**	0.426*
Urine P-32						1.000	$\bar{0}$.404*			0.386*	$\bar{0}$.402*	0.537**
Feces Ca							1.000	0.774**	0.498**	$\bar{0}$.557**		$\bar{0}$.361*
Feces P								1.000		$\bar{0}$.474**		
Femur ash									1.000			
Liver Ca-45										1.000		0.779**
Liver P-32											1.000	

*Value significant ($P < 0.05$).

**Value significant ($P < 0.01$).

associated with the contents in the forestomachs. Treatments three-a, seven-a, and 13a replaced treatments three, seven, and 13 in the three-dimensional design.

The results from Table VII indicate that coefficients of variation on the responses described by regression equations varied from a low of 4.80 to a high of 58.25. Those values with low coefficients (i.e. femur ash per cent, plasma calcium, and phosphorus) may be considered as directly dependent upon the dietary variables, whereas those responses with higher coefficients may be secondary. Dietary calcium and phosphorus expressed a significant linear effect on most of the responses. No linear effects were shown for vitamin D_3. A significant linear effect was apparent for vitamin D_3 in the uptake of Ca-45 in blood but due to only 26 hours in the metabolism period, this effect could not be quantitated.

Response equations are of considerable importance in predicting responses for any untried combination of experimental variables within the limits of the experimental design. If a statistically non-significant deviation from regression (Table V) of the model from the experimental data exists reliable predictions can be made (Table VI). The results presented are described with a non-significant deviation from regression.

The dietary effects upon growth are presented in Figure 1. Dietary phosphorus expressed a negative effect upon growth ($P<0.05$). The effects expressed by calcium and vitamin D_3 were non-significant. The depressing effects of dietary phosphorus are greater at low calcium

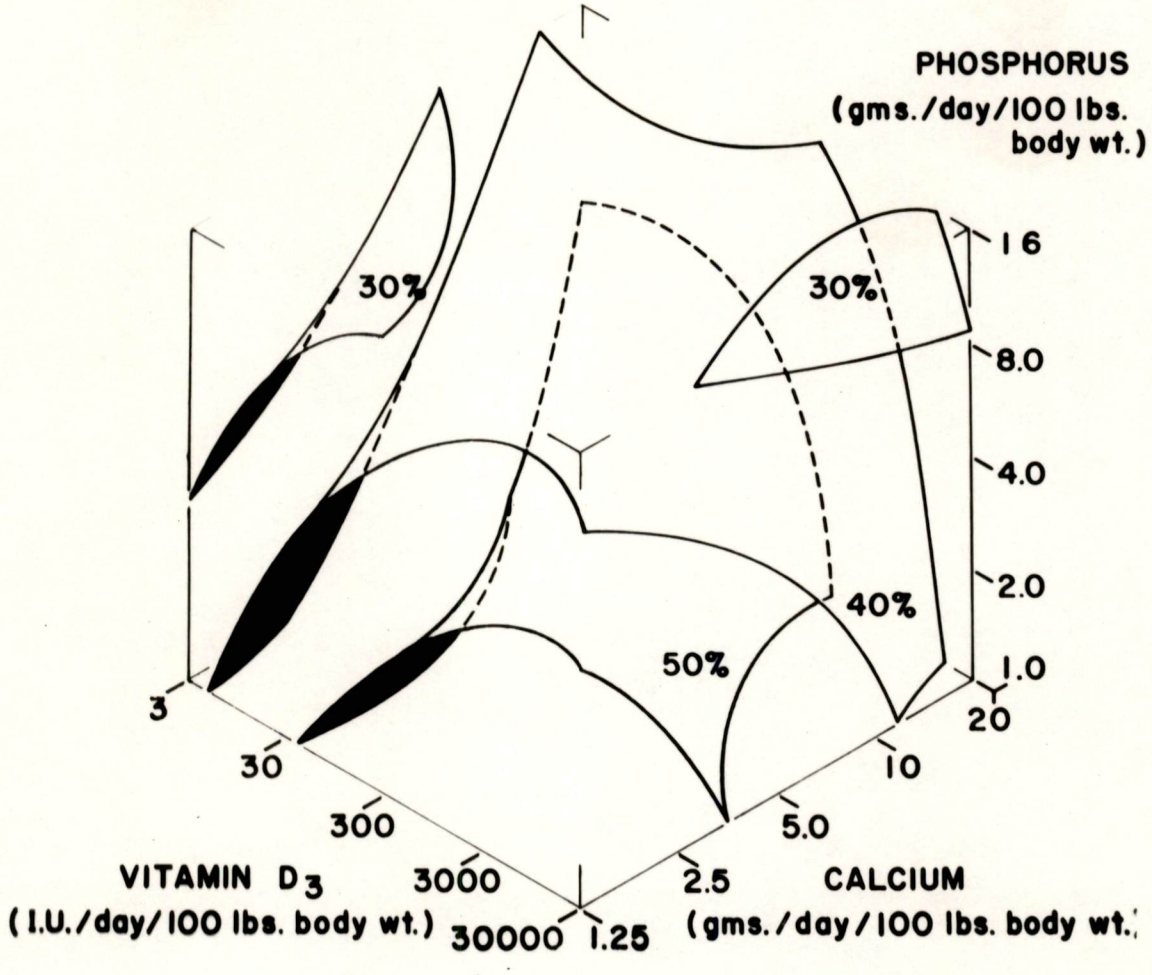


Figure 1. The per cent gain in weight for the 5-week period.

and vitamin D or high calcium and vitamin D levels. A 50 per cent and greater increase in growth is observed over approximately 25 per cent of the experimental area. The 40 per cent contour covers approximately 80 per cent of the area.

Plasma calcium and phosphorus levels are given in Figures 2 and 3. Plasma calcium was affected significantly only by dietary calcium whereas both dietary calcium and phosphorus affected plasma phosphorus. At low levels of vitamin D and high levels of phosphorus, increasing the dietary calcium caused a decrease in plasma calcium whereas at high vitamin D and low phosphorus the opposite was observed (Figure 2). It can be observed from Figure 3 that dietary calcium expressed its greatest depressing effect upon plasma phosphorus at low dietary phosphorus levels. At high levels of dietary calcium, dietary phosphorus expressed a greater increasing effect than is present at lower levels. The 16 per cent contour covered approximately 40 per cent of the total experimental area.

The dietary effects upon urine are presented in Figures 4, 5, 6, and 7. Dietary calcium and phosphorus significantly affected the concentration of calcium and phosphorus in urine. Increased amounts of calcium caused an increase in urinary calcium and a decrease in urinary phosphorus ($P < 0.01$) whereas, increased amounts of phosphorus caused a decrease in urinary calcium ($P < 0.05$) and an increase in urinary phosphorus ($P < 0.01$). Dietary combination that resulted in high concentrations of urinary calcium (50 mg per cent) had approximately 1.2 per cent of the Ca-45 dose in 26 hours (Figures 4 and 5). Those

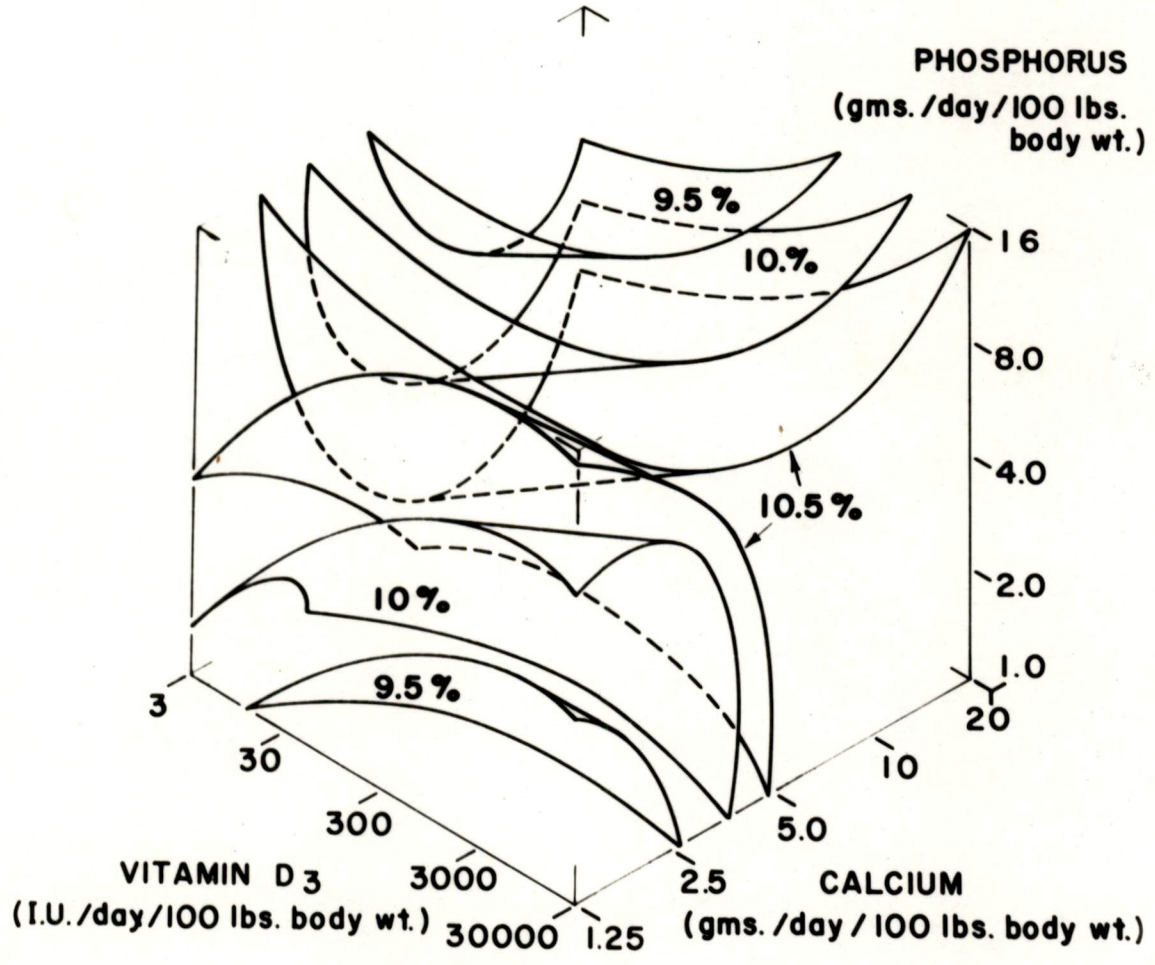


Figure 2. The average plasma calcium concentration (mg %) for the 5-week period.

