A STUDY OF THE CALCIUM AND PHOSPHORUS REQUIREMENTS OF YOUNG PIGS REARED UNDER ARTIFICIAL ENVIRONMENTAL CONDITIONS

By

JAMES ARTHUR COALSON

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Abilene Christian College

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Thesis Approved:

J. C. Hillier
Thesis Adviser

Eldon Nelson

D. D. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Calcium and phosphorus have long been recognized as essential elements in skeletal formation, and growth and maintenance of physiological functions in swine. Calcium and phosphorus requirements for young pigs have recently been under investigation because of increased growth rates due to diet formulation, antibiotics and sanitation procedures. It was thought that specific-pathogen-free (SPF) pigs might have higher calcium and phosphorus requirements than conventionally reared pigs because of lowered disease levels, but this has not been proven.

This investigation was undertaken to study the effects of four calcium and phosphorus levels upon young SPF pigs and to see if the pathological condition known as atrophic rhinitis can be initiated on a low calcium and phosphorus diet.

In the establishment of standard requirements for calcium and phosphorus, major emphasis has been placed almost consistently on growth and feed efficiency. In this study, more emphasis will be placed on various blood components and slaughter data information.

CHAPTER II

LITERATURE REVIEW

Calcium and Phosphorus Functions

Calcium and phosphorus are very essential since they make up over 70 percent of the ash of the animal body (Maynard and Loosli, 1962). As a part of the total ash, calcium makes up approximately 2 percent of the animal's weight and phosphorus about 3 percent (Ruch and Fulton, 1960). About 99 percent of the calcium and 80 percent of the phosphorus are present in the bones. The dried bone is composed of from 30 to 40 percent organic substances and 60 to 70 percent inorganic material. The inorganic material is composed of two highly insoluble calcium salts: $Ca_3(PO_4)$, 85 percent and $CaCO_3$, 10 percent; plus a small amount of magnesium. The ash portion contains about 36 percent calcium and 17 percent phosphorus, resulting in an approximate 2 to 1 ratio (Maynard and Loosli, 1962). The 1.0 percent body calcium which occurs outside the bones is widely distributed throughout the organs and tissues. The large amounts of phosphorus, existing outside the bones, are present mostly in organic combinations such as phosphoprotein, nucleoprotein, phospholipids and phosphocreatine.

Calcium Studies

The concentration of calcium in sow's milk (1 to 3 weeks lactation) consistently falls within the range of 0.8 to 1.0 percent, on a dry-matter basis (Jylling, 1960 and Perrin, 1965). This same concentration in synthetic milk diets will not support optimum skeletal development, thus, the calcium supplied in such manner must have lower availability (Blair, 1963). According to Blair and Benzie (1964), a calcium level of 1.6 percent gave the most rapid skeletal development.

Studies were made with 40 baby pigs, using a synthetic milk diet, with the phosphorus level maintained at 0.5 percent and calcium varied from zero to 1.6 percent of dietary solids (Miller et al., 1962). Optimal skeletal development, as measured by bone density, ash content, breaking strength and the absence of rachitic symptoms, occurred in pigs consuming 1.0 percent of calcium. Maximal calcium retention occurred at this dietary level also. Calcium content of the liver, heart and kidneys was directly related to calcium intake. Levels of serum phosphorus and alkaline phosphatase were inversely related to dietary and serum calcium levels.

This evidence would suggest a higher calcium requirement than 0.8 percent recommended for the 4.5 kg. pig (N.R.C., 1964).

A total of 202 pigs weaned at 14 days of age were used in five experiments by Combs and Wallace (1962) to study the influence of increasing increments of calcium with phosphorus held constant at 0.44 percent. Calcium was increased from 0.40 to 0.80 percent by increments of 0.10 percent. These levels resulted in a decreasing linear response with respect to daily gains and feed efficiency.

Sixteen crossbred pigs were used by Rutledge et al. (1961) to study the calcium requirement of 3- to 9-week-old pigs using practical type diets containing 0.4, 0.6, 0.8, or 1.0 percent of dietary calcium and 0.6 percent phosphorus. Levels of dietary calcium had no consistent effect on rate of gain, efficiency of feed utilization, or on blood serum calcium and phosphorus. There were no marked differences in ash, calcium and phosphorus contents among femur samples due to treatment. Radiographs of the femurs and humeri indicated marked increases in degree of calcification and in bone density with increased dietary calcium. Rutledge et al. suggested that 0.8 percent dietary calcium is near the minimum required for normal bone development in pigs between 3 and 9 weeks of age.

Using a purified diet, Dudley et al. (1961) fed a total of 96 fourteen day old pigs for five to six weeks to investigate their response to dietary calcium levels. Pigs fed 0.1 percent calcium and 0.8 percent phosphorus developed weak legs and pasterns. Maximum efficiency and rate of gain were obtained with 0.2 percent calcium, while the ash content of the femur increased with all increments of calcium up to 2.0 percent of the diet.

The above review of the literature points up the need for re-evaluation of present calcium requirements for young pigs. The work of Miller et al. (1962) indicate that the calcium requirement may be higher than 0.80 percent for baby pigs. Rutledge et al. (1961) suggests that 0.80 percent calcium is near the minimum for normal bone development, while Dudley et al. (1960) reports that 0.20 percent calcium will support maximum efficiency and rate of gain without any reference to status of bone or skeletal development.

Phosphorus Studies

The phosphorus content of sow's milk, for the first three weeks, is consistently near 0.60 percent. This is, perhaps, the principle reason for the requirement for the 4.5 kg. pig to be stated as 0.60 percent (N.R.C., 1964).

Bethke et al. (1933) asserted that the minimal phosphorus requirement for swine to be 0.60 percent, irrespective of the amount of calcium present. Using a total of 144 baby pigs (fed from three to seven weeks of age), Zimmerman et al. (1961) reported that phosphorus at 0.44 percent of the ration was inadequate for maximum gains regardless of the calcium level. Combs et al. (1962) maintained that 0.44 percent phosphorus was adequate for young pigs from 2 to 7 weeks of age.

Using a synthetic milk diet, Miller et al. (1964) maintained the calcium level at 0.8 percent and varied the phosphorus concentration form 0.20 to 0.80 percent of dietary solids. Growth rate and food consumption were depressed only in those pigs receiving less than 0.40 percent phosphorus. Serum alkaline phosphatase concentration was elevated only in the severely phosphorus-deficient pigs. Kidney phosphorus concentration was somewhat increased in phosphorus-deficient pigs. Humeral ash, calcium and phosphorus concentrations were maximal in pigs receiving 0.60 percent of dietary phosphorus. It was concluded that 0.40 percent of dietary phosphorus is adequate to prevent gross rachitic symptoms and to achieve optimal rate of body weight gain and economy of food utilization. These results indicate that 0.50 percent is adequate to maintain normal concentrations of serum calcium, phosphorus and alkaline phosphatase and to provide for an adequate rate of skeletal development. Calcium and phosphorus balance studies were conducted by Miller _et al_. (1964) on 29 baby pigs receiving a synthetic milk diet containing phosphorus levels of 0.20, 0.40, 0.50, 0.60, 0.70 and 0.80 percent with 0.80 percent calcium. Growth rate, food intake and mineral retention were greatly depressed in pigs receiving 0.20 percent of phosphorus. Increasing dietary phosphorus levels to 0.50 percent resulted in increased phosphorus retention and percentage phosphorus retention. To obtain maximal strength of bone and to insure the absence of rachitic lesions it appears necessary to provide the baby pig with 0.60 percent or more of dietary phosphorus.

The phosphorus recommendations for baby pigs range from 0.44 percent (Zimmerman _et al_., 1961; Combs _et al_., 1962) to 0.60 percent (Miller et al., 1964). Recommendations seem to vary according to which set of criteria are used; growth and feed efficiency or blood and skeletal analysis. Further studies need to be conducted to help clearify this situation.

Calcium-Phosphorus Ratio

Early work on calcium and phosphorus requirements emphasized the desirability of providing a favorable ratio of the two elements. It was thought that the most desirable calcium to phosphorus ratio was between 1.5:1 and 2:1. The present research data on calcium requirements have tended to approximate 1.5 times the value for phosphorus. In fact the N.R.C. requirements for a 4.5 kg. pig represents a 1.33:1 ratio (N.R.C., 1964). A large excess of either calcium or phosphorus interferes with the absorption of the other. With an excess of either one, the other tends to become tied up as the insoluble tricalcium phosphate. This explains the importance of a suitable ratio (Cunha, 1957). With a sufficient supply of Vitamin D in the ration, the ratio becomes of less importance, and more efficient utilization is made of the amounts of the elements present.

Zimmerman et al. (1963) found that calcium to phosphorus ratios of 1.6:1 or wider adversely influenced gains. Calcium and phosphorus appeared to independently influence the efficiency of feed utilization. High calcium levels (above 0.80 percent) reduced the efficiency, while phosphorus up to approximately 0.60 percent improved the efficiency of feed utilization. This data suggests a ratio of approximately 1.33:1 for pigs between two and seven weeks of age.

Although the exact required ratio is not known, we can conclude that the phosphorus requirement is slightly less than calcium and the ratio will depend on the availability of each element plus the presence of adequate Vitamin D.

Calcium-Phosphorus: Studies and Recommendations

Calcium and phosphorus requirements have been established using a variety of criteria, i.e., growth rate, feed conversion, total ash, calcium and phosphorus content of various bones, breaking strength, specific gravity, blood clotting time and levels of calcium, phosphorus and alkaline phosphatase in blood serum. Brown et al. (1966) stated that generally serum alkaline phosphatase activity was inversely related to serum calcium, but the increase-decrease was somewhat delayed compared to the decrease-increase of serum calcium. Serum alkaline phosphatase appears to be an adequate indicator of the magnitude of the serum calcium level.

Carroll, Krider and Andrews (1962) stated the following as general clinical signs of a dietary calcium deficiency: slow or interrupted growth, reduced appetite, poor hair and skin condition, lameless and stiffness, and weaken bone structure. The phosphorus deficiency symptoms listed are: slow or interrupted growth, reduced appetite, lameness and stiffness, and weakened bone structure.

A total of 144 baby pigs (fed from three to seven weeks of age) were used by Zimmerman et al. (1961) to study the calcium and phosphorus requirements in a ration high in milk products. A 4 X 4 factorial arrangement of 0.4, 0.5, 0.6 and 0.7 percent total phosphorus and 0.50, 0.65, 0.80 and 0.95 percent calcium in the ration was used with randomized incomplete blocks arranged in such a way that one-third of the calcium X phosphorus interaction was confounded. With regard to gains, the calcium X phosphorus interaction was highly significant. Calcium-phosphorus ratios greater than approximately 1.6:1.0 suppressed the growth rate. Phosphorus at 0.4 percent was inadequate regardless of calcium level. Increasing the phosphorus level significantly improved feed utilization, and conversely, increasing the calcium level significantly decreased the same. Efficiency of feed utilization became progressively poorer as the calcium-phosphorus ratio widened. Zimmerman et al. (1963) reported that calcium levels above 0.80 percent reduced efficiency, while phosphorus up to 0.60 percent improved efficiency of feed utilization. These results suggest a maximum calcium level of 0.80 percent and a minimum phosphorus level of 0.60 percent to assure maximum performance and adequate skeletal development. This is in close agreement with the National Research Council's recommendations of 1964.

Utilizing pigs of 3.6 and 11.3 kg. live weight, Blair and Benzie (1964) attempted to determine the dietary levels of calcium and phosphorus that would promote adequate bone development in pigs weaned at ten days of age. The synthetic diets contained 0.4, 0.8, 1.2 and 1.6 percent calcium and 0.6, 0.9, 1.2 and 1.5 percent phosphorus. Raising the calcium and phosphorus levels caused a significant increase in the dry fat-free weight, ash content and radiographic density of the bones studied. These workers concluded that the 3.6 to 11.3 kg. pig requires at least 1.3 percent available calcium and 1.0 to 1.2 percent phosphorus. These suggested amounts are higher than any other recent recommendations.

The calcium and phosphorus in bone are in a state of dynamic equilibrium with the calcium and phosphorus of the blood. With an adequate dietary intake of calcium and phosphorus, apposition of bone exceeds resorption in the growing animal; this is the basis of skeletal growth. If serum calcium is lowered, resorption exceeds apposition and generalized osteitis fibrosa results. Serum alkaline phosphatase appears to be quite active in this resorption process. Thus, it is essential that the animal have available all needed calcium and phosphorus to permit proper development of the skeleton. These requirements decrease with additional weight gain. The current National Research Council's recommendations for swine are presented in Table I.

Recommendations on the levels of calcium and phosphorus needed in diets for pigs of various ages are few in number. As a minimum level, Zimmerman <u>et al</u>. (1963) recommends 0.80 percent calcium and 0.60 percent phosphorus. This is quite different from 1.3 percent calcium and 1.0 percent phosphorus as recommended by Blair and Benzie (1964). This certainly indicates a need for additional work to help standardize the calcium and phosphorus requirements for baby pigs.

TABLE I

1964 NATIONAL RESEARCH COUNCIL'S REQUIRED LEVELS OF DIETARY CALCIUM, PHOSPHORUS AND VITAMIN D[1]

Pig Weight (kg.)	Calcium(Ca) (% of diet)	Phosphorus(P) (% of diet)	Ca:P ratio	Vitamin D (I.U. per kg.)
4.5	0.80	0.60	1.33:1.0	220
11.3	0.65	0.50	1.30:1.0	198
22.7	0.65	0.50	1.30:1.0	198
34.0 to 102.0	0.50	0.40	1.25:1.0	132
Breeding stock	0.60	0.40	1.50:1.0	220

[1]N.R.C. (1964).

Disease Interrelationships

The incidence of atrophic rhinitis or a condition resembling rhinitis has been linked to improper levels of calcium and phosphorus by Cornell University workers (Brown _et al._, 1965; Krook _et al._, 1965; Pond _et al._, 1965). Brown, Krook and Pond (1966) state: "It is obvious that the worst cases of atrophic rhinitis can be expected if the dietary calcium is considerably below normal but not low enough to cause any severe retardation of growth."

It has been suggested that SPF pigs have higher than normal requirements for calcium and phosphorus, but the report by Seerley et al. (1963) indicated that higher levels than N.R.C. (1964), when fed to SPF pigs, gave poor feed efficiency.

Summary

The recommended allowances of calcium and phosphorus for pigs have been increased gradually over the past twenty years. It is believed that this increase has been required because of the rapid growth rate in pigs which has been made possible by increased protein intake, improved sanitary conditions, a reduced incidence of infectious diseases and selection for greater genetic growth potential. For this reason, the current calcium and phosphorus recommendations are possibly not keeping pace with the increased growth rate in young pigs and need to be investigated thoroughly.

CHAPTER III

MATERIALS AND METHODS

General Procedure

One trial was conducted using 40 purebred Yorkshire baby pigs (13 males and 27 females) and 5 purebred Hampshire pigs (3 males and 2 females). These pigs were maintained under Specific-Pathogen-Free (SPF) conditions, thus minimizing the confounding effects of certain environmental conditions on the response of the pigs to the dietary treatments.

These pigs were obtained by allowing gravid gilts and sows to farrow normally at term under aseptic conditions. The pigs were collected in sterile plastic bags as they emerged from the birth canal and were then placed in a pre-sterilized plastic isolator. They were transported to the Swine Nutrition Laboratory, given pasteurized sow colostrum by stomach tube, placed in individual cardboard rearing units and fed a fortified cow's milk diet. At two weeks of age they were moved to individual, open-topped, solid-sided metal pens and assigned to one of four experimental purified diets. The period from 14 to 21 days of age was used to adjust the pigs to their respective test rations. The experimental period covered 42 days (21 to 63 days of age).

Individual weekly feed consumption was recorded and live body weights were taken at weekly intervals. Blood samples were obtained when the pigs were 21, 42 and 63 days of age. Four pigs from each of four rations were sacrificed and various response measurements were obtained.

Animal Rooms and Equipment

The Swine Nutrition Laboratory consisted of three separate temperature controlled rearing rooms, the incubator room, nursery room and grower room, where the pigs were housed from birth to two, two to six and six to nine weeks of age, respectively.

Incubator Room. The incubator facilities consisted of one room 6.1 X 3.05 X 2.68 m., and an anteroom 3.05 X 3.05 X 2.68 m., for changing clothes and preparing the liquid diet (Table II). Forty individual disposable cardboard incubators[1] were used to hold the pigs during the first two weeks of life. The incubators were designed to provide each baby pig with dry, heated, filtered and sterilized air. Inside dimensions were 54.61 X 26.67 X 21.12 cm. Each had an observation port on top, an opening for a feed tray in front and was connected to an overhead air input supply system by plastic tubes. The input air was sterilized[2] and dehumidified[2], forced over thermostatically controlled

[1]Fort Dodge Container Corporation, Fort Dodge, Iowa.

[2]Kathabar, Surface Combustion Division, Midland-Ross Corporation, Toledo, Ohio.

heaters, taken through three or four layers of cotton filters which covered four square openings (11.05 X 11.05 cm.) in the top of the incubator and passed out of the incubator and into the room. This air was exhausted from the building and was not recycled through the incubators. The air outflow port of the incubators located in the front of the boxes consisted of a series of 25 holes (17.78 mm. diameter) in a five X five arrangement and covered with 3 or 4 layers of cotton.

The interior of each incubator was kept at a positive air pressure. This minimized the possibility of air-born contamination.

A grill of 6.35 mm. wire mesh raised 53.34 mm. above the bottom of the incubator kept the piglet free of its urine and feces. Each incubator was quilon coated on the inside liner to help condition the cardboard to withstand moisture.

A closely fitted metal feeding tray was taped into place in such a way that it could not be overturned. This tray measured 14.73 X 10.16 X 4.57 cm.

Each incubator had a 17.78 X 12.70 cm. observation opening in the top which was covered with a plexi-glass material after the pig was placed inside to prevent the piglet from jumping out.

Temperature within the incubator room itself was maintained at 26°C. Control of the temperature within the incubator boxes was of vital importance. Provision of an environmental situation that reduces heat loss and provides an immediately available supply of dietary energy is critical to the survival of newborn pigs. This was achieved by putting a 100°C. thermometer into each rearing chamber. Each heater was thermostatically controlled so that the temperature in the box could be adjusted to any required level between 25° and 70°C.

Feed trays and wire mesh bottoms were steam sterilized (121°C. for 30 minutes) before being placed within the incubators. The units were allowed to stand at least 30 minutes before air flow was started through the filters. The cotton air filters were dipped in 1.0 percent mercuric chloride, then dried. Thus, if they became wet, the germicide would be activated and kill vegetative bacteria.

Individual cardboard incubators were used for control of physical factors such as temperature, humidity, airflow, sound and light. The methods used in handling the animals were designed to provide isolation from each other, from other pigs, from the caretakers and to keep immediate surroundings as clean as possible. Meticulous attention to detail was essential. The personnel who cared for the pigs changed clothes in the anteroom, putting on clean overalls and rubber boots. They also stepped into a disinfectant[3] pan when entering and leaving the incubator room.

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[3]Nolvasan-S, Fort Dodge Laboratories, Fort Dodge, Iowa.

Nursery Room. The nursery room was 9.37 X 3.05 X 3.05 m. and contained 36 solid-sided, open-topped pens. Each pen had a perforated, reinforced, galvanized expanded metal floor, was 76.2 X 45.7 X 58.4 cm. in size and was equipped with an adjustable self-feeder and an automatic watering device. The feeder was fitted with a heavy lip around the inside to minimize feed loss. A waste feed tray (34.92 X 25.40 X 13.97 cm.) was located under the self feeder.

Grower Room. The grower room contained 25 pens similar to those described for the nursery. Each pen was 11.76 X 59.69 X 56.69 cm. in dimensions and provided adequate space for each pig up to nine weeks of age. A room adjacent to the grower room provided space for storage of the purified ration ingredients, ration preparation and mixing, and some ration storage.

All laboratory rearing rooms had concrete floors and the incubator and nursery rooms contained no windows, thus eliminating the entrance of sunlight.

The air conditioning of each room was controlled individually and conditions could be varied as desired. Ventilation is required to remove the moisture produced by the animals as well as to control temperature and odors. Normally, the temperature in all rooms was maintained at 26°C. and 35 percent relative humidity. To avoid contamination, the pressure in all of the rooms was maintained slightly higher than the environmental pressure so that air swept out of the rooms when doors were opened.

The rooms were prepared for the pigs a few days before the pigs were to enter the facilities. The rooms and pens were aseptically cleaned with a detergent and disinfectant[4] and thoroughly steamed. Then all the equipment necessary to last throughout the experiment was put in place, i.e., incubators were assembled and placed in the incubator room. The rooms were sealed, dampers in the air conditioning system were closed, relative humidity increased by allowing steam to flow into the rooms, and the rooms fumigated with formaldehyde gas.[5] After 12 hours the dampers were opened and the rooms areated for at least 8 hours before use.

Ultraviolet germicidal lamps were used as aids in cleaning the air in the incubator and nursery rooms. These were located on the wall approximately 50.8 cm. down from the ceiling and were complete with a shield to protect the pigs from any direct ultraviolet rays. All lamps were cleaned each week by wiping with an alcohol solution. Lamps were turned off each time personnel entered the room.

Pig Isolator. A rigid molded acrylic isolator[6] was used for collection and transport of the baby pigs to the laboratory. The complete isolator consisted of the basic housing which was in two sections. Both top and bottom sections were molded of 10.28 mm. clear acrylic with radiused

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[4]Klenzade XY-12, Klenzade Products, Beloit, Wisconsin.

[5]Formaldegen, Vineland Poultry Laboratories, Vineland, New Jersey.

[6]The Germfree Laboratories, Incorporated, Miami, Florida.

crevice-free edges and corners for easy cleaning and decontamination. The flat working area was 121.92 cm. X 76.2 cm. The overall outside dimensions were 137.2 cm. X 91.44 cm. The top and bottom were both 38.1 cm. high and hinged so that either side could be opened or the top completely removed.

Shoulder length (81.29 cm.) black neoprene gloves were attached to the isolator by means of three complete turns of plastic film tape and a clamp so arranged that only neoprene was exposed to the interior of the isolator.

Both the air input and the output ports were fitted with four layers of Owens-Corning Fiberglas PF 115 filter material, and the plastic structure was maintained under a positive air pressure to reduce or eliminate the migration of contaminants through any leaks in the wall.

A 1/30 horsepower, 3,020 rpm, 140 cfm., detachable electric centrifugal blower motor[7] forced fresh air into the unit through the four layers of filter material, and the exhaust air passed through a similar filter arrangement which prevented the back flow of air into sterile environment, which could occur during a sudden withdrawal from the gloves.

The air filter units (input and output) were sterilized in an autoclave at 150°C. for 90 minutes and attached to the isolator. The interiors of the isolator were cleaned with

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[7]Model 20610, Dayton Electric Mfg. Co., Chicago, Illinois.

copious detergent, dried, sprayed with a 1.0 percent mercuric chloride solution and allowed to dry. This was followed by formaldehyde gas[8] sterilization. Ordinary burlap feed sacks were sterilized and placed on the isolator floor to permit firm footing.

Isolator space was quite adequate for a large litter. Good visibility and ease of transportation were also definite advantages of the isolator. Two distinct disadvantages were the lack of a germicide trap and lack of an air heater.

Pig Diets

Sow Colostrum. Approximately 200 ml. of sow colostrum (Table II) were given each pig during the first 24 to 36 hours of life. This colostrum was obtained just prior to or during parturition or within 12 hours after onset of parturition. To obtain large volumes, the sow was restrained by placing a running noose on the upper jaw, and approximately 20 U.S.P. units of pituitary oxytocic principle[9] were injected into an ear vein to accomplish colostrum let-down. After a few seconds, colostrum was obtained from several teats by manual extrusion. Up to 900 ml. were obtained from a single milking. The colostrum from several sows was pooled, pasteurized (61.7 - 62.8°C., 30 minutes), cooled and frozen at -17°C. until needed.

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[8]Formaldegen, Vineland Poultry Laboratories, Vineland, New Jersey.

[9]P.O.P., Armour Pharmaceutical Co., Kankakee, Illinois.

TABLE II

ANALYZED CHEMICAL COMPOSITION
OF POOLED SOW COLOSTRUM

Nutrient	Liquid[a] Basis	Dry Matter Basis
	%	%
Total solids	23.44	- -
Crude protein (N X 6.38)	13.63	58.15
Fat	7.80	33.28
Solids-not-fat	15.64	66.72
Nitrogen-free extract	1.20	5.12
Ash	0.814	3.47
Calcium	0.118	0.50
Phosphorus	0.134	0.57

[a]All mean values are based on six determinations.

<u>Liquid Milk Diet</u>. The liquid diet fed the pigs from birth to two weeks of age consisted of pasteurized, homoggeized cows' milk fortified with dried whole milk, dried whey, minerals and vitamins (Table III). The dried whole milk and whey were added in sufficient quantity to approximately double the dry matter content of the cows' milk. The liquid diet was formulated to resemble sow's milk rather than cows' milk in order to provide more energy per unit volume. The analyzed and calculated chemical composition of the diet is presented in Tables IV and V, respectively. On a dry matter basis, calcium and phosphorus content was 1.319 and 0.983 percent, respectively. Antibiotic material was added at a level to supply 132.3 mg. of active antibiotic per kg. of dry matter.

The liquid diet was prepared in the following manner: One and one-half liters of cows' milk were poured into a two liter Waring Blender, the remaining ingredients were added in appropriate quantities (Table III) and the resultant mixture was homogenized by constant stirring at low speed (15,500 rpm) for approximately five minutes.

The remaining 2.29 liters of milk were added and the diet was blended for an additional five minutes. Although foaming of the mixture occurred, the foam disappeared on cooling or on warming while stirring. It was not considered necessary to homogenize the final preparation since the diet formed a rather stable emulsion upon mixing.