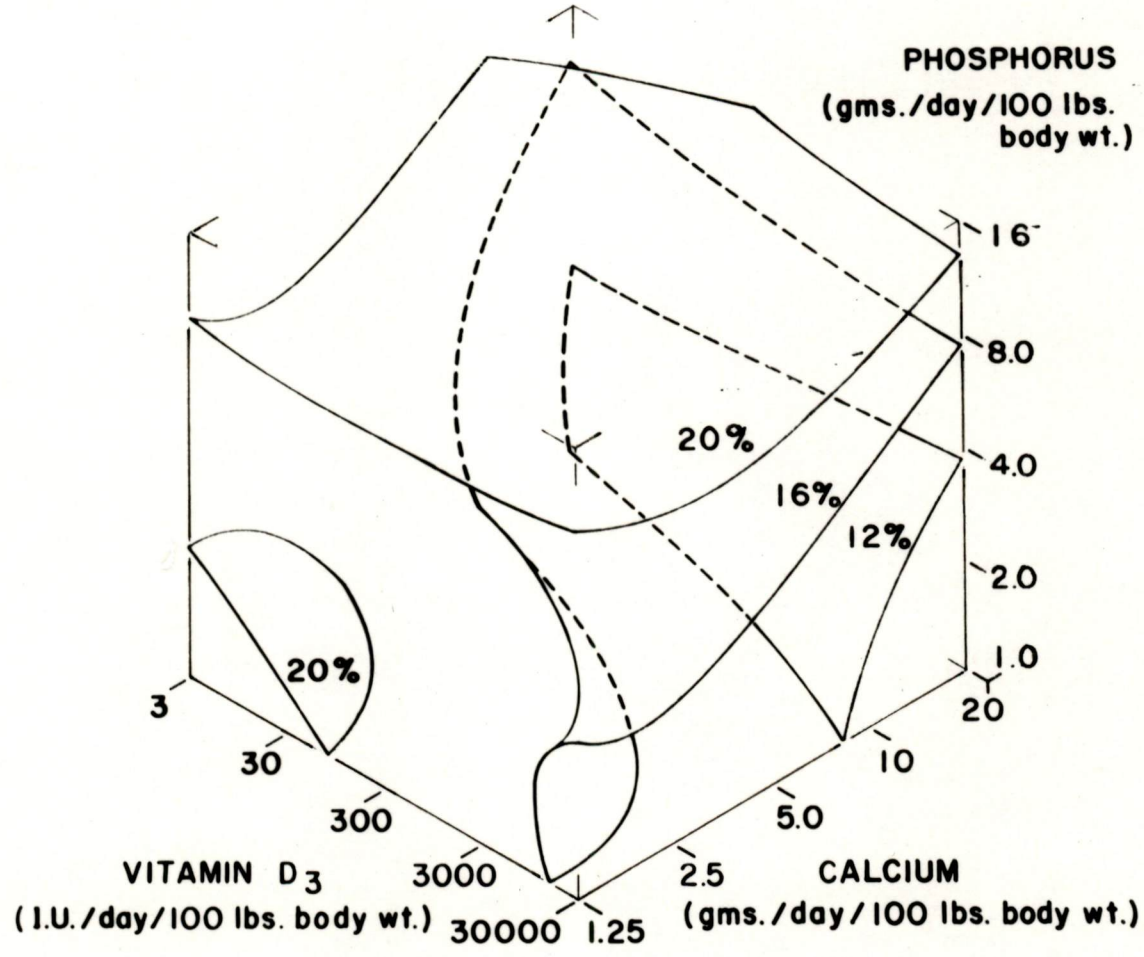


Figure 3. The average plasma phosphorus concentration (mg %) for the 5-week period.

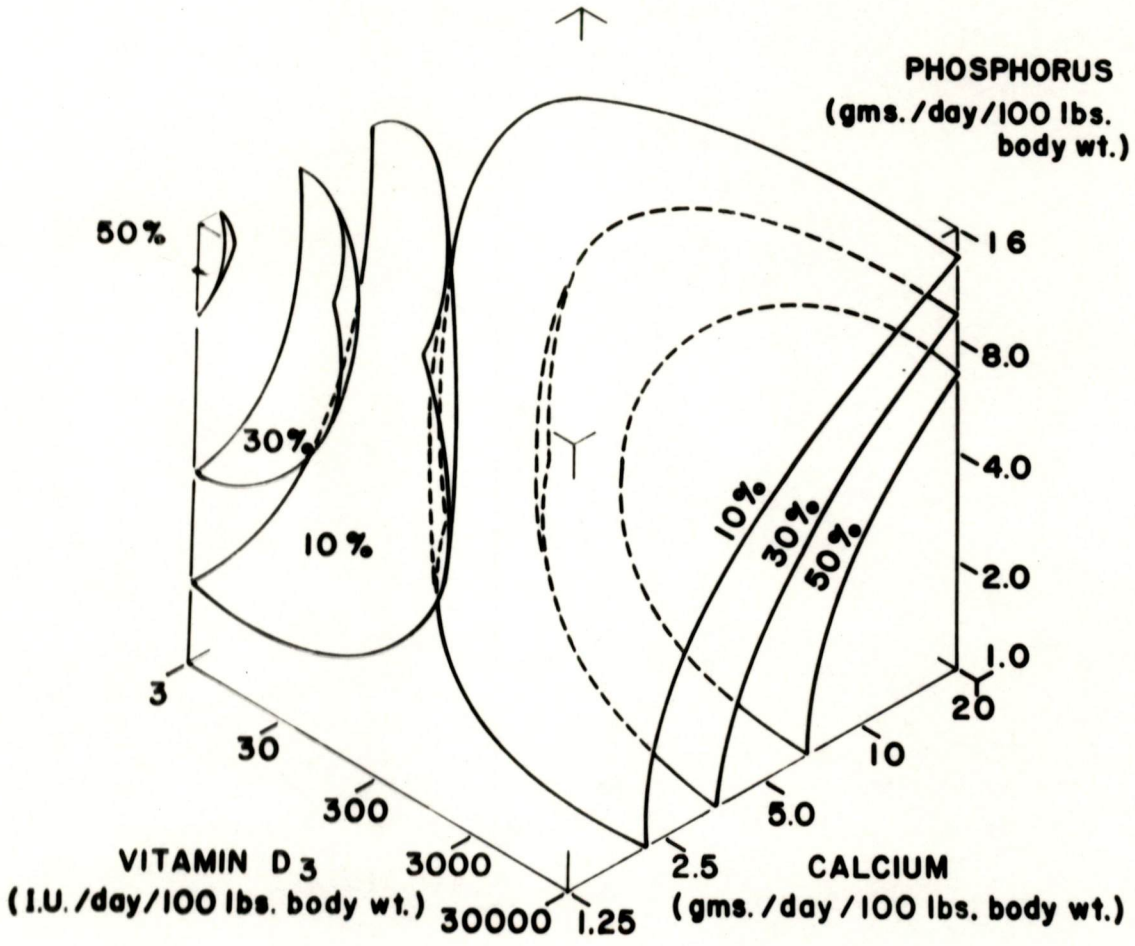


Figure 4. The average urine calcium concentration (mg %) for the 26-hour period.

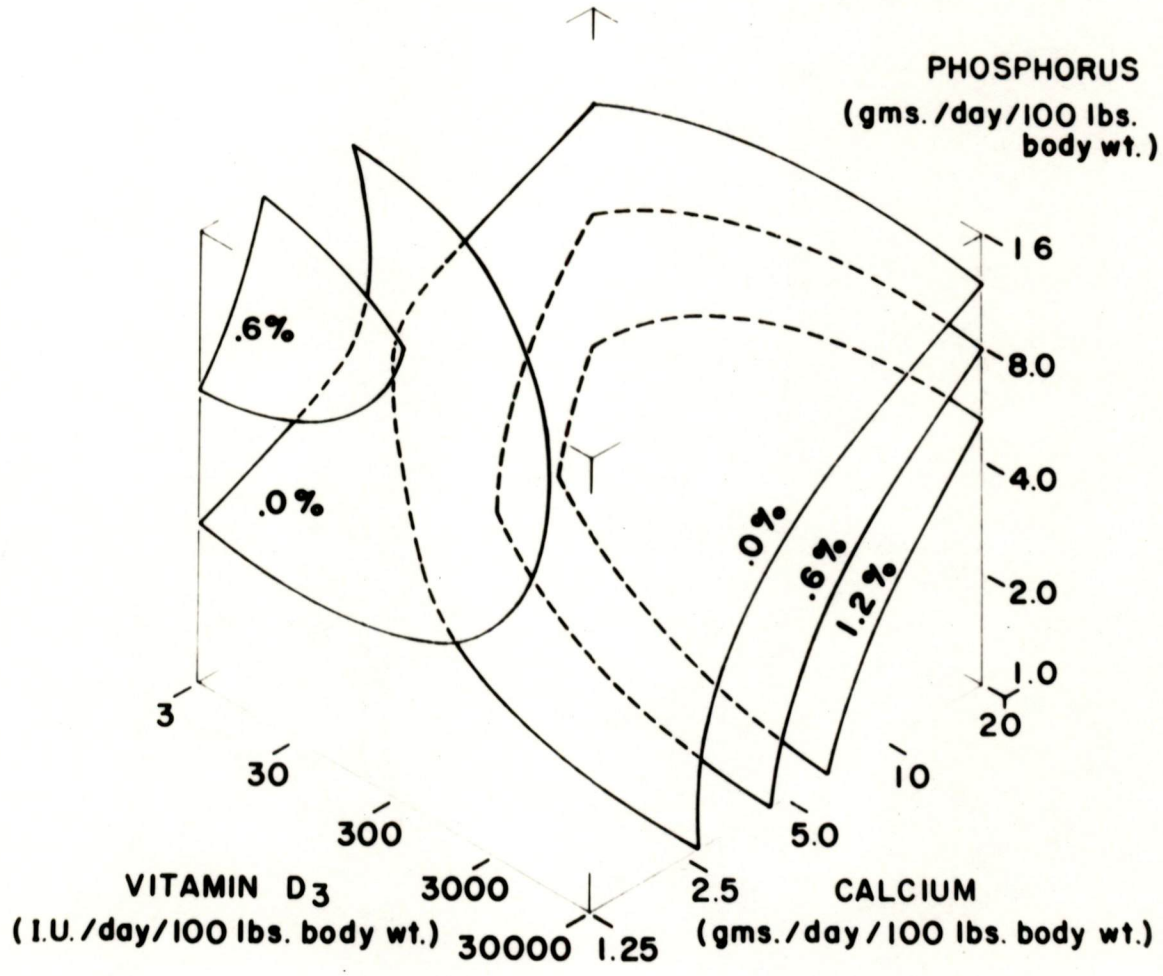


Figure 5. The total per cent of dose of Ca-45 for the 26-hour period.