TABLE III

COMPOSITION OF THE FORTIFIED COWS' MILK DIET

Ingredient	Composition
Homogenized cow milk	1 gal. (3,785.3 ml.)
Dried whole milk	515.87 gm.
Dried whey (50% lactose)	260.04 gm.
Mineral mixture[a]	20.00 ml.
Sulfamethazine-antibiotic[b]	1.50 gm.
Citric acid	5.00 gm.
Niacin	16.67 mg.
Vitamin K	3.039 mg.
Vitamin D_2	100 I.U.
Vitamin E	10.7 mg.

[a]Contributed the following per gallon:

21.47 gm. $FeSo_4 \cdot 7H_2O$

15.19 gm. $ZnSO_4 \cdot 7H_2O$

9.27 gm. $MnCl_2 \cdot 4H_2O$

18.07 mg. KI

2.5 ml. conc. HCl

[b]Aureo S-P250, a trademark for a premix of chlortetracycline (44.1 gm. per kg.), sulfamethazine (4.4%) and penicillin (22.05 gm. per kg.), American Cyanamid Company, Princeton, New Jersey.

TABLE IV

ANALYZED CHEMICAL COMPOSITION OF THE FORTIFIED COWS' MILK DIET

Nutrient	Liquid[a] Basis	Dry Matter Basis
	%	%
Total milk solids	25.02	- -
Crude protein (N X 6.38)	6.23	24.90
Fat	4.90	19.58
Solids-not-fat	20.12	80.42
Nitrogen-free extract	11.86	47.40
Ash	2.03	8.11
Calcium	0.330	1.319
Phosphorus	0.246	0.983

[a]All mean values are based on six determinations.

TABLE V

CALCULATED NUTRIENT COMPOSITION OF THE FORTIFIED COWS' MILK DIET

Nutrient	Quantity
Minerals[a], mg.	
Magnesium	880.39
Iron	195.38
Zinc	55.56
Manganese	44.54
Copper	11.31
Iodine	0.22
Vitamins[b]	
Vitamin A	9,513.20
Vitamin D	230.20
Vitamin E	8.60
Vitamin K	2.45
Choline	2,988.33
Inositol	621.79
Pantothenic acid	26.86
Niacin	24.50
Riboflavin	12.99
Pyridoxine	6.44
Thiamine	3.77
Folic acid	0.34
Biotin	0.33
B_{12}	0.33

[a]All minerals are expressed as gm. per kg. of diet dry matter.

[b]Vitamins A and D are expressed as International Units (I.U.)/kg. while all other vitamins are expressed as mg./kg.

The liquid diet was placed in a pasteurization unit[10] and heated at a temperature of 61.7°C., for 30 minutes. This heat process assured a negative phosphatase test which is the officially recognized test for satisfactory pasteurization. It was then rapidly cooled, transferred to sterile containers and either maintained at 2°C. until fed or frozen at -17°C. until needed. The frozen diet was thawed by permitting it to stand at 20°C., followed by heating and shaking just prior to dispensing to the piglets.

Since the diet was not subjected to the high temperature of steam sterilization, heat labile vitamins were not supplemented.

Feedings were at four hour intervals, starting at six a.m. and ending at ten p.m. Although a less frequent feeding schedule would have reduced labor and time required to feed the pigs, it was felt that five times a day feeding would restrict the quantity of diet consumed at a given time and accomplish a greater total daily dietary intake without complicating management because overfeeding diarrhea would be absent or at least minimal. With this regimen diarrhea was a minor problem and was usually controlled by limiting the dietary intake of the pig until the diarrhea was alleviated. When diarrhea was severe, a single oral treatment

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[10]Home Health milk and cream pasteurizer, Model PA-52A, two gallon size, Waters Conley Company, Inc., Rochester, Minnesota.

with neomycin sulfate[11] gave excellent results.

The fortified milk diet was heated to approximately 38°C. during the initial 14 days of the test by placing it in a water bath maintained at 53°C.

Tray feeding was initiated approximately 4 to 6 hours after the final pigs had been collected. The pigs started with a quantity of 20 to 25 ml. of diet per feeding. The volume of diet allotted to each pig was determined on an individual basis. This was estimated at each feeding by considering the condition of the animal and how well its previous feeding was consumed. By this technique, the feedings of the pigs appeared to be essentially on an *ad libitum* basis. The pigs obtained their water requirements from the liquid diet that was used. The quantity of diet was increased approximately 5 ml. at successive six a.m. feedings except for individual pigs that did not consume the previous feeding. Normally, pigs were consuming approximately 160 ml. per feeding at 14 days of age. Dietary consumption by individual pigs was accurately measured and recorded.

Calcium-Phosphorus Purified Rations. The physical compositions of the calcium-phosphorus purified diets are presented in Table VI and the proximate analysis and analyzed calcium and phosphorus levels for each diet are presented in Table VII. On the basis of the literature, two

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[11]Liquid Biosol-M, The Upjohn Company, Kalamazoo, Michigan.

TABLE VI

PERCENTAGE COMPOSITION OF EXPERIMENTAL PURIFIED DIETS

Ingredient[a]	Diet A	Diet B	Diet C	Diet D
	%	%	%	%
Casein[b]	25.58	25.58	25.58	25.58
Corn starch[c]	46.98	45.41	43.92	42.32
Glucose monohydrate[d]	11.30	11.30	11.30	11.30
α-Cellulose[e]	6.00	6.00	6.00	6.00
Corn oil[f]	5.30	5.30	5.30	5.30
Mineral mixture[g]	3.00	3.00	3.00	3.00
Dicalcium phosphate[h]	0.345	2.0116	3.6783	5.345
Calcium carbonate[i]	0.4282	0.2528	0.0773	0.00
Water with water soluble vitamins[j]	1.00	1.00	1.00	1.00
Fat soluble vitamins[k]	0.0041	0.0041	0.0041	0.0041
Antioxidant[l]	0.0125	0.0125	0.0125	0.0125
Sulfamethazine-antiobiotic[m]	0.125	0.125	0.125	0.125
Total	99.98	99.996	99.997	99.98

TABLE VI CONTINUED

[a]Ingredients are expressed on an air dry feed basis.

[b]Borden's New Zealand Lactic Casein, 83.9% crude protein by analysis, The Borden Company, New York, New York.

[c]Corn Products Company, Argo, Illinois.

[d]Cerelose 2001, Corn Products Company, Argo, Illinois.

[e]Solka-Floc, BW-100, Brown Company, Berlin, New Hampshire.

[f]Mazola, Corn Products Company, Argo, Illinois.

[g]See Table VIII. Supplied 50 ppm of supplemental zinc as well as other minerals.

[h]Contained 21.62% elemental calcium and 18.34% elemental phosphorus by analysis.

[i]Contained 39.89% elemental calcium by analysis. Courtesy Calcium Carbonate Company, Quincy, Illinois.

[j]Supplied 3 mg. thiamine, 6 mg. riboflavin, 40 mg. niacin, 30 mg. pantothenic acid, 2 mg. pyridoxine, 13 mg. para-aminobenzoic acid, 80 mg. ascorbic acid, 130 mg. inositol, 1.3 gm. choline, 260 mcg. folic acid, 50 mcg. biotin and 100 mcg. cyanocobalanin per kg. of total ration. Courtesy Hoffman-Taff, Inc., Springfield, Missouri.

[k]Supplied 10 mg. alpha-tocopherol, 1.5 mg. vitamin A, 40 mcg. 2 methyl. 1,4 naptho-quinone and 12.5 mcg. of vitamin D_2 per kg. of total ration.

[l]Santoquin liquid, 1,2 dihydro-6-ethoxy,2,2,4,trimethyl quinoline, Monsanto Chemical Company, St. Louis, Missouri.

[m]Aureo S.P 250, a trademark for a premix of chlortetracycline (44.1 gm. per kg.), sulfamethazine (4.4%) and penicillin (22.05 gm per kg.), American Cyanamid Company, Princeton, New Jersey.

TABLE VII

CHEMICAL COMPOSITION OF THE PURIFIED DIETS
FED FROM THREE TO NINE WEEKS OF AGE

Analysis	Diet A	Diet B	Diet C	Diet D
Proximate analysis, %				
Dry matter	90.12	89.84	90.10	89.21
Crude protein (N X 6.25)	21.73	21.43	21.62	21.10
Ether extract	6.09	5.81	5.85	5.85
Crude fiber	2.72	3.00	3.14	3.42
Nitrogen-free extract	56.75	55.70	54.48	52.74
Ash	2.83	3.90	5.01	6.10
Minerals				
Calcium, %	0.27	0.57	0.95	1.25
Phosphorus, %	0.14	0.44	0.73	1.05
Phosphorus to calcium ratio	1:2.00	1:1.28	1:1.30	1:1.10

dietary calcium-phosphorus levels (Diets A and B) were below the current recommendation (N.R.C., 1964) and two dietary levels (Diets C and D) were above the current recommendations. Due to an error, the calcium to phosphorus ratio was not constant for all diets.

The protein, cellulose, fat, calcium-phosphorus free basal mineral mixture (Table VIII) and vitamin portions of the diet were held constant in all rations and only the quantity of cornstarch, calcium carbonate and dicalcium phosphate were altered to form the four dietary treatments studied.

The rations were formulated to be adequate in all other nutrients, particularly vitamin D and zinc, and they contained 125 gm. of antibiotic activity per ton. The vitamin levels used in making the diets are shown in Table VI. These were based on the best estimates of the requirements of the pig as determined from the current literature.

The rations were mixed for 15 minutes in a horizontal mixer and stored in large plastic containers with lids and identifying labels.

Experimental Pigs

Collection and Handling. The sows and gilts used had been maintained on a ration (Table IX) designed to meet their nutritive needs as defined by the N.R.C. (1964). This ration contained 0.80 percent calcium and 0.62 percent phosphorus and was hand-fed at the level of 2.7 kg. per head per day, fed in equal portions twice daily (a.m. and p.m.). It provided more calcium (0.20 percent) and phosphorus (0.22 percent) than is currently recommended by the National Research Council (1964).

At 110 days of gestation, selected gilts and sows were taken to clean surroundings and retained in farrowing crates to await the appearance of milk in the udder.

The newborn pigs were protected from bacterial contamination and respiratory infection by catching each pig in a sterilized plastic bag as the sow farrowed normally. The vulva and buttocks of the sow were first cleansed with a mild antiseptic[12] and the bag opening was held against the vulva. After a pig dropped into a bag, the top was immediately closed and the bag immersed in an antiseptic solution[12] and the pig was passed via the entrance lock directly into the body of the previously described isolator unit.

Working through the rubber gloves, thoroughly drenched with a two percent tinture of iodine, each pig was quickly dipped into a germicidal solution[12]. The nose and the mouth of the pig were wiped free of membranes and mucous. Each pig was dried vigorously by wiping with sterile paper towels, and encouraged to breathe as needed by a gentle massage.

Oxytocin[13] was administered into a marginal auricular

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[12]Nolvasan-S, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

[13]P.O.P., Armour Pharmaceutical Company, Kankakee, Ill.

TABLE VIII

COMPOSITION OF BASAL MINERAL MIXTURE[a]

Mineral		Percent
$NaHCO_3$[b]		50.00
K_2SO_4[b]		23.37
$MgCO_3$[b]		4.10 (1,861.4)[c]
$FeSO_4 \cdot 2H_2O$[b]		1.40 (635.6)[c]
$ZnSO_4 \cdot H_2O$[b]		0.80 (363.2)[c]
$MnSO_4 \cdot H_2O$		0.30 (136.2)[c]
$CuSO_4$		0.20 (90.8)[c]
$CoCO_3$		0.20 (90.8)[c]
KI		0.004 (1.816)[c]
Cerelose[d]		19.63
	Total	100.004

[a]Fed at the level of three percent of the purified diets.

[b]Supplied through the courtesy of Calcium Carbonate Company, Quincy, Illinois.

[c]Gm.

[d]Cerelose 2001, Corn Products Company, Argo, Illinois.

TABLE IX

PERCENTAGE COMPOSITION OF THE SOW RATION FED DURING GESTATION

Ingredient	Percent in Ration
Milo, western yellow, ground (8.0% protein)[a]	79.62
Soybean meal (50% protein)	10.43
Tankage (60% protein)	2.50
Alfalfa meal (17% protein)	5.00
Dicalcium phosphate (28% Ca - 18% P)	1.25
Calcium carbonate (38% Ca)	0.50
Salt (trace mineral)	0.50
Zinc sulfate[b]	0.02
Vitamin B_{12} supplement[c]	0.012
Vitamin B supplement[d]	0.06
Total	99.89
Calculated chemical composition	
Crude protein	13.93
Total digestible nutrients	76.44
Calcium	0.80
Phosphorus	0.62

[a]Crude protein content chemically determined.

[b]Zinc added to supply a supplement of 50 ppm in diet.

[c]Contained 88.2 mg. B_{12} per kg. of supplement.