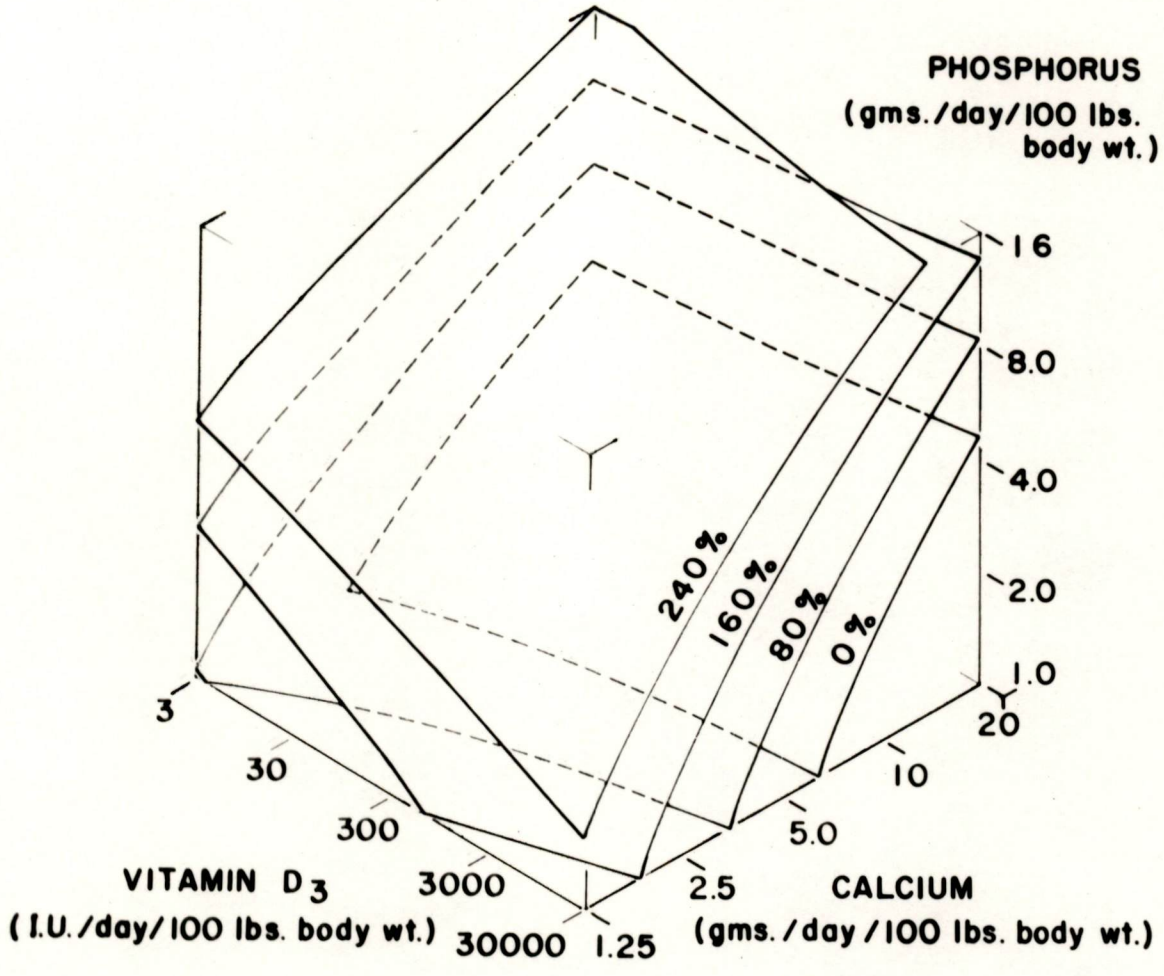


Figure 6. The average urine phosphorus concentration (mg %) for the 26-hour period.

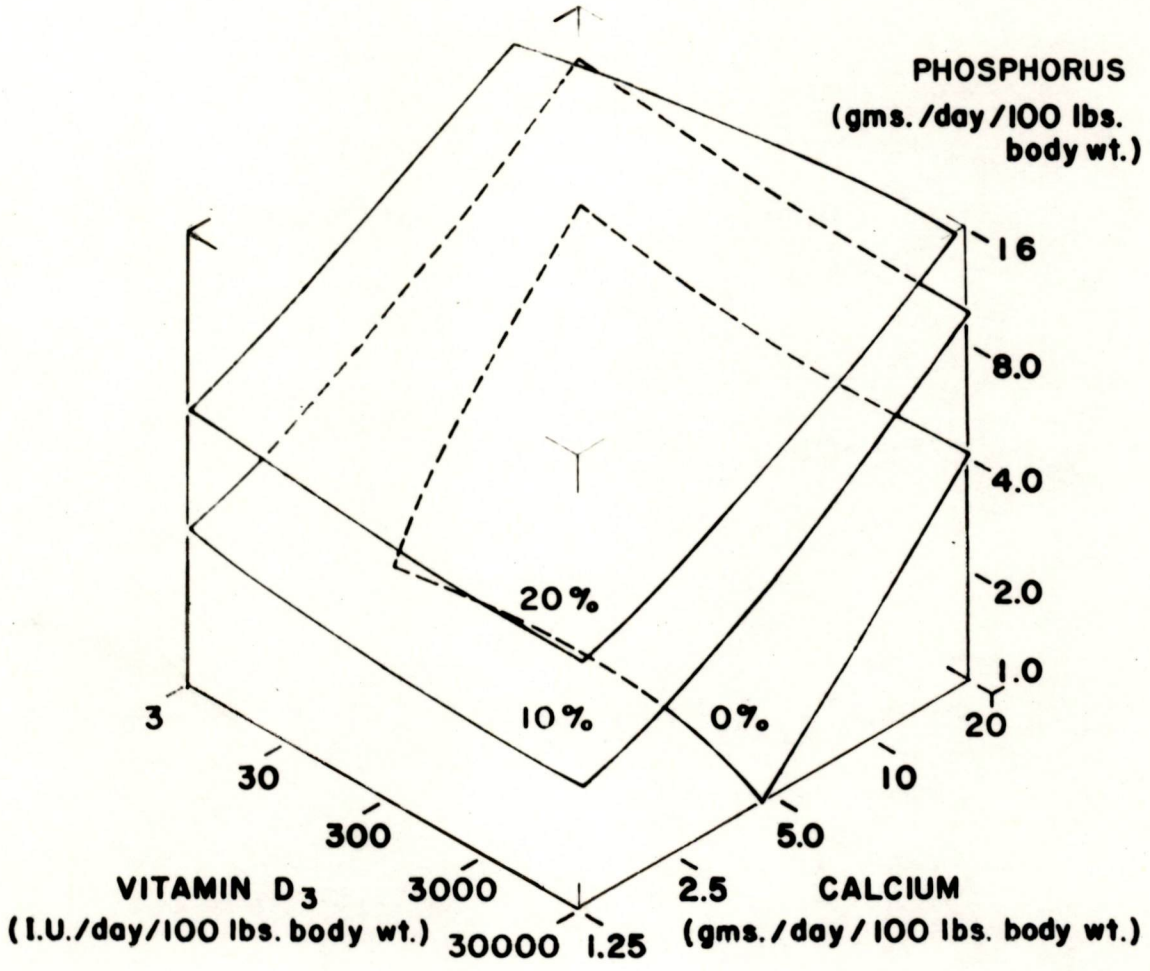


Figure 7. The total per cent of dose of P-32 for the 26-hour period.

dietary combinations that resulted in high urinary phosphorus (240 mg per cent) were associated with approximately 20 per cent of the P-32 dose in 26 hours (Figures 6 and 7). The P-32 concentration was directly dependent on stable phosphorus and Ca-45 concentration was not directly dependent upon stable calcium. Zero per cent dose of P-32 was associated with zero mg per cent phosphorus and zero per cent dose of Ca-45 was associated with 10 mg per cent calcium.

Fecal calcium and phosphorus concentrations are illustrated in Figures 8 and 9. Fecal calcium was affected significantly only by dietary calcium ($P < 0.01$) and fecal phosphorus was affected by dietary calcium ($P < 0.01$) and phosphorus ($P < 0.05$).

The dietary effects upon femur ash per cent are given in Figure 10. Dietary calcium increased ($P < 0.01$) femur ash per cent. A coefficient of variation of 4.80 per cent indicates a sensitive measurement. The effect of increased calcium was the greatest at low levels of phosphorus and high levels of vitamin D.

Liver Ca-45 and P-32 uptakes are presented in Figures 11 and 12. Ca-45 uptake in liver was decreased as dietary calcium was increased ($P < 0.01$). Increased dietary calcium with concomitant decreases in vitamin D_3 results in small changes. The highest per cent uptakes were observed with dietary combinations of high calcium, low phosphorus, and high vitamin D or low calcium, medium to high phosphorus and low vitamin D. The uptake of P-32 in liver was increased as dietary calcium was increased ($P < 0.01$), and decreased as dietary phosphorus was increased ($P < 0.05$). Dietary calcium expressed greater increasing

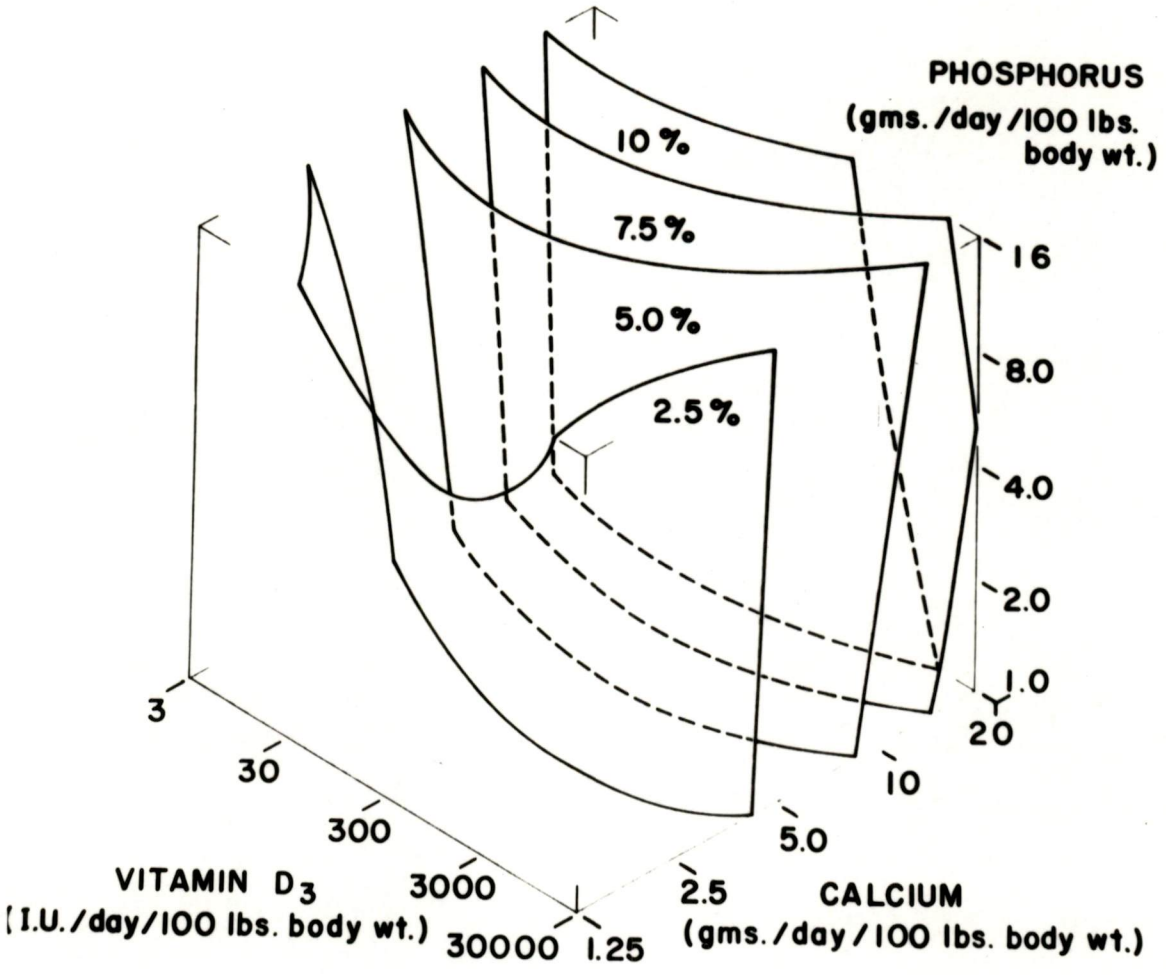


Figure 8. The average feces calcium concentration (per cent) for the 26-hour period.

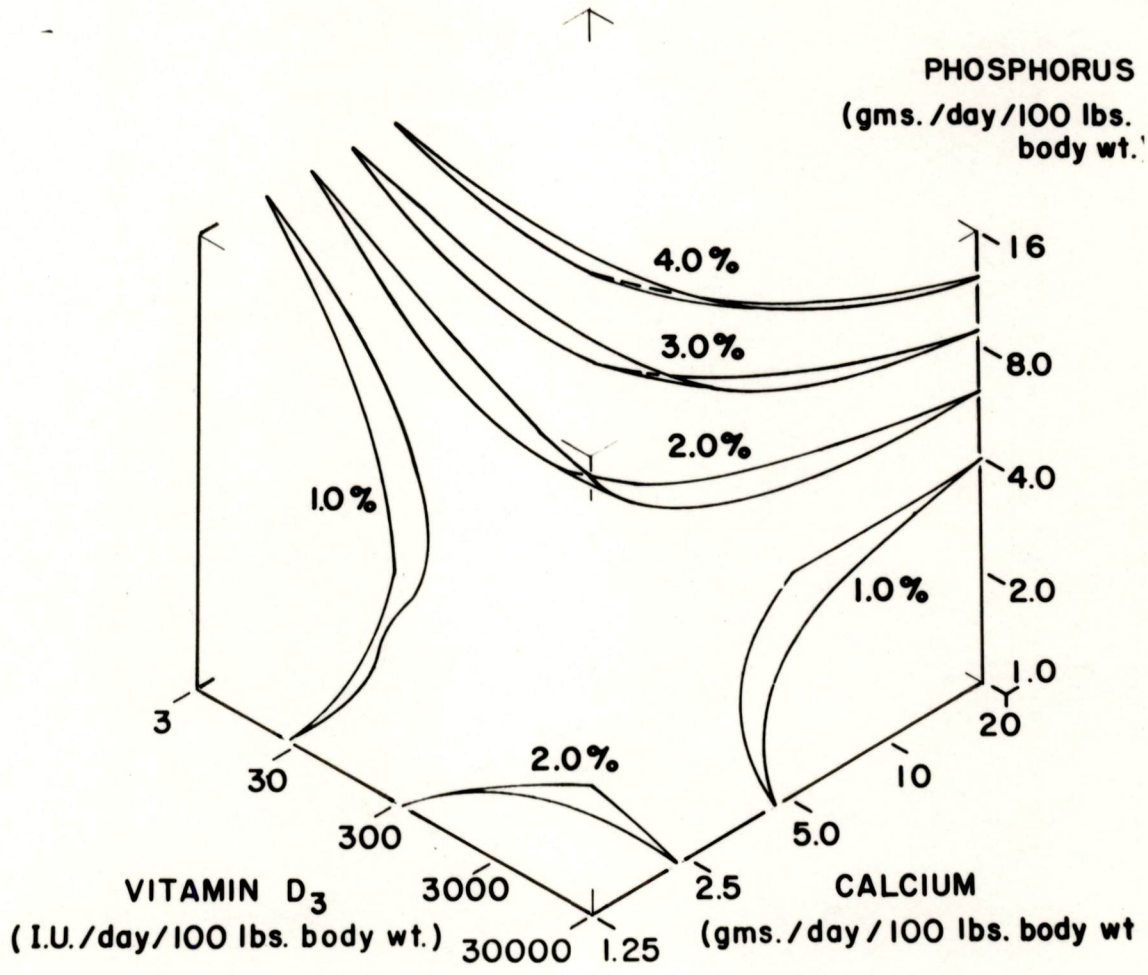


Figure 9. The average feces phosphorus concentration (per cent) for the 26-hour period.