[d]Contained riboflavin, 4.4 gm.; pantothenic acid, 8.8 gm.; nicotinic acid, 19.8 gm.; and choline chloride, 198 gm. per kg. of supplement.

vein in cases where farrowing was slow and/or difficult.

After the entire litter had been collected and treated, the pig laden isolator was transported from the procurement location to the Swine Nutrition Laboratory, which is isolated from the swine barn and from personnel in contact with the swine herd.

Before removing the pigs from the isolator, personnel donned sterile 30.5 cm. rubber gloves, freshly laundered coveralls, disposable masks, and thoroughly disinfected rubber boots. A ten percent formalin solution was sprayed around the entrance lock before the front plastic cover was removed. Only one pig was removed at a time and the operators' gloves were dipped in a disinfectant solution[14] before handling each pig.

Pigs were removed at random from the isolator unit. Sex, birth weight (to the nearest gm.) and strength score were recorded. The umbilical cord was ligated approximately 2.5 cm. from the navel, severed and the proximal end was swabbed with a two percent solution of tinture of iodine. Needle teeth were not clipped and no iron injections were given.

Each baby pig received 25 ml. of pasteurized sow colostrum (composition shown in Table II). A 35 cc. plastic syringe with an attached 4.0 mm. diameter flexible rubber tube was used to feed the colostrum. Pigs were held vertically

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[14]Nolvasan-S, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

by their head and their jaws were forced open with the fingers and thumb. The rubber tube was directed down the esophagus to the stomach. After the tube was in place, the colostrum was put into the stomach by pressing the plunger on the syringe. The danger of getting colostrum into the lungs was minimized by placing the end of the tube near the entrance of the stomach.

Each pig was then assigned to an individual disposable cardboard rearing unit located in the incubator room. The rearing units were kept at 33°C. for three or four days, then the temperature was gradually reduced to 27°C.

Assignment to Treatment. At 14 days of age, the pigs were removed from their individual sealed incubator boxes and transported to the nursery room where they remained for four weeks. The pigs were weighed to the nearest 50 gm., individually identified (ear notched) and placed in individual metal pens. Pigs were randomly assigned to each treatment diet.

Adjustment of the pigs to the purified diet in dry meal form was facilitated by means of mixing the dry meal with reducing quantities of the fortified milk ration in shallow metal feed trays. Two sets of feeding trays were used; while one set was in use, the other set was soaking in a detergent and disinfectant solution.[15] Before use, the trays were rinsed with hot water. After feeding, the used

[15]Nolvasan-S, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

trays were thoroughly cleaned with hot water and placed in the disinfectant solution to soak until the next feeding.

By the end of the third week the animals were completely adjusted to the dry diet. The animals were fed *ad libitum*, with the feeders refilled as necessary.

Data Obtained and Chemical and Statistical Analysis

All pigs were weighed at birth, at three weeks of age and weekly thereafter to nine weeks of age. Weekly feed consumption was recorded.

Blood samples were collected by anterior vena cava puncture as described by Carle and Dewhirst (1942) at three, six and nine weeks of age. A 10 ml. plastic disposable syringe fitted with a 38.1 mm. 20-gauge needle was used to withdraw approximately 6 ml. of blood. A total of 0.05 ml. of a heparin solution (2.5 mg. per ml.) was added to 1.5 ml. of the blood sample. Hemoglobin, hematocrit (packed cell volume) and red and white cell counts were determined immediately.

The remaining blood sample (approximately 4.5 ml.) was placed in a plastic centrifuge tube. The sample was then held at 20°C. for approximately 40 minutes to promote syneresis. Separation of serum and clot was completed in a refrigerated centrifuge at 3,000 rpm for 15 minutes. The resulting serum was removed by decantation, placed in sterile stoppered vials and stored at -17°C. for the later determination of levels of serum calcium, inorganic phosphorus and alkaline phosphatase.

Hemoglobin was determined using the cyanmethemoglobin method of Cannan (1958). Hematocrit was determined in duplicate according to the micromethod described by McGovern et al. (1955). Blood samples were centrifuged for five minutes at 10,000 rpm in an International "Hemacrit" centrifuge.

Erythrocytes were counted in duplicate from a single filling of a "zero error" pipette using Hayem's solution as the diluting fluid. Cells were counted in a hemocytometer with Neubauer ruling using a National Bureau of Standards certified cover glass. Acceptable duplicate counts differed no more than ten percent of the lower count.

Total leukocyte counts were made in duplicate on a hemocytometer with a National Bureau of Standards certified cover glass from one dilution with a "zero cover" Hellige pipette. Turk's acetic acid solution was used for the diluting fluid. Counts were considered acceptable if the higher value was no greater than 110 percent of the lower value.

Mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.) and mean corpuscular hemoglobin concentration (M.C.H.C.) were calculated by the following equations as described by Wintrobe (1961):

$$\text{M.C.V. } (u^3) = \frac{\text{vol. packed red blood cells, ml. per 1,000 ml.}}{\text{red blood cells, millions per mm}^3}$$

$$\text{M.C.H. (micro-mcg.)} = \frac{\text{hemoglobin, gm. per 1,000 ml.}}{\text{red blood cells, millions per mm}^3}$$

$$M.C.H.C.(\%) = \frac{\text{hemoglobin, gm. per 100 ml. X 100}}{\text{vol. packed red blood cells, ml. per 100 ml.}}$$

Serum calcium was determined in duplicate by atomic absorption spectrophothometry using a Perkin-Elmer Model 303 with a Boling (total consumption) burner and an air-acetylene flame. Serum phosphorus determinations were made by the method of Tausskey and Shorr (1953). Serum calcium to phosphorus ratios and the product of calcium times phosphorus were calculated.

Serum alkaline phosphatase activity was determined by a colorimetric procedure developed by Klein *et al.* (1960), as outlined by General Diagnostics (1965). Phosphatase activity was expressed in Klein-Babson-Read units per 100 ml. serum. One unit is defined as the amount of enzyme that will liberate 1.0 mg. of phenolphthalein in 30 minutes at 37°C. Since 0.2 ml. of serum was used in the assay, the activity, expressed as units per 100 ml. of serum, was numerically equal to one-half the amount (in mcg.) of phenolphthalein released.

At the conclusion of the experiment, four pigs (two males and two females) from each ration were selected at random and sacrificed by exsanguination. Feed, but not water, was withdrawn approximately eight hours prior to slaughter.

Various bones, organs and glands were removed, blotted to remove excess blood and weighed. The heart was dissected free from the great veins. The arteries were severed at the point of emergence from the heart, where their color changes from deep red to white and blood was removed before weighing. The lungs were dissected free of the pleural membranes, the trachea and the chief bronchi. The liver was freed of the gall bladder and cystic duct. The spleen was removed and closely trimmed at the hilus. The kidneys were removed from their retroperitoneal position, and the arteries, veins and ureters were cut at the hilus. The renal capsule was left intact, and the two kidneys were weighed separately. The stomach was dissected from from the esophagus and duodenum, emptied of its contents, washed with water and blotted dry. All these organs were weighed immediately after excision and the heart, liver and left kidney, along with a sample of hair taken from the back region, were stored in sealed polyethylene bags at -17°C. until analyzed for calcium and phosphorus. Suitable aliquots for analysis were obtained after thawing, chopping and hemogenizing the entire organ.

The right humerus, femur, ulna-radius and eighth rib were dissected free of muscles, ligaments and periosteum, weighed and the maximum length was measured by means of a vernier scale slide-calipers. The length of the humerus (paralled to the axis) was from head to condyle. The femur length was from head to condyle while the ulna-radius was measured from the proximal end of the ulna to the distal end of the ulna-radius. The eighth rib was measured from the head directly to the sternal extremity. Diameter or width of each of the bones was recorded and the specific gravity was found using the equation by Whiteman et al. (1953). These four bones were then stored in polyethylene bags at -17°C. until analyzed for calcium and phosphorus.

Bones were extracted with 95 percent ethanol for 16 hours, followed by a petroleum ether extraction for an additional three hours. The fat-free bones were dried, ground and along with the left kidney, liver, heart and hair were ashed at 550°C. for 16 hours, with the ashes being dissolved in 4 N HCL to a known volume.

Bone and tissue phosphorus were analyzed in duplicate by the method of Fiske and SubbaRow (1925). Calcium analysis was performed in duplicate using a Perkin-Elmer Atomic Absorption Spectrophotometer by methods set forth by the manufacturer.

A cross-section of the snout through the first maxillary premolar teeth was grossly examinated by Veterinary Pathology personnel for any evidence of nasal turbinate atrophy.

Proximate analysis values were determined by the methods of A.O.A.C. (1955).

Analyses of variance, calculation of standard errors and orthogonal comparisons among treatment totals were conducted according to the methods outlined by Steel and Torrie (1960). Orthogonal comparisons (linear and quadratic) were conducted only when analysis of variance for the various criteria were significant ($P<.05$).

CHAPTER IV

RESULTS AND DISCUSSION

Forty-five baby pigs were collected in preparation for this study. At two weeks of age each pig was randomly assigned to one of four purified diets and given one week to adjust to its dry ration.

The data on body weight gains and feed efficiencies are presented in Table X. There was a significant difference ($P<.05$) in body weight gains in all weeks except one. The heaviest pigs were those on Ration C (0.95 percent calcium and 0.73 percent phosphorus) and the lightest pigs were on Ration A (0.27 percent calcium and 0.14 percent phosphorus). The growth data are shown graphically in Figure 1. Good growth rates were obtained when rations B, C and D were fed.

The growth rates obtained were appreciably higher than those reported by Ashton and Crampton (1943), using farm conditions and a ration consisting of 15 percent protein, and approximately the same as those reported by Wood and Groves (1965).

Total gain, total feed intake and feed efficiency for the complete experimental period are presented in Table XI. There was a significant difference ($P<.05$) among the

TABLE X

BODY WEIGHT, WEEKLY WEIGHT GAIN AND FEED EFFICIENCY OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS(P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D	SE[a]	SD[b]
No. of Pigs	11	11	11	12		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05		
Initial Weights	2.49	3.00	3.27	2.98	0.22	
21 to 28 days						
28 day weight[c]	3.64	4.21	4.88	4.72	0.32	*
Body weight gain[c]	1.12	1.21	1.62	1.75	0.20	NS
Feed efficiency[d]	2.04	2.01	1.44	2.23	0.23	NS
28 to 35 days						
35 day weight	5.36	5.98	6.51	7.17	0.50	NS
Body weight gain	1.72	1.77	1.63	2.44	0.23	NS
Feed efficiency	2.02	2.04	2.82	1.53	0.40	NS
35 to 42 days						
40 day weight	7.13	8.96	9.64	9.59	0.66	*
Body weight gain	1.77	3.01	3.13	2.42	0.28	*
Feed efficiency	2.48	1.58	1.50	2.34	0.15	*
42 to 49 days						
49 day weight	9.26	12.10	12.81	12.32	0.80	*
Body weight gain	2.13	3.11	3.16	2.73	0.23	*
Feed efficiency	2.02	2.20	1.82	2.03	0.24	NS

TABLE X CONTINUED

49 to 56 days						
56 day weight	11.87	15.52	16.77	15.73	0.94	*
Body weight gain	2.61	3.41	3.96	3.40	0.25	*
Feed efficiency	1.72	1.97	1.60	1.76	0.14	NS
56 to 63 days						
63 day weight	15.27	18.89	20.81	19.82	1.11	*
Body weight gain	3.40	3.37	4.04	4.09	0.36	NS
Feed efficiency	2.38	1.88	1.86	2.32	0.19	NS

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

[c]All body weights and body weight gains recorded in Kg.

[d]Kg. of feed solids per Kg. of body weight gain.