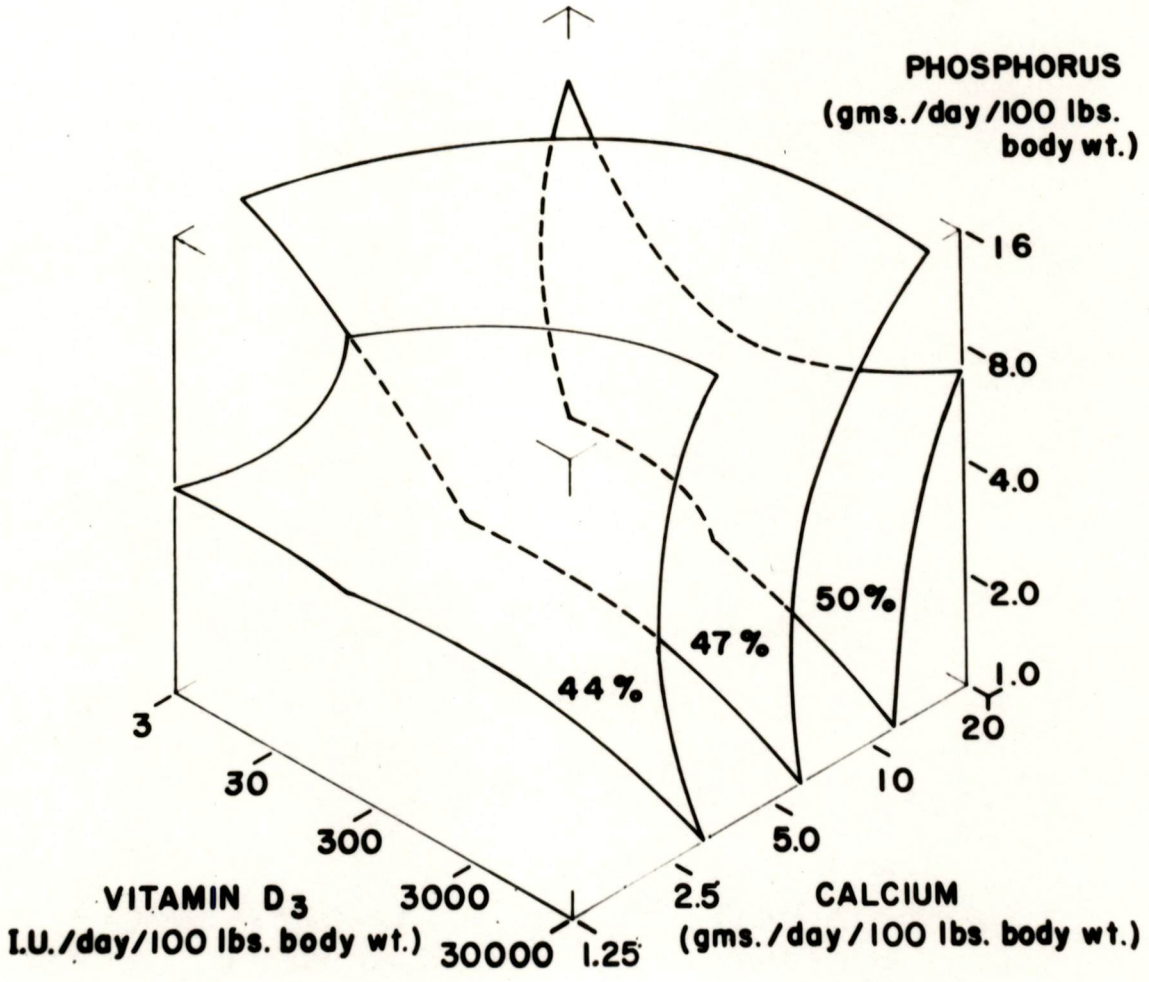


Figure 10. The per cent ash in femur.

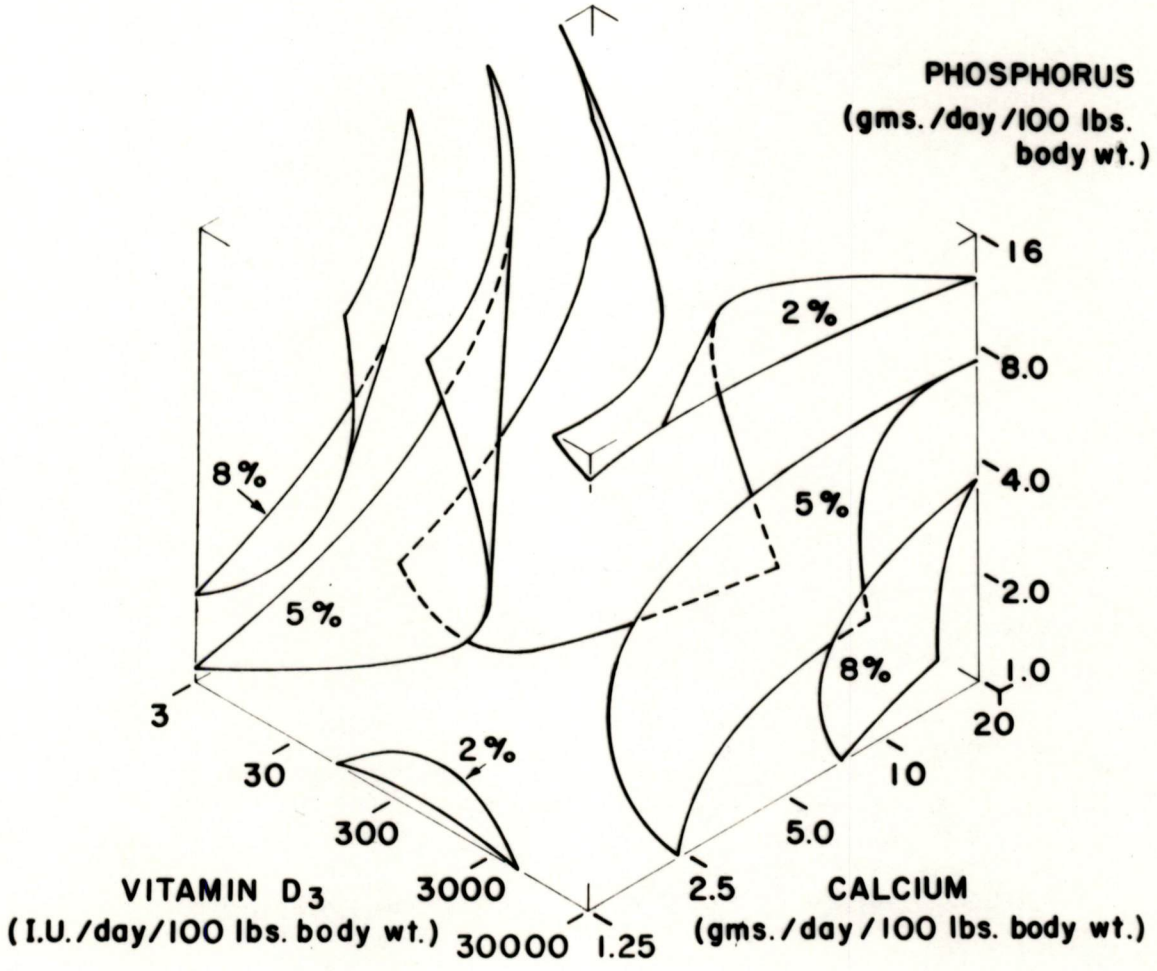


Figure 11. The liver Ca-45 per cent dose X 10^5 per gram.

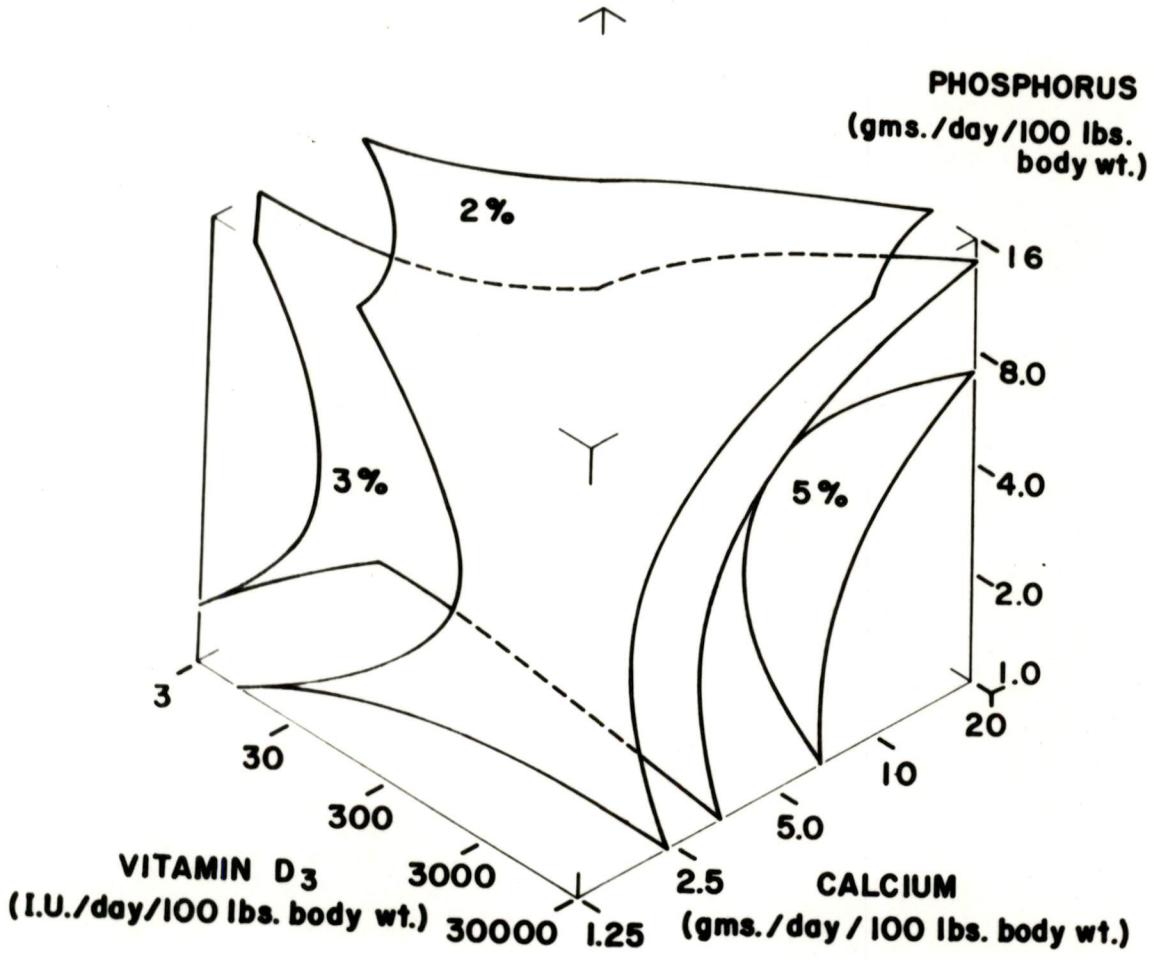


Figure 12. The liver P-32 per cent dose X 10^3 per gram.

affects at low phosphorus levels. The highest per cent uptake of P-32 was observed at high calcium, low to medium phosphorus and high vitamin D_3.

Calcium-45 uptake by muscle as affected by the dietary variables is given in Figure 13. Increases in dietary calcium decrease uptake ($P < 0.05$) and increases in dietary phosphorus increased uptake ($P < 0.01$). The higher per cent uptakes of Ca-45 were associated with dietary combinations that were low in calcium, medium to high in phosphorus and low in vitamin D_3.

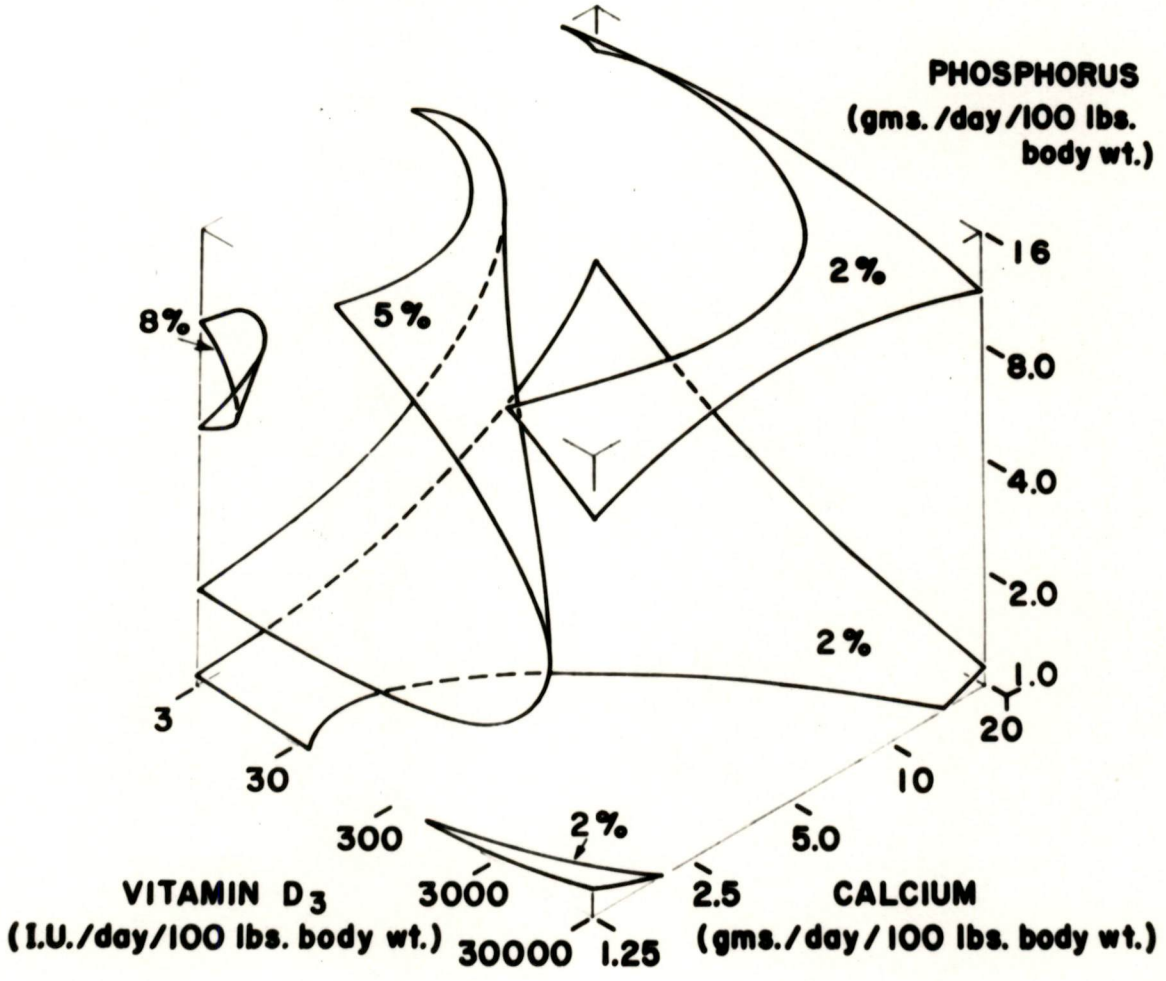


Figure 13. The muscle Ca-45 per cent dose X 10^5 per gram.

CHAPTER V

DISCUSSION

In this experiment the effects of vitamin D upon calcium and phosphorus metabolism were small. This was observed in a previous experiment of this type with rats (13). In the rat study, sources of vitamin D other than dietary were not removed and no attempt was made to deplete the animals of body stores. In the reported study, the calves were housed throughout the entire experiment and for 3 weeks pre-experimental in a closed darkened barn. Also, a 7-day period during which no vitamin D was added to the ration was conducted before the experimental feeding period. According to other work, definite responses of measurements such as plasma calcium and phosphorus and bone ash per cent should have responded to dietary vitamin D (15, 22, 62). The fact that the animals did not respond could be due to a significant amount of vitamin D in the basal ration. No vitamin D assay was conducted. Also the removal from the diet of calcium and phosphorus along with vitamin D_3 during the 7-day pre-experimental period could have lowered the stress that was placed on the animals. There were indications that the animals were beginning to become responsive to vitamin D_3 toward the end of the feeding period. This was indicated by the significant effect of vitamin D_3 upon Ca-45 uptake in plasma. However, due to the length of the metabolism period (26 hours), this response could not be quantitatively

expressed.

The performance of calves on rations containing abnormally low calcium:phosphorus ratios seems to have been related to the total intake of calcium and phosphorus. From Table IV it can be observed that treatments three, three-a, seven, seven-a, ten, 13, and 13a have Ca:P ratios below one, (calves died on treatments three, seven, and 13). The Ca:P ratio and total intake, respectively, for treatments three and seven were 0.40 and 13.15 grams. Calves on treatments (three-a and seven-a) with a higher ratio (0.53) and lower salt intake (10.81 grams) grew normal. Treatment 13 resulted in a ratio of 0.36 and a total intake of 23.65 grams. Calves performed normally when the ratio was raised to 0.66 and the salt (calcium and phosphorus) intake lowered to 15.65 grams, (treatment 13a). These results support the conclusions that calves in this study were able to perform normally at high levels of salt (approximately 15 grams), only when the ratio was 0.50 or above. They could tolerate abnormally low ratios only at reduced salt intakes (treatment ten).

It has been reported with calves (20) and with pigs (11, 46) that high ratios decrease growth rate and feed consumption. This was attributed to excess calcium because these high ratios were obtained by adding calcium to a constant phosphorus ration. Calcium has been postulated to interfere with the digestion and/or absorption of nutrients and mineral elements other than phosphorus (19, 20). In the present study, dietary intake was controlled and held at a unit weight. Any effects expressed would be the result of digestion and/or

absorption. It can be observed from the response equation in Table VII and from Figure 1 that calcium did not significantly affect growth rate. The decreasing effect observed for dietary phosphorus may be due to the formation of abnormally low ratios as phosphorus increases. The deviation from regression of this response equation approached significance at the 0.05 level (F value = 2.58 and F value $P < 0.05 = 2.96$).

Plasma calcium and phosphorus levels were directly related to the dietary level. It has been demonstrated with sheep (50) that the amounts of calcium and phosphorus absorbed are significantly dependent on dietary calcium and phosphorus, respectively. The response equation for plasma phosphorus (Table VII) expresses a linear and quadratic relationship to dietary calcium and phosphorus. An explanation for the decreased stable phosphorus when animals were fed high calcium levels may be through a competition between the cations (Ca^{++}) on the mucosal side of the gut with anions on the serosal side, one of these being PO_4^{---}.

Shelling (60) showed that excess calcium is excreted through the kidneys until its concentration in the urine reaches a maximum and the remainder is excreted through the gut as the insoluble phosphate salt. Excess phosphorus follows a path similar to calcium, first through the kidneys, which are able to excrete phosphorus to a limited concentration, and then the remainder through the bowel as the calcium salt, thus removing calcium from the body. The results with calves support the effects of excessive dietary calcium. However, dietary phosphorus was found to not affect fecal calcium. This

is in agreement with other work (1, 50, 65). The kidney of the calf was able to excrete large quantities of phosphorus (Figures 6 and 7) and urinary phosphorus and calcium were negatively correlated (Table VIII).

It has been reported that cattle normally excrete 0.5 per cent or less of a Ca-45 dose by way of urine (33, 35). The animals in these studies were fed rations containing normal amounts of calcium and phosphorus. From Figure 5, it can be observed that approximately 75 per cent of the experimental area covered was associated with 0.6 per cent or less urinary Ca-45 dose. As the Ca:P ratio becomes greater than one and the total salt intake above 10 grams, urinary excretion of Ca-45 is one per cent or larger.

When the urinary excretion of calcium and Ca-45 are compared (Figures 4 and 5), it is found that the experimental area associated with 10 mg per cent calcium shows no Ca-45 excretion. The urinary calcium excreted in these areas is from sources other than dietary. Therefore 10 mg per cent of calcium in urine would be an estimate of urinary metabolic calcium.

The results from urine are also in agreement with those reported by Vermeulen (67). Concentrations of calcium in the urine of rats were reported to approach 100 mg per cent on high calcium diets. From Figure 4 it can be observed that dietary combinations of high calcium and medium to low phosphorus resulted in a 50 mg per cent or greater concentration. A reciprocal relationship between urinary calcium and phosphorus similar to the one described in rats (67) was shown to exist in the calf. Urine volume did not vary significantly

($P < 0.05$) and the concentrations expressed in Figures 4 and 6 are an indication of actual excretion. The urinary excretion of calcium and phosphorus was dependent upon the plasma concentrations of the two elements. The correlation coefficients from Table VIII were positive ($P < 0.01$) for plasma calcium to urinary calcium and Ca-45 and plasma phosphorus to urinary phosphorus and P-32.

The equation derived for femur ash per cent in calves is in agreement with the one derived for rats (13). A smaller coefficient of variation is associated with the calf equation. This could be due to the analytical procedure for bone ash determination. The calf bones were analyzed on a fat free basis while fat was not removed from the rat bones. The responses of bone ash per cent and fecal calcium were similar. They are significantly affected only by dietary calcium and are positively correlated (Tables VII and VIII).

The equation describing Ca-45 in muscle is of interest when one examines the quadratic terms. All of the terms are negative so therefore a partial derivative of the equation with respect to X_1, X_2, and X_3 and a simultaneous solution result in a stationary point which is a maximum (10). The maximum value is found to be 9.8×10^{-5} per cent of dose at 0.11 grams of calcium, 17.58 grams of phosphorus, and zero I.U. of vitamin D_3. Inspection of the curves in Figure 13 indicates a movement toward a maximum in that direction.

In the study reported by Chandler and Cragle (13), only those responses describing fecal and urinary measurements were described with a non-significant deviation from regression. The rats in the

study were fed ad libitum and no correction was made for differences in dietary intake. Controlled dietary intake in the calf experiment made it possible to describe other measurements with non-significant deviation from regression.