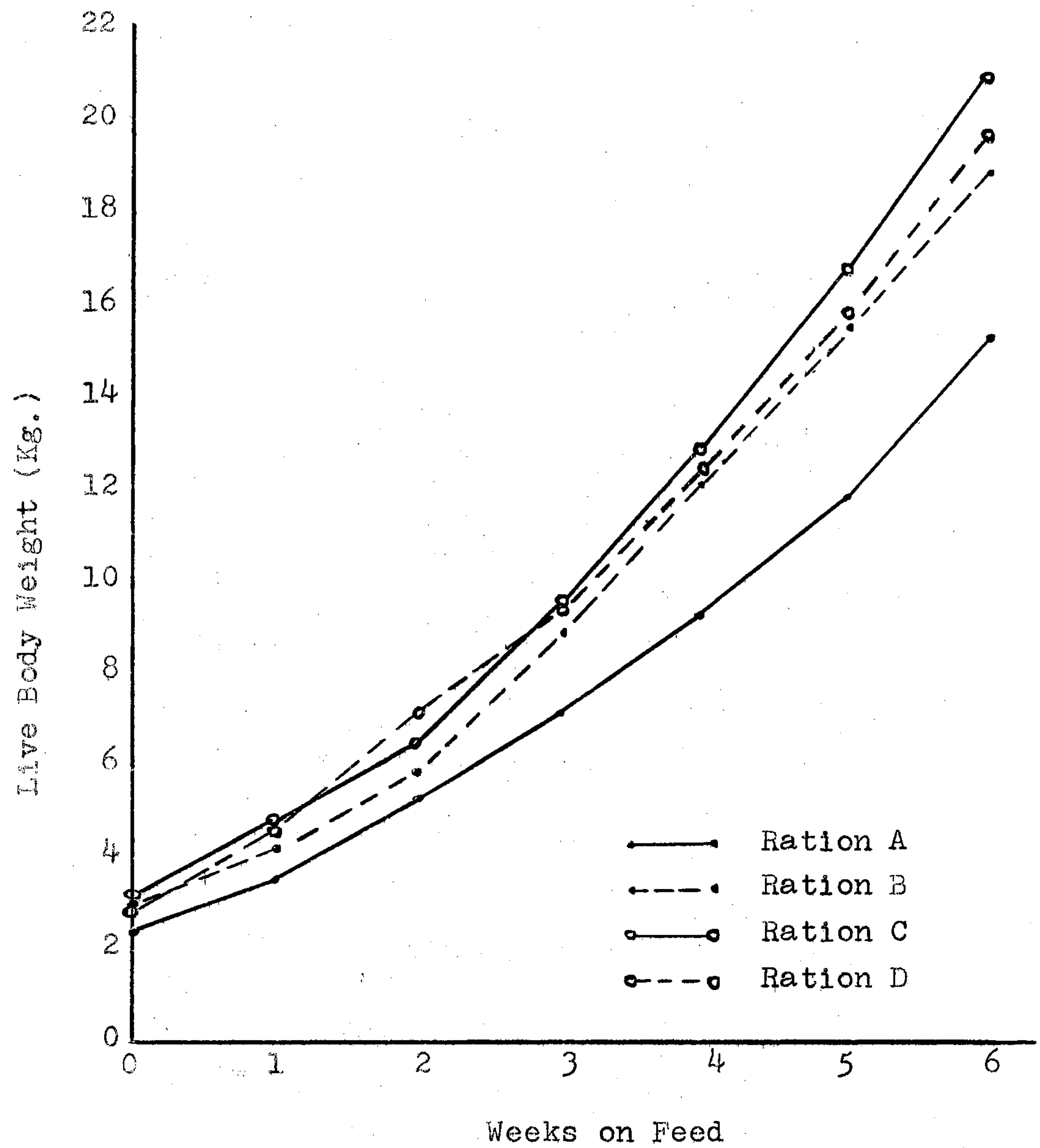


Figure 1. Growth Curves of Pigs Fed Four Levels of Calcium and Phosphorus from Three to Nine Weeks of Age

TABLE XI

TOTAL GAIN, FEED INTAKE AND FEED EFFICIENCY OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 11 0.27 0.14	B 11 0.57 0.44	C 11 0.95 0.73	D 12 1.25 1.05	SE[a]	SD[b]
Kg. gain	12.75	15.89	17.54	16.84	1.01	*
Kg. feed	25.14	28.01	29.41	32.72	1.70	*
Feed efficiency[c]	2.03	1.80	1.67	1.97	0.08	*

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

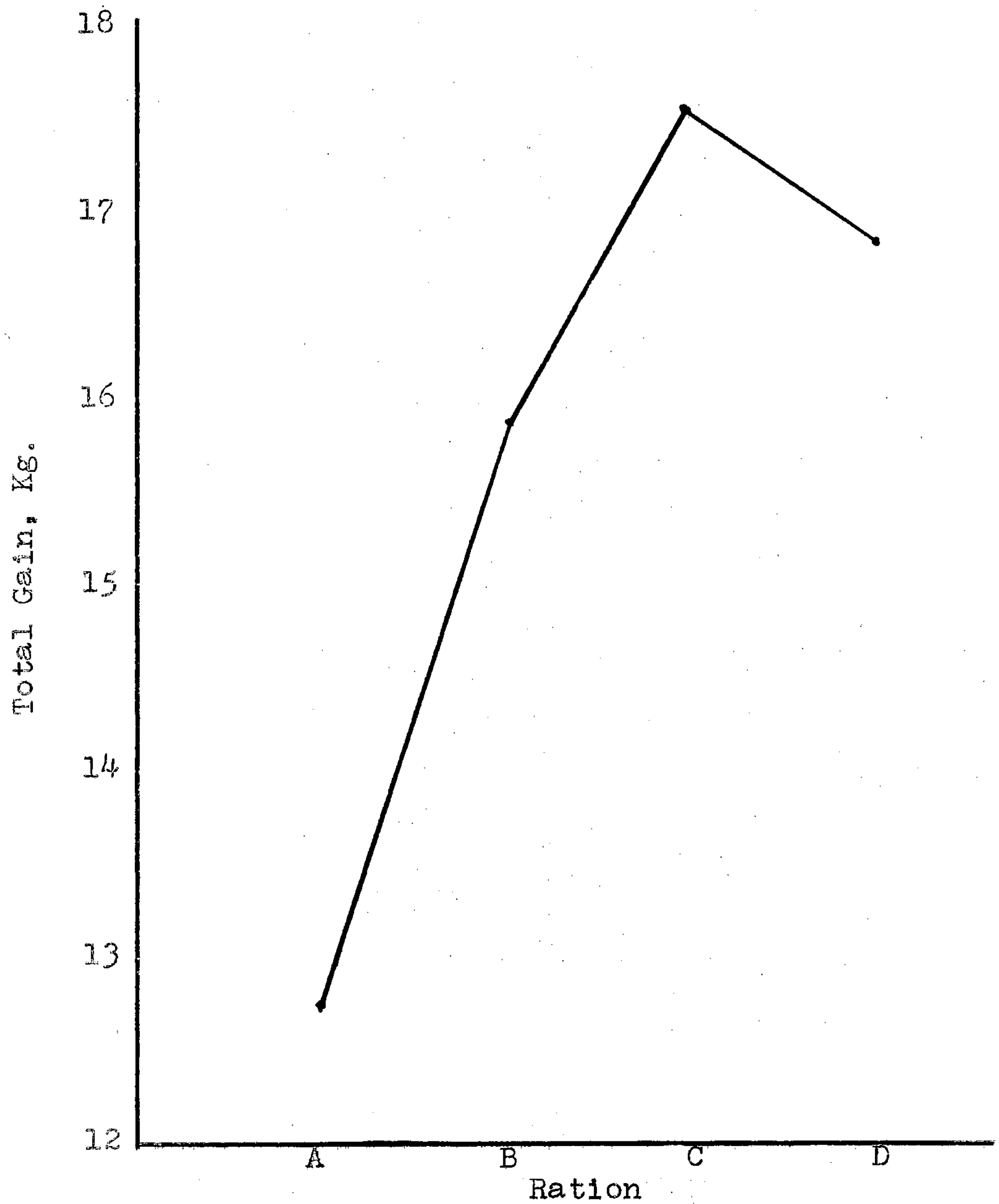
[c]Kg. of feed solids per Kg. of body weight gain.

treatment means for each of the three variables. Pigs on Ration C (0.95 percent calcium and 0.73 percent phosphorus) grew the fastest and were most efficient in converting their diet into gain. Total gain and feed efficiency are graphically shown in Figures 2 and 3, respectively. There was a linear increase in total feed intake starting with Ration A and concluding with Ration D. This would indicate that the high mineral levels of Ration D (1.25 percent calcium and 1.05 percent phosphorus) did not severely limit feed intake.

Data in Table XI suggests that phosphorus levels have an effect on growth and feed efficiency but phosphorus level is confounded with calcium level and it is, therefore, impossible to separate the two. Newman _et al_. (1964) using rations containing 0.60 percent calcium and 0.35, 0.45, 0.55 and 0.65 percent phosphorus found that the levels of phosphorus used had no effect on growth rate, feed consumption or feed utilization.

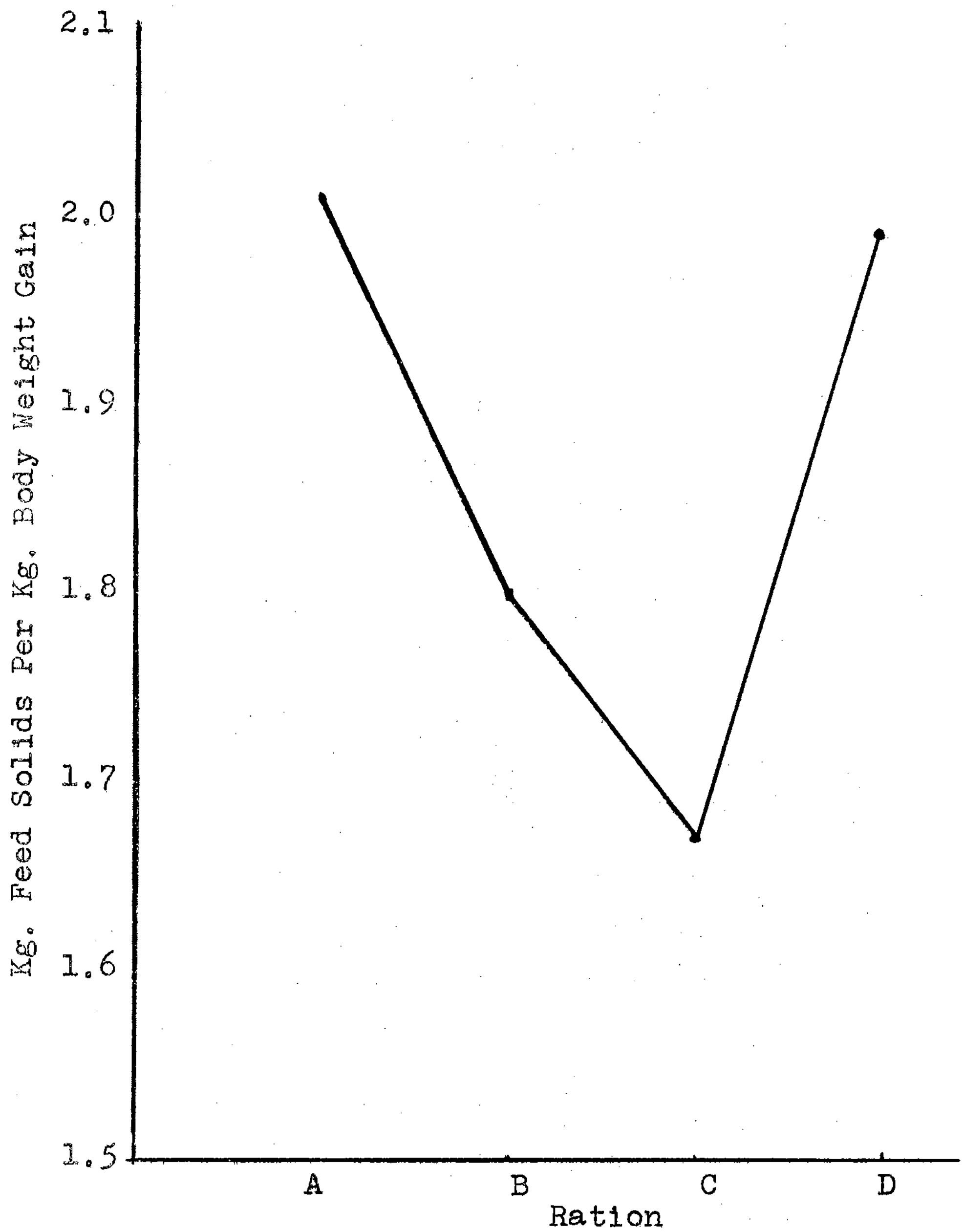
Results obtained in this experiment indicate that 0.95 percent calcium (at least above 0.57 percent) is needed for maximum growth and feed efficiency. This amount is considerably higher than that recommended by Dudley _et al_. (1961) which indicated that for maximum rate and efficiency of gain the calcium requirement was no higher than 0.20 percent of the diet. Miller _et al_. (1960) presented growth and feed consumption data to indicate that 0.60 percent calcium was needed for maximum growth and feed efficiency.

Results (Table XI) show that gains were decreased when



Test for Significant Linear and Quadratic Effects.
Linear (P<.005)
Quadratic (P<.10)

Figure 2. Total Gains (Kg.) of Pigs Fed Four Levels of Calcium and Phosphorus from Three to Nine Weeks of Age



Test for Significant Linear and Quadratic Effects.
Quadratic ($P<.005$)

Figure 3. Feed Efficiency of Pigs Fed Four Levels of Calcium and Phosphorus from Three to Nine Weeks of Age

the calcium level was raised above 0.95 percent of the diet and that the level of 1.25 percent calcium did not effect feed intake in pigs from three to nine weeks of age. When the level of calcium was increased from 0.48 to 0.88 and then to 1.32 percent in the diet of young pigs, Combs et al. (1966b), reported that average daily gain and efficiency were significantly decreased, but there was no significant effect on feed intake among pigs two to eight weeks of age.

Mean serum calcium levels (Table XII) ranged from 11.03 to 12.54 mg. per 100 ml. These values were considered to be normal since the concentration of calcium in blood serum of the pig varies from 9 to 15 mg. per 100 ml. (Dukes, 1955). Differences in serum calcium levels after either three or six weeks on test were not significant. After six weeks pigs on Ration C had the highest serum calcium levels.

The ability of these pigs to maintain normal serum calcium levels over a wide range in dietary levels of calcium might be explained by the function of parathyroid hormone in maintenance of serum calcium homeostasis (Harrison, 1966). The primary function of parathyroid hormone is the maintenance of a normal level of calcium in the blood. The mechanism by which parathyroid hormone is able to maintain a normal blood calcium level is not yet completely understood. At one time there was considerable argument over the question of whether parathyroid hormone exerted its action mainly through the kidney, or by a direct effect on the bone. It now appears that the hormone acts

TABLE XII

SERUM CALCIUM AND PHOSPHORUS LEVELS OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 11 0.27 0.14	B 11 0.57 0.44	C 11 0.95 0.73	D 12 1.25 1.05	SE[a]	SD[b]
Weeks on feed	Serum calcium, mg./100 ml.					
0	11.23	11.20	11.57	11.28	0.33	NS
3	11.57	12.54	12.27	11.48	0.33	NS
6	11.03	11.44	11.63	11.61	0.27	NS
	Serum inorganic phosphorus, mg./100 ml.					
0	6.29	7.10	8.19	7.62	0.28	*
3	7.34	8.85	9.19	12.03	0.48	*
6	7.99	8.67	9.17	12.64	0.50	*
	Amount of Change[c]					
0-3	1.04	1.74	0.99	4.40	0.51	*
3-6	0.65	0.18	0.01	0.60	0.38	NS

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

[c]Represents mean differences, 0-3 and 3-6 weeks.

at both sites (Best and Taylor, 1966).

Bronner and Albert (1965) also reported that blood calcium concentrations remained constant over a wide range of daily calcium intakes. Measurements of the calcium deposition and resorption rates in bones indicated that calcium resorption from bone appeared to play the major role in regulating the blood calcium level. Ullrey et al. (1967) studied the serum calcium levels of pigs at birth and at the following pospartum intervals: 24, 48 and 72 hours; 5 and 7 days; 2, 3 and 5 weeks; and 2, 3, 4 and 5 months. Mean calcium values did not vary appreciably with age, and ranged from 9.3 to 11.8 mg. per 100 ml.

The concentration of inorganic phosphorus (determined as phosphate but calculated as phosphorus) in blood serum is reported in Table XII. The mean values ranged from 6.29 mg. to 12.64 mg. per 100 ml. These values represent a greater range than the values of 5 to 8 mg. per 100 ml. reported by Dukes, 1955.

Ullrey et al. (1967) found inorganic phosphorus concentrations of 5.3 mg. per 100 ml. at birth, 11.6 mg. per 100 ml. at two weeks, followed by a gradual decline to 7.1 mg. per 100 ml. at 5 months of age.

There was a significant difference ($P<.05$) in the initial inorganic phosphorus values, with pigs on Ration A having the lowest values. This could be the result of the one week adjustment period for each pig before the initial bleeding. There were also significant differences at three and six weeks. Therefore, an analysis of variance was made on the amount of change (Table XII) from zero to three weeks and three to six weeks, respectively. There was a significant difference ($P<.05$) in the change from zero to three weeks, but no significant differences in the change from three to six weeks.

Data in this experiment shows a linear increase from 7.99 mg. per 100 ml. on Ration A to 12.64 mg. per 100 ml. on Ration D. This is in agreement with Miller et al. (1964b) who reported that serum inorganic phosphorus concentration is related to dietary phosphorus intakes. The influence of dietary calcium and phosphorus levels on serum calcium to serum inorganic phosphorus ratios is presented in Table XIII. A significant difference was indicated at both three and six weeks.

Mean serum alkaline phosphatase values are reported in Table XIII and graphically shown in Figure 4. Serum alkaline phosphatase activity is, in part, a reflection of osteoblastic activity. Serum alkaline phosphatase activity is highest in the pig at birth, falls to approximately one-half by the fifth day, levels off at about 28 days and then gradually declines to 56 days post-partum (Young and Underdahl, 1948).

Long et al. (1965) determined serum alkaline phosphatase activity in baby pigs at intervals from birth to four weeks of age. Serum alkaline phosphatase activity was negatively correlated (-0.53) with body weight ($P<.05$). It

TABLE XIII

SERUM CALCIUM TO PHOSPHORUS RATIOS AND SERUM ALKALINE PHOSPHATASE VALUES FOR PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	11	11	11	12		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Weeks on feed	Ratio of serum calcium to inorganic phosphorus					
0	1.79	1.58	1.43	1.51	0.06	NS
3	1.62	1.41	1.34	1.01	0.02	*
6	1.39	1.34	1.27	0.94	0.06	*
	Serum alkaline phosphatase, Klein-Babson-Read units					
0	32.94	30.19	26.38	27.69	1.76	NS
3	33.63	19.22	14.21	18.75	1.88	*
6	21.79	11.80	9.79	12.35	1.81	*

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

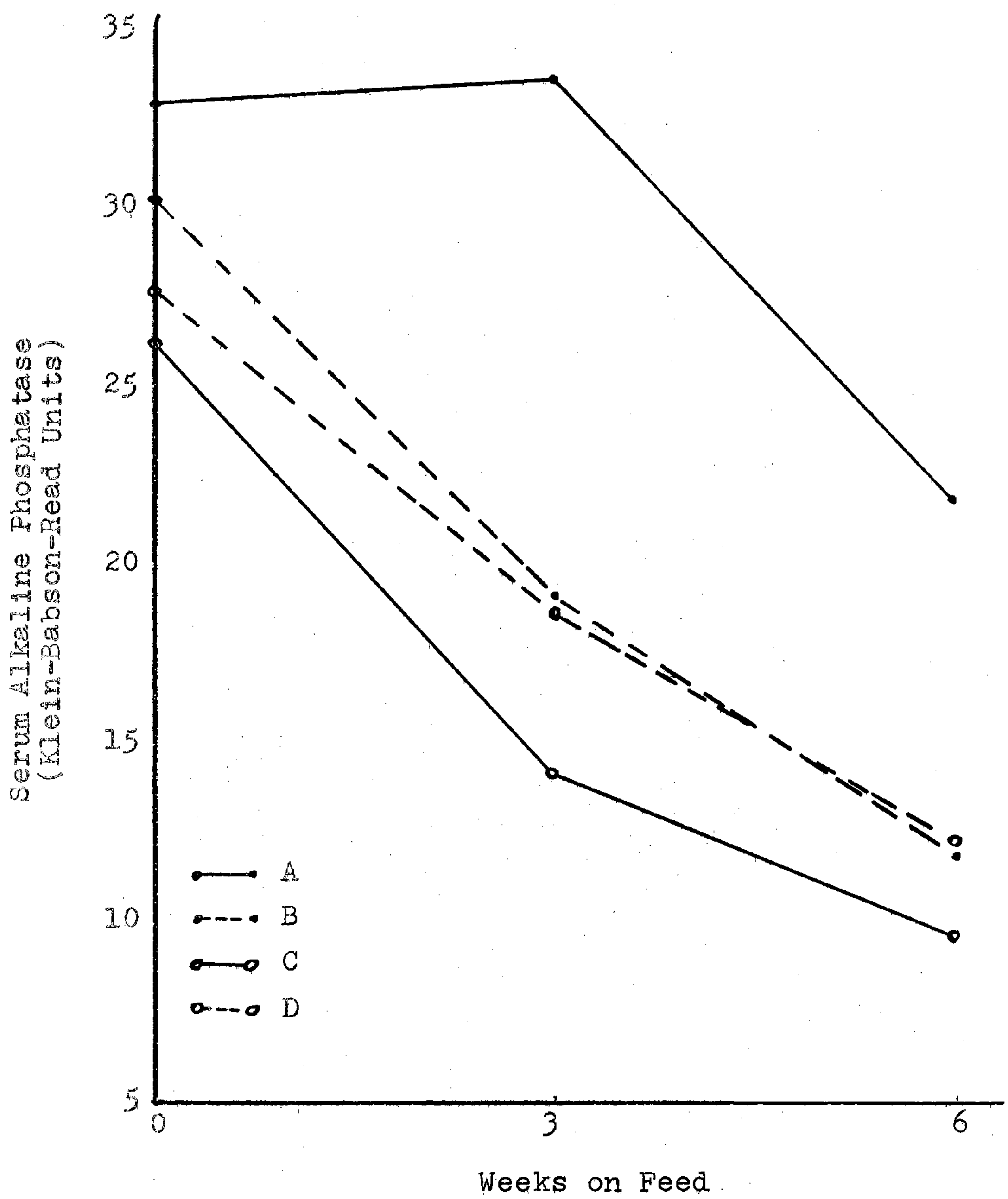


Figure 4. Influence of Dietary Calcium and Phosphorus Levels on Serum Alkaline Phosphatase Values

would appear that as body mass increased, the phosphatase available for maintenance of serum levels declined.

In this experiment, pigs on Ration A (0.27 percent calcium and 0.14 percent phosphorus) produced elevated serum alkaline phosphatase values. After six weeks, pigs on Ration A had the highest mean values and pigs on Ration C had the lowest mean serum phosphatase activity. The mean value for Ration A was significantly different ($P<.05$) from all others.

Miller et al. (1962b and 1964b) reported elevated serum alkaline phosphatase levels in baby pigs receiving less than 0.80 percent dietary calcium and less than 0.60 percent dietary phosphorus, respectively.

The mean values of hematocrit and hemoglobin are presented in Table XIV. There was a significant difference ($P<.05$) in initial hematocrit readings, but when analyzed as amount of change from zero to three weeks and three to six weeks, the differences were not significant. The differences in hemoglobin concentrations were not significant in this study. All values were within the reported normal values.

The mean values of erythrocyte population and leukocyte counts are shown in Table XV. No noticable differences or trends among these values were observed.

Mean corpuscular volume (M.C.V.), mean corpuscular hemoglobin (M.C.H.) and mean corpuscular hemoglobin concentration (M.C.H.C.) were calculated and are shown in Table

TABLE XIV

BLOOD HEMATOCRIT AND HEMOGLOBIN LEVELS OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	11	11	11	12		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Weeks on feed			Hematocrit, %			
0	38.49	32.49	32.36	36.12	1.65	*
3	44.45	39.77	37.63	41.74	2.12	NS
6	44.09	38.45	31.99	38.08	2.37	*
			Amount of Change[c]			
0-3	5.95	7.27	5.27	5.62	2.07	NS
3-6	0.36	1.31	5.63	3.66	1.52	NS
			Hemoglobin, gm./100 ml blood			
0	11.23	10.60	11.22	11.04	0.54	NS
3	12.68	12.07	12.43	12.10	0.56	NS
6	12.22	12.28	10.86	10.99	0.58	NS

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

[c]Represents mean differences, 0-3 and 3-6 weeks

TABLE XV

RED BLOOD CELL AND WHITE BLOOD CELL COUNTS OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	11	11	11	12		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Weeks on feed	Red Blood Cells, millions/cubic mm.					
0	5.15	5.11	5.47	4.84	0.30	NS
3	5.73	5.30	5.78	5.23	0.29	NS
6	6.34	5.65	5.06	5.58	0.33	NS
	White blood cells, thousands/cubic mm.					
0	9.64	8.91	9.80	8.40	1.21	NS
3	6.74	9.90	9.49	9.37	1.02	NS
6	7.89	11.94	7.69	9.78	1.30	NS

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences, ($P<.05$). NS = non-significant ($P>.05$).

XVI. The average M.C.V. value when averaged over treatments was 68.10 cubic microns. This is higher than the 51.1 cubic microns reported by Miller et al. (1961b) and the 56.0 cubic microns reported by Swenson et al. (1958). There is no obvious explanation for these higher values. The M.C.H. value, averaged over all treatments, was 21.4 micro-mcg. This is in close agreement with the 18.7 micro-mcg. reported by Swenson et al. (1958) and Miller et al. (1961b). The M.C.H.C. value, averaged over all treatments, was 33.8 percent. This is identical to the values reported by Miller et al. (1961b) and Swenson et al. (1958). M.C.H.C. was determined by dividing the gm. hemoglobin per 100 ml. of blood by the packed cell volume per 100 ml. and expressing the value as a percentage. This value is never greater than normal but may be normal (normochromic) or less than normal (hypochromic). Significant differences ($P<.05$) were observed at three and six weeks with pigs on Ration C having the highest percentages and those on Ration A having the lowest percentages.

At the end of the six week experimental period, four animals were randomly selected from each treatment and slaughtered. Various organs and bones were removed for gross observations and chemical analysis.

At slaughter, the nasal turbinates were grossly observed for evidence of atrophy. Turbinates were evaluated from the standpoint of amount of bone present, distortion of dorsal and ventral turbinates and deviation of the septum.