In this experiment with calves, the experimental error between duplicate samples within calves expressed as a coefficient of variation was approximately 5 per cent. A comparison of this value with the coefficient of variation for the various measurements between calves gives an indication of the magnitude of the variation associated with a measurement exclusive of analytical variation. This variation is an important clue as to the nature of the system being measured. For example, the measurements of femur ash per cent, plasma calcium, and plasma phosphorus have coefficients of 4.80, 5.74, and 7.70, respectively (Table VII). The mechanisms involved with these measurements can be thought of as being direct and not influenced appreciably by other mechanisms. However, the calcium and phosphorus concentrations in feces, where coefficients of variation are 48.21 and 58.25, respectively, it is obvious that alternate metabolic possibilities exist (13).

CHAPTER VI

SUMMARY

Thirty Holstein male calves were used to determine the effects of dietary calcium, phosphorus, and vitamin D_3 upon growth and responses related to growth. In addition, the validity of a mathematical expression of biological systems was investigated.

A three-dimensional central composite experimental design with five levels of each of the three variables in 15 treatments was employed. Dietary calcium and phosphorus were varied from 1.25 to 20 and 1 to 16 grams per day per 100-pound calf, respectively. Vitamin D_3 was varied from 3 to 30,000 I.U. per day per 100-pound calf.

Growth rate was found to be dependent only on dietary phosphorus in that growth was decreased as phosphorus increased. Calcium and phosphorus ratios less than 0.50 could be tolerated only at reduced intakes of calcium and phosphorus (approximately 8 grams). Calves were able to perform normally at high levels of salt (approximately 15 grams), only when the ratio was 0.50 or above.

The urinary excretion of calcium and phosphorus were found to be related in a reciprocal manner. In urinary excretion, high phosphorus concentration was associated with low calcium and high calcium was associated with low phosphorus. The urinary excretion was highly correlated with plasma concentrations and concentrations were directly dependent upon dietary intakes of calcium and phosphorus.

Coefficients of variation were the smallest on measurements such as femur ash per cent, plasma calcium, and phosphorus. This indicates a direct mechanism, not being influenced appreciably by other mechanisms.

The observed data for 13 responses were fitted to a quadratic response model. Lack of fit of the model for other responses was associated with large errors between calves and variation due to uncontrolled factors.

GRADUATE CREST

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APPENDIX

TABLE IX

PLASMA AND GROWTH VALUES FOR INDIVIDUAL CALVES

Treatment no.	Calf no.	Plasma Ca[c] mg %	Plasma P[c] mg %	Plasma Ca-45 26 hr.[a]	Plasma P-32 26 hr.[a]	Plasma Ca-45 3 hr.[a]	Plasma P-32 3 hr.[a]	Growth Wt. 4 wks.[b]	Growth Wt. 9 wks.[b]	Growth % gain
1	340	10.05	19.6	13.02	13.18	1.94	1.95	88	137	55
	118	9.28	16.8	13.11	24.16	1.32	1.97	120	183	53
2	342	9.81	15.8	12.10	10.04	1.17	1.06	99	148	49
	124	11.11	13.2	9.11	18.09	1.37	1.71	105	152	45
3a	347	9.69	22.5	14.14	20.00	2.09	1.88	94	115	22
	119	9.90	18.4	15.49	14.18	2.51	1.38	109	143	31
4	344	9.90	21.3	11.30	13.26	1.27	1.62	97	140	44
	128	9.16	14.4	11.06	10.25	2.01	1.83	105	158	50
5	325	8.89	18.4	11.00	16.11	1.21	1.39	123	173	40
	121	9.89	14.3	11.78	11.32	1.58	1.62	116	175	51
6	318	10.52	13.7	9.49	11.72	1.66	1.63	92	138	50
	114	11.28	10.0	17.10	21.77	2.56	2.70	109	169	55
7a	110	9.40	20.4	9.57	14.56	1.95	2.48	111	171	54
	120	9.87	16.2	13.78	15.07	1.83	1.45	104	142	37
8	317	10.19	19.6	13.35	10.89	2.22	1.50	120	173	44
	116	9.92	16.9	16.47	29.52	1.38	2.72	94	111	18
9	314	10.03	20.1	12.19	11.01	2.49	1.73	117	169	44
	111	10.17	17.8	17.82	24.11	4.05	2.82	100	141	41

TABLE IX (continued)

Treatment no.	Calf no.	Plasma Ca[c] mg %	Pc mg %	Ca-45 26 hr.[a]	P-32 26 hr.[a]	Ca-45 3 hr.[a]	P-32 3 hr.[a]	Growth Wt. 4 wks.[b]	Wt. 9 wks.[b]	% gain
10	312	10.22	21.1	11.61	7.60	1.34	1.01	97	148	53
	112	9.87	15.8	15.76	27.70	3.18	2.95	114	163	43
11	337	9.97	13.3	8.79	5.80	1.43	0.86	106	164	55
	123	11.62	13.8	6.73	11.04	1.16	1.69	108	158	46
12	345	10.68	18.1	14.73	18.54	1.74	3.11	108	167	54
	127	9.87	17.3	11.06	9.88	1.06	1.14	110	163	48
13a	343	9.10	21.0	14.78	13.93	2.02	1.62	99	142	43
	125	10.08	18.1	9.12	10.67	1.20	1.49	115	183	59
14	324	9.93	18.6	15.57	12.10	1.84	1.38	103	141	37
	115	9.89	17.0	20.25	23.39	2.23	2.18	119	163	37
15	315	10.90	20.9	19.89	17.24	3.60	2.43	98	129	32
	113	10.14	15.4	20.91	23.40	3.43	3.17	95	146	53

[a]Uptake - square inches of area under a curve of concentration vs. time.

[b]Weight in pounds.

[c]Average for 5-week period.

TABLE X

F VALUES FOR PLASMA AND GROWTH MEASUREMENTS

Source	Degree of freedom	Plasma Ca	Plasma P	Plasma Ca-45 26 hr.	Plasma P-32 26 hr.	Plasma Ca-45 3 hr.	Plasma P-32 3 hr.	% gain
Groups	1	0.77	44.93**	1.54	5.04*	0.83	1.86	0.03
Treatments	14	1.31	6.07**	2.65*	0.54	2.66*	0.65	1.61
X_1	1	5.90*	29.93**	2.92	1.22	2.00	0.31	0.20
X_2	1	2.69	21.63**	0.56	0.01	2.14	0.02	5.15*
X_3	1	1.15	2.16	0.77	0.25	4.06	2.48	0.20
X_1^2	1	1.41	11.02**	9.55**	9.84	2.88	1.26	1.13
X_2^2	1	1.21	4.92*	4.15	0.57	9.55**	0.12	0.33
X_3^2	1	0.11	0.00	8.08*	0.54	0.02	0.47	1.05
X_1X_2	1	2.83	1.18	0.07	0.02	0.32	0.00	0.14
X_1X_3	1	1.48	0.43	4.49	2.07	2.00	0.86	1.43
X_2X_3	1	0.04	1.89	0.01	0.48	0.39	0.04	0.00
Dev. from reg.	5	0.30	2.36	1.30	0.32	2.77	0.70	2.58

*= $P < 0.05$.

**= $P < 0.01$.

TABLE XI

URINE AND FECES VALUES FOR INDIVIDUAL CALVES

Treatment no.	Calf no.	Urine					Feces[c]	
		Volume[a] 26 hr.	Ca mg %	P mg %	Ca-45[b] 26 hr.	P-32[b] 26 hr.	Ca %	P %
1	340	1705	31.4	44.0	0.106	3.47	1.67	1.18
	118	1875	18.3	125.7	0.042	8.48	2.17	1.44
2	342	1884	29.1	2.7	0.406	0.12	9.88	3.16
	124	2882	33.6	1.8	0.980	0.18	7.33	2.10
3a	347	1536	17.0	195.0	0.053	10.57	2.84	1.39
	119	3034	17.3	205.3	0.236	22.01	1.75	1.18
4	344	2462	18.5	187.0	0.029	14.93	6.12	4.18
	128	2410	21.8	90.0	0.031	8.68	10.38	5.65
5	325	2065	11.9	242.0	0.031	10.53	1.95	0.60
	121	1929	15.2	105.7	0.042	3.79	3.00	2.01
6	318	1869	46.1	3.4	0.656	0.20	7.75	0.69
	114	2013	88.2	3.4	2.036	0.62	6.38	1.67
7a	110	1696	19.1	324.0	0.104	15.50	1.18	0.78
	120	4023	18.9	151.7	0.316	20.43	2.31	1.63
8	317	2783	15.0	176.0	0.023	9.93	7.55	4.84
	116	1514	14.2	185.0	0.063	17.78	4.63	1.32

TABLE XI (continued)

Treatment no.	Calf no.	Urine					Feces[c]	
		Volume[a] 26 hr.	Ca mg %	P mg %	Ca-45[b] 26 hr.	P-32[b] 26 hr.	Ca %	P %
9	314	2739	17.3	134.8	0.096	8.93	6.40	2.96
	111	2118	13.3	133.3	0.070	9.59	1.93	0.90
10	312	1928	15.6	323.3	0.027	17.89	1.03	0.28
	112	2373	12.0	288.7	0.028	19.41	2.66	1.81
11	337	1967	38.1	2.7	0.654	0.18	15.68	4.36
	123	3781	69.8	1.2	2.133	0.10	7.34	1.99
12	345	1464	7.8	2.4	0.036	0.07	5.57	1.95
	127	2609	39.5	1.8	0.843	0.18	2.47	0.61
13a	343	2604	19.9	230.0	0.078	16.65	10.03	4.71
	125	3391	7.7	340.3	0.030	27.00	3.48	2.06
14	324	2188	22.1	154.7	0.051	10.71	4.76	1.38
	115	1647	13.0	136.7	0.034	7.07	0.98	0.57
15	315	1948	13.6	175.0	0.030	9.46	5.25	3.21
	113	2897	12.3	113.0	0.036	10.99	1.58	0.92

[a]Volume in ml.