TABLE XVI

MEAN CORPUSCULAR VOLUME, MEAN CORPUSCULAR HEMOGLOBIN AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION OF PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	11	11	11	12		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Weeks on feed	Mean corpuscular volume, cubic microns					
0	76.05	66.29	60.28	76.92	4.78	NS
3	77.73	75.26	67.62	80.32	3.86	NS
6	70.00	68.23	63.86	70.30	4.24	NS
	Mean corpuscular hemoglobin, micro-mcg.					
0	21.98	21.49	20.91	23.43	1.38	NS
3	22.15	22.98	22.39	23.28	1.00	NS
6	19.40	22.10	21.96	20.14	1.06	NS
	Mean corpuscular hemoglobin concentration,%					
0	29.25	33.69	35.59	30.47	1.77	NS
3	28.52	31.03	34.54	28.98	1.48	*
6	27.74	32.58	35.96	28.86	1.26	*

[a]Standard error of treatment means. Standard error when twelve per treatment is the reported standard error times $\sqrt{11/12}$.

[b]Significant differences *(P<.05). NS = non-significant (P>.05).

None of the pigs were diagnosed as having evidence of atrophic rhinitis, although imperfect formations were noted.

Absolute and relative weights of the eighth rib, humerus, femur and ulna-radius from the right half of the carcass are presented in Table XVII. The absolute bone weights are shown graphically in Figure 5. There was a significant difference ($P<.05$) among all treatments for each of the bones. Ration C consistently produced the heaviest and Ration A the lighest bone weights. This would be expected since Ration C produced the heaviest animals and Ration A the lightest animals.

Due to the difference in slaughter weights, it seemed advantageous to adjust for body weights by expressing bone weights on a relative basis, in gm. per kg. of body weight at slaughter. There were no significant differences when bone weights were put on a relative basis (Table XVII), but pigs on Ration C did produce the heaviest relative weights of all four bones. The absolute weights of fresh bone are in contrast to those of Miller et al. (1962b) who obtained maximum mean fresh eighth rib, femur and humerus weights when the ration calcium levels were above 1.20 percent of the diet.

Absolute bone diameter and length data are presented in Table XVIII. Absolute bone diameters are graphically shown in Figure 6. A significant difference ($P<.05$) was found among all treatments. The bones from animals on Ration C had the largest absolute diameter in all cases.

TABLE XVII

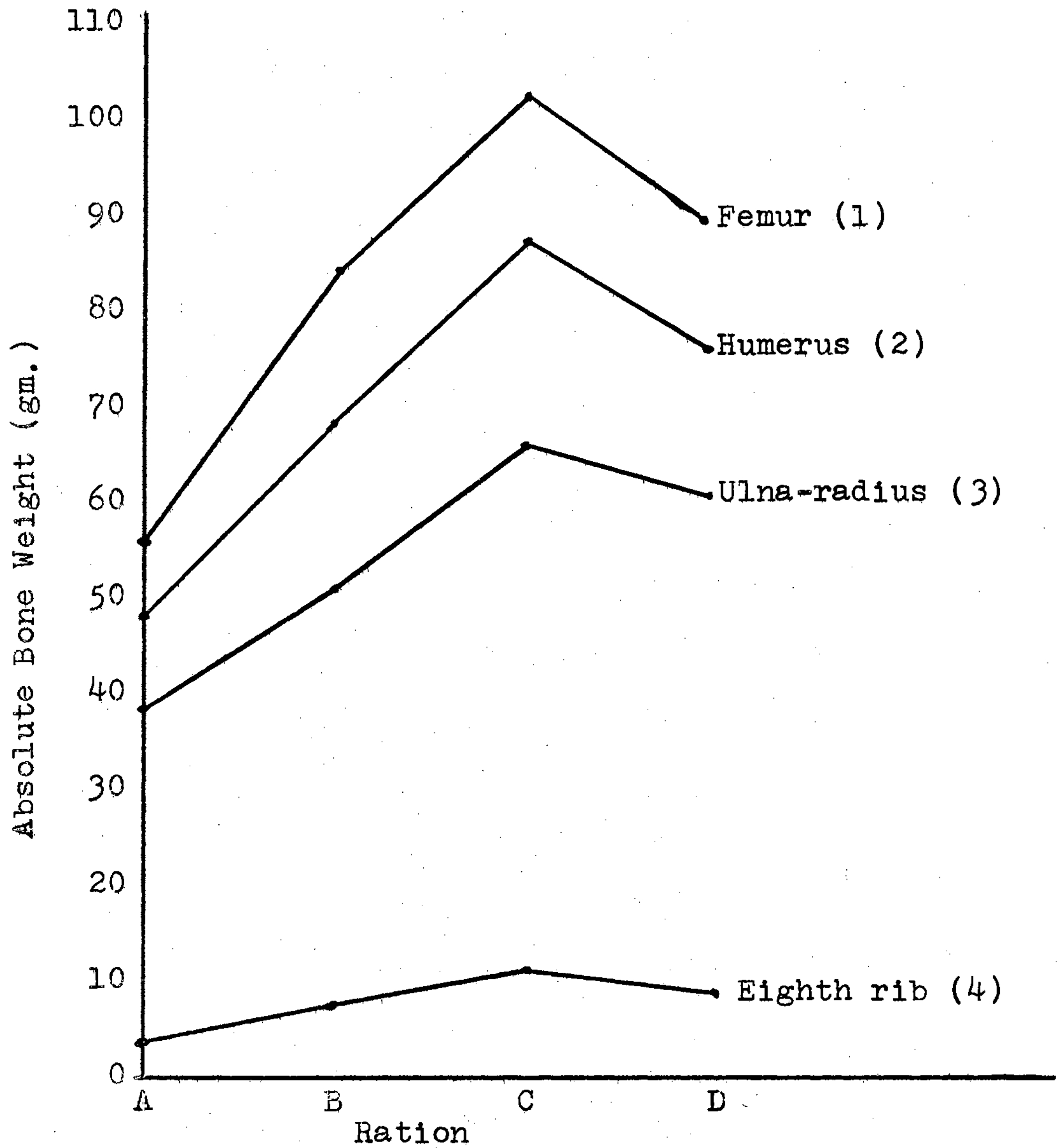
INFLUENCE OF DIETARY CALCIUM (Ca) AND PHOSPHORUS (P) ON ABSOLUTE AND RELATIVE BONE WEIGHTS OF PIGS SACRIFICED AT NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
			Absolute weights, gm.			
Eighth rib	3.9	7.7	11.2	8.8	1.5	*
Humerus	47.9	68.4	87.2	76.6	7.6	*
Femur	55.8	84.3	102.0	89.4	8.9	*
Ulna-radius	38.6	51.1	65.8	60.9	6.0	*
			Relative weights, units[c]/Kg. live body wt.			
Eighth rib	280.5	396.0	518.2	407.8	59.0	NS
Humerus	3.4	3.8	4.0	3.7	0.3	NS
Femur	4.0	4.6	4.8	4.3	0.4	NS
Ulna-radius	2.8	2.8	3.0	2.0	0.2	NS

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

[c]Unit for eighth rib is mg. and all other units reported are in gm.



Test for Significant Linear and Quadratic Effects.

(4) Linear ($P<.025$), Quadratic ($P<.10$)
(2) Linear ($P<.05$), Quadratic ($P<.05$)
(1) Linear ($P<.025$)
(3) Linear ($P<.025$)

Figure 5. Effect of Ration Calcium and Phosphorus Levels on Absolute Bone Weight of Pigs Slaughtered At Nine Weeks of Age

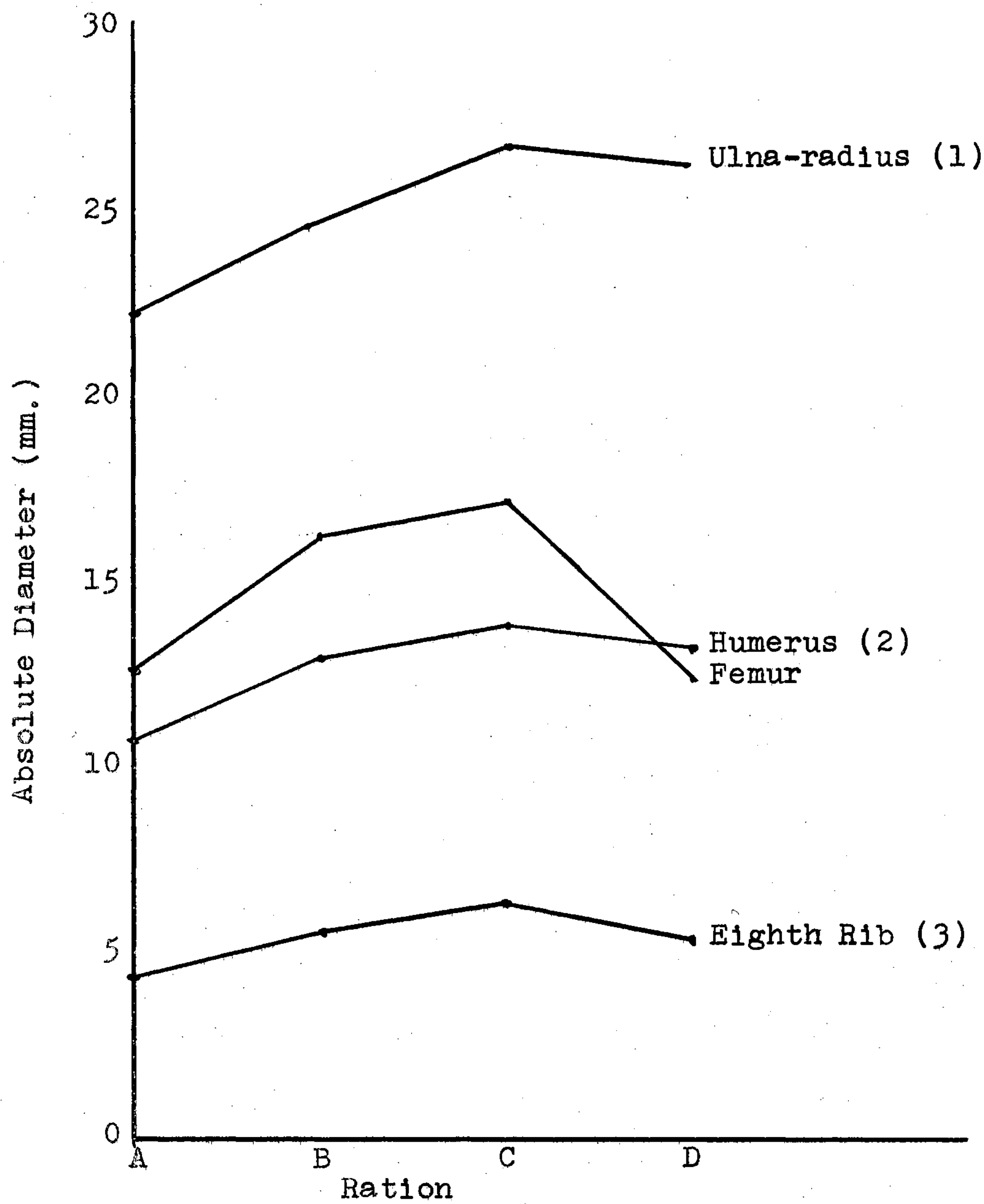
TABLE XVIII

INFLUENCE OF DIETARY CALCIUM (Ca) AND PHOSPHORUS (P) LEVELS ON ABSOLUTE BONE DIAMETER AND LENGTH OF PIGS SACRIFICED AT NINE WEEKS OF AGE.

Ration	A	B	C	D		
No. of Pigs	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
			Absolute diameter, mm.			
Eighth rib	4.3	5.6	6.3	5.4	0.4	*
Humerus	10.7	13.0	13.9	13.2	0.7	*
Femus	12.7	16.3	17.1	12.4	1.4	*
Ulna-radius	22.1	24.6	26.8	26.4	0.9	*
			Absolute length, mm.			
Eighth rib	93.9	106.5	113.0	114.4	5.5	*
Humerus	87.9	100.9	105.7	104.2	4.3	*
Femur	98.7	110.2	122.1	111.9	3.6	*
Ulna-radius	109.2	116.0	127.0	120.7	4.8	NS
			Ratio of absolute diameter to length			
Eighth rib	1:22.7	1:19.2	1:18.0	1:20.2	1.5	NS
Humerus	1: 8.3	1: 7.8	1: 7.7	1: 8.0	0.4	NS
Femur	1: 7.8	1: 6.8	1: 7.1	1:8.4	0.4	NS
Ulna radius	1: 4.9	1: 4.7	1: 4.7	1:4.6	0.1	NS

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).



Test for Significant Linear and Quadratic Effects.
(3) Linear (P<.10, Quadratic (P<.05)
(2) Linear (P<.05), Quadratic (P<.10)
(1) Linear (P<.005)

Figure 6. Effect of Ration Levels of Calcium and Phosphorus on Mean Bone Diameters of Pigs Slaughtered at Nine Weeks of Age

All bones, except the ulna-radius, revealed a significant difference ($P<.05$) in absolute length.

Due to the differences in slaughter weights, bone diameter and length were put on a relative basis (mm. of bone per kg. live body weight). When reported on a relative basis, differences in diameter or length, as shown in Table XIX, were not significant although pigs on Ration A consistently had the largest relative values.