[b]Per cent dose.

[c]Dry basis.

TABLE XII

F VALUES FOR URINE AND FECES MEASUREMENTS

Source	Degree of freedom	Urine					Feces	
		Volume	Ca	P	Ca-45	P-32	Ca	P
Groups	1	4.19	1.26	1.21	5.09*	1.71	5.18*	2.25
Treatment	14	0.58	3.63*	7.58**	3.25*	7.37**	3.28*	1.86
X_1	1	0.52	17.31**	44.52**	17.77**	29.40**	39.97**	10.52**
X_2	1	2.32	7.89*	52.33**	7.48*	66.90**	0.16	6.25*
X_3	1	0.28	0.12	1.28	0.42	0.53	0.15	0.19
X_1^2	1	0.09	6.80*	0.01	8.14*	0.73	3.01	0.00
X_2^2	1	0.02	1.30	0.76	0.71	2.43	0.37	2.20
X_3^2	1	0.81	0.76	0.01	1.01	0.16	2.57	0.18
X_1X_2	1	0.80	3.37	0.00	5.38*	0.90	0.14	1.42
X_1X_3	1	1.25	2.82	0.65	0.75	0.00	0.62	1.79
X_2X_3	1	0.15	2.52	0.01	0.86	0.09	0.08	0.00
Dev. from reg.	5	0.39	1.58	1.32	0.59	0.41	0.46	0.71

* = $P < 0.05$.

** = $P < 0.01$.

TABLE XIII

BONE[a] VALUES FOR INDIVIDUAL CALVES

Treatment no.	Calf no.	5th rib		Vertebra[b]	Femur	Bone ash[c]				
		Prox. % ash	Mid % ash	% ash	% ash	% ash	Ca %	P %	Ca-45[d]	P-32[d]
1	340	16	47	27	38	32	36.0	21.0	17.1	12.5
	118	14	53	31	47	36	34.6	16.6	11.7	6.9
2	342	22	53	37	47	40	36.2	17.4	8.3	7.8
	124	29	67	37	47	45	34.4	17.7	8.4	6.0
3a	347	29	53	30	48	40	36.3	17.8	4.8	4.8
	119	18	54	33	46	38	36.8	17.9	10.5	7.4
4	344	25	50	31	47	38	35.2	17.6	10.1	7.0
	128	21	52	33	45	38	35.5	19.8	11.4	6.0
5	325	24	54	32	47	39	35.1	18.3	10.2	5.5
	121	23	52	29	44	37	36.1	17.4	11.4	7.2
6	318	23	50	34	48	39	37.4	18.8	9.3	8.3
	114	22	56	31	47	39	36.5	18.5	14.1	10.3
7a	110	26	56	36	46	41	36.1	18.6	8.2	5.5
	120	19	53	33	46	38	38.1	18.9	8.7	6.3
8	317	27	57	40	49	43	37.1	18.7	7.6	4.1
	116	21	56	32	48	39	37.4	18.3	5.9	7.1
9	314	25	53	45	46	42	37.8	18.9	12.1	6.2
	111	17	53	30	45	36	37.6	18.3	14.7	12.5

TABLE XIII (continued)

Treatment no.	Calf no.	5th rib Prox. % ash	5th rib Mid % ash	Vertebra[b] % ash	Femur % ash	Bone ash[c] % Ash	Bone ash[c] Ca %	Bone ash[c] P %	Ca-45[d]	P-32[d]
10	312	22	49	29	38	35	36.6	18.7	13.9	7.5
	112	20	51	34	43	37	36.9	18.1	14.1	19.4
11	337	26	50	40	52	42	39.0	19.2	7.9	5.7
	123	24	57	37	51	42	37.1	19.7	8.0	6.3
12	345	21	52	26	45	36	35.6	16.4	10.6	6.9
	127	23	51	34	45	38	35.8	18.3	11.1	7.1
13a	343	20	51	25	45	35	34.7	17.3	15.3	7.1
	125	21	50	31	45	37	35.5	18.1	12.1	5.8
14	324	16	52	37	50	39	38.7	19.3	12.8	7.3
	115	18	54	32	47	38	35.0	17.8	12.9	7.7
15	315	23	51	39	44	39	36.4	18.8	14.6	7.8
	113	22	49	29	45	36	36.5	17.2	14.7	11.8

[a] Dry fat free basis.

[b] Transverse process of lumbar vertebra.

[c] Average amount in all bone samples.

[d] 10^2 X % dose.

TABLE XIV

F VALUES FOR BONE MEASUREMENTS

	Degree of freedom	% ash prox. rib	% ash mid rib	Lumbar vertebra % ash	% ash femur	Av. % ash	% Ca	% P	% Ca-45 dose	% P-32 dose
Group	1	3.55	2.90	0.79	0.01	0.26	0.64	0.48	0.42	2.40
Treatments	14	1.53	1.42	1.00	2.75*	2.24	1.79	0.53	3.92**	1.19
X_1	1	4.18	3.28	4.14	23.00**	1.31	1.04	0.52	8.37*	3.40
X_2	1	0.12	0.09	0.19	0.50	0.21	1.19	0.67	0.98	0.65
X_3	1	3.33	1.48	0.06	0.23	0.11	1.60	0.01	0.00	0.10
X_1^2	1	1.41	0.00	0.66	0.02	1.01	3.06	1.66	2.44	1.36
X_2^2	1	0.47	0.38	3.11	0.91	1.40	3.46	0.83	3.45	1.39
X_3^2	1	0.26	0.47	0.04	0.11	0.16	0.13	0.00	0.11	0.03
X_1X_2	1	1.87	1.42	0.13	0.41	2.45	0.40	0.64	2.29	0.00
X_1X_3	1	2.21	0.73	0.31	0.05	1.35	1.80	0.18	0.31	0.81
X_2X_3	1	0.24	3.35	1.35	0.21	0.84	0.00	0.00	1.10	0.01
Dev. from reg.	5	1.47	2.01	0.80	2.62	2.17	2.46	0.59	7.16**	1.79

* = $P < 0.05$.

** = $P < 0.01$.

TABLE XV

SOFT TISSUE[c] VALUES FOR INDIVIDUAL CALVES

Treatment no.	Calf no.	Liver		Kidney		Muscle	
		Ca-45[b]	P-32[a]	Ca-45[b]	P-32[a]	Ca-45[b]	P-32[a]
1	340	5.20	2.74	6.18	1.99	4.88	0.49
	118	5.21	3.38	6.97	2.02	5.94	0.96
2	342	3.37	4.71	5.73	1.75	3.35	0.52
	124	3.28	3.79	4.21	2.06	3.84	0.78
3a	347	8.03	2.72	12.92	1.64	8.42	0.43
	119	8.66	3.20	16.91	1.58	7.90	0.63
4	344	3.91	2.28	7.03	1.58	6.14	0.39
	128	4.34	2.81	5.18	1.66	4.96	0.68
5	325	5.84	3.28	7.94	1.43	6.36	0.84
	121	3.86	2.42	6.56	1.38	4.52	0.60
6	318	4.44	4.94	5.32	1.65	4.66	1.00
	114	7.24	6.18	8.80	2.60	5.10	1.61
7a	110	3.23	1.90	4.40	1.15	4.86	0.32
	120	7.48	3.16	16.02	1.58	9.04	0.80
8	317	1.74	2.18	5.79	1.48	4.29	0.48
	116	6.15	5.22	11.17	2.55	6.58	1.10
9	314	3.62	2.84	4.04	1.48	4.76	0.78
	111	7.42	3.65	11.70	2.34	9.12	1.04

TABLE XV (continued)

Treatment no.	Calf no.	Liver		Kidney		Muscle	
		Ca-45[b]	P-32[a]	Ca-45[b]	P-32[a]	Ca-45[b]	P-32[a]
10	312	5.42	2.32	7.38	1.48	5.32	0.83
	112	7.26	2.96	12.68	2.82	7.34	0.66
11	337	1.74	3.22	4.41	1.05	4.10	0.59
	123	2.66	3.60	6.14	2.03	3.82	0.90
12	345	2.67	2.57	5.42	1.62	3.93	1.08
	127	2.46	3.34	5.70	1.97	0.46	0.64
13a	343	3.45	2.52	35.02	1.46	5.94	0.70
	125	3.44	1.98	8.02	1.38	3.79	0.54
14	324	7.58	3.40	9.34	1.93	6.23	1.12
	115	7.66	3.24	5.19	1.78	6.42	1.10
15	315	6.40	3.55	6.58	1.40	4.59	0.91
	113	6.40	3.44	9.96	2.20	5.70	0.71

[a] 10^3 X % dose.

[b] 10^5 X % dose.

[c] % dose/gram wet tissue.

TABLE XVI

F VALUES FOR SOFT TISSUE MEASUREMENTS

Source	Degree of freedom	Liver Ca-45	Liver P-32	Kidney Ca-45	Kidney P-32	Muscle Ca-45	Muscle P-32
Groups	1	5.36*	3.57	0.05	12.58*	0.62	2.99
Treatments	14	3.54*	2.77*	1.00	1.09	1.80	1.60
X_1	1	15.46**	9.91**	1.60	0.00	6.56*	1.00
X_2	1	2.48	6.76*	4.48	1.30	9.35**	4.05
X_3	1	0.84	1.22	0.02	0.11	0.29	0.25
X_1^2	1	1.46	0.00	0.63	0.27	0.05	0.35
X_2^2	1	12.87**	5.59*	0.44	0.32	4.06	2.51
X_3^2	1	3.12	0.03	0.94	0.18	0.34	0.37
X_1X_2	1	1.65	1.58	1.32	0.00	0.00	0.13
X_1X_3	1	4.55	4.98*	0.48	4.26	0.57	3.50
X_2X_3	1	4.05	0.11	0.11	0.51	0.81	1.25
Dev. from reg.	5	0.61	1.55	0.80	1.66	0.64	1.79

* = $P < 0.05$.

** = $P < 0.01$.