Bone specific gravity was positively related to the ration calcium and phosphorus level (Table XX). Without exception, specific gravity values were increased with successive increments of dietary calcium and phosphorus (Figure 7). There was a significant difference ($P<.05$) among all treatments for each of the bones examined. These data are in close agreement with those of Miller _et_ _al_. (1962b) who reported that humerus, femur and eighth rib specific gravity values increased with increasing levels of calcium up to 1.20 percent.

Maximum specific gravity of the femur was obtained in pigs receiving 1.25 percent dietary calcium. This is in contrast to the work of Newman _et_ _al_. (1967) who reported obtaining maximum femur specific gravity in pigs receiving 0.60 percent dietary calcium.

Results of this experiment show that maximum humerus and eighth rib specific gravity values occurred when dietary phosphorus level was 1.05 percent. Miller _et_ _al_. (1961c) showed rib and femur specific gravity to be positively

TABLE XIX

INFLUENCE OF DIETARY CALCIUM (Ca) AND PHOSPHORUS (P) LEVELS ON RELATIVE DIAMETER AND LENGTH OF BONES OF PIGS SACRIFICED AT NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 4 0.27 0.14	B 4 0.57 0.44	C 4 0.95 0.73	D 4 1.25 1.05	SE[a]	SD[b]
	Relative diameter, mm./Kg. live body wt.					
Eighth rib	0.30	0.32	0.28	0.26	0.02	NS
Humerus	0.78	0.75	0.64	0.62	0.07	NS
Femur	0.94	0.95	0.79	0.59	0.11	NS
Ulna-radius	1.64	1.45	1.24	1.27	0.16	NS
	Relative length, mm./Kg. live body wt.					
Eighth rib	6.98	6.29	5.15	5.57	0.67	NS
Humerus	6.51	5.93	4.89	5.04	0.65	NS
Femur	7.33	6.48	5.66	5.42	0.73	NS
Ulna-radius	8.13	6.74	5.88	5.85	0.67	NS

[a]Standard error of treatment means.

[b]NS = non-significant ($P > .05$).

TABLE XX

SPECIFIC GRAVITY OF BONES TAKEN FROM NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 4 0.27 0.14	B 4 0.57 0.44	C 4 0.95 0.73	D 4 1.25 1.05	SE[a]	SD[b]
			Specific gravity			
Eighth rib	1.11	1.19	1.22	1.28	0.02	*
Femur	1.12	1.16	1.20	1.23	0.01	*
Humerus	1.13	1.16	1.20	1.23	0.01	*
Ulna-radius	1.14	1.18	1.21	1.25	0.01	*

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05).

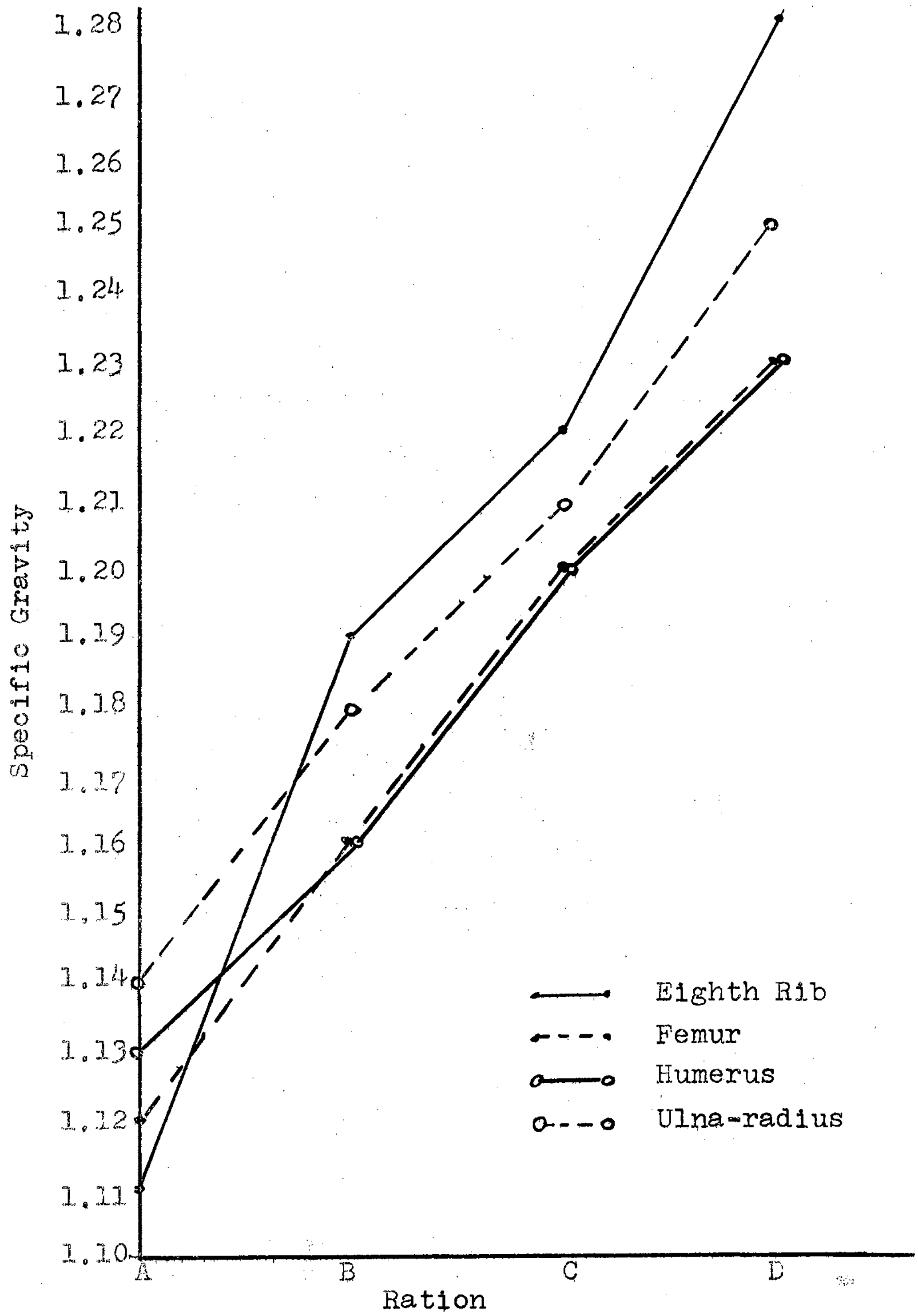


Figure 7. Influence of Ration Levels of Calcium and Phosphorus on Specific Gravities of Bones Taken from Pigs Slaughtered at Nine Weeks of Age

related to dietary phosphorus levels. Maximum humerus and eighth rib specific gravity values were obtained when the dietary phosphorus levels were 0.50 and 0.60 percent, respectively (Miller et al., 1964b).

Mean values of bone ash, calcium, phosphorus and bone calcium: phosphorus ratio are presented in Table XXI. The precent ash content of all bones is graphically presented in Figure 8. There was a significant difference ($P<.05$) in percent ash in all bones tested, except for the humerus. Ration C produced the largest ash content for all bones, except the humerus, and Ration A produced the lowest ash content. This is in agreement with results obtained by numerous workers who have found that baby pig rations low in calcium and phosphorus tend to produce bones with low ash values (Eggert et al., 1959; Vandepopuliere et al., 1959; Miller et al., 1960; Zimmerman et al., 1961; Blair and Benzie, 1964).

Ration C produced the highest percent of calcium and phosphorus in bone ash in all cases. This would be expected since Ration C usually had the largest ash values. The calcium and phosphorus content of all bones examined is shown in Figures 9 and 10, respectively.

The influence of calcium and phosphorus levels on actual and relative organ and gland weights is presented in Tables XXII and XXIII, respectively. Except in the case of the lungs, all differences in absolute organ weights were non-significant. A significant difference in absolute lung

TABLE XXI

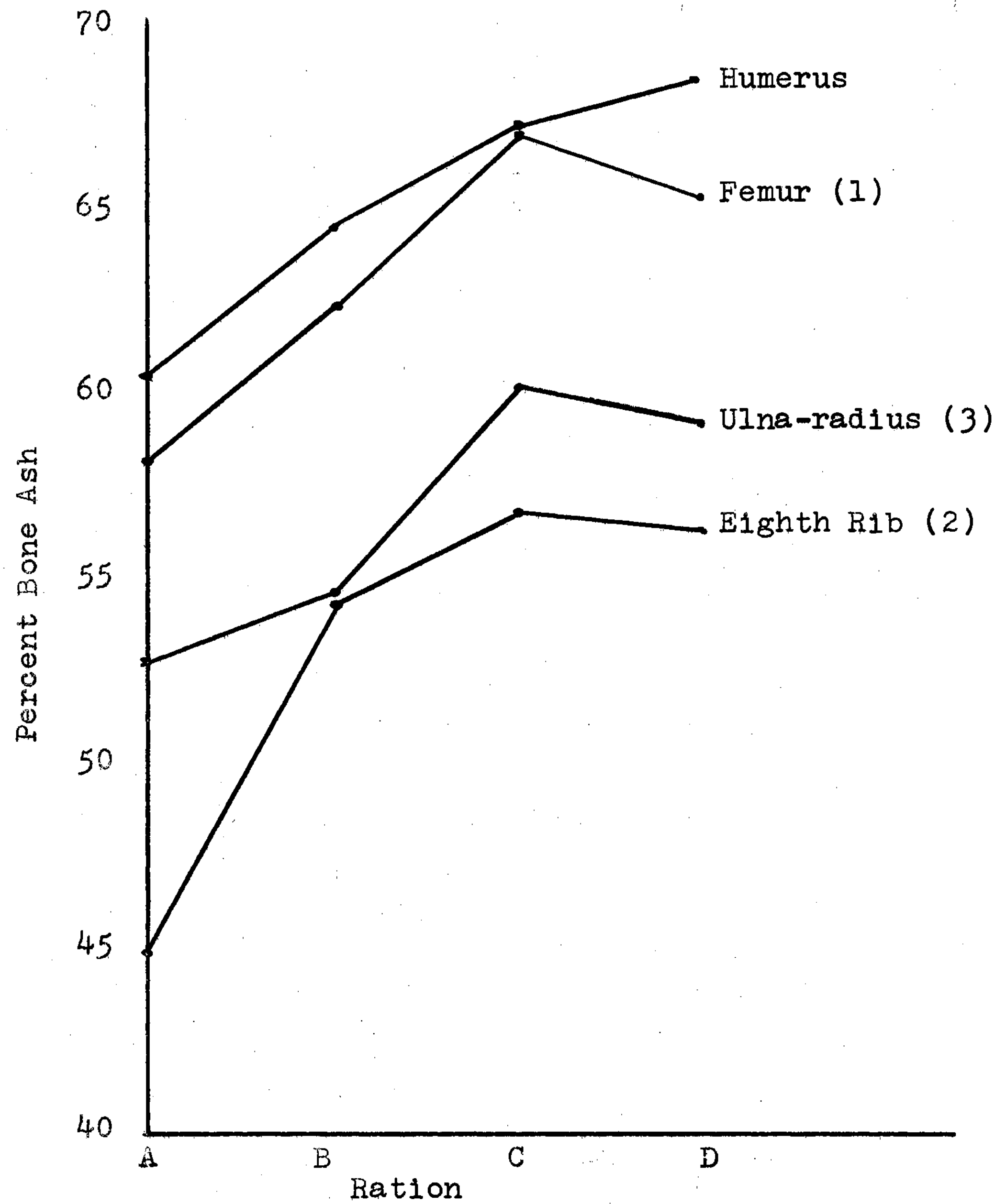
ASH, CALCIUM AND PHOSPHORUS CONTENT OF BONES TAKEN FROM NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM (Ca) AND PHOSPHORUS (P) FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
			Eighth rib ash[c]			
Ash, %	44.98	54.23	56.85	56.23	1.35	*
Calcium, %	17.80	21.88	23.21	22.66	0.46	*
Phosphorus, %	8.15	9.08	10.87	10.41	0.32	*
Ca/P	2.17	2.42	2.13	2.17	0.07	*
			Humeral ash[c]			
Ash, %	60.49	64.43	67.37	68.44	2.62	NS
Calcium, %	24.77	26.50	26.71	25.73	0.51	NS
Phosphorus, %	10.36	11.89	12.32	12.23	0.15	*
Ca/P	2.38	2.22	2.16	2.09	0.04	*
			Femur ash[c]			
Ash, %	58.37	62.45	67.10	65.36	1.68	*
Calcium, %	23.63	25.60	26.73	26.05	0.59	*
Phosphorus, %	11.15	11.80	13.20	13.15	0.35	*
Ca/P	2.12	2.17	2.02	1.97	0.04	*
			Ulna-radius ash[c]			
Ash, %	52.79	54.68	60.22	59.20	1.64	*
Calcium, %	21.67	22.52	24.72	23.39	0.92	NS
Phosphorus, %	9.07	9.84	11.30	10.77	0.33	*
Ca/P	2.39	2.28	2.19	2.17	0.09	NS

[a]Standard error of treatment means.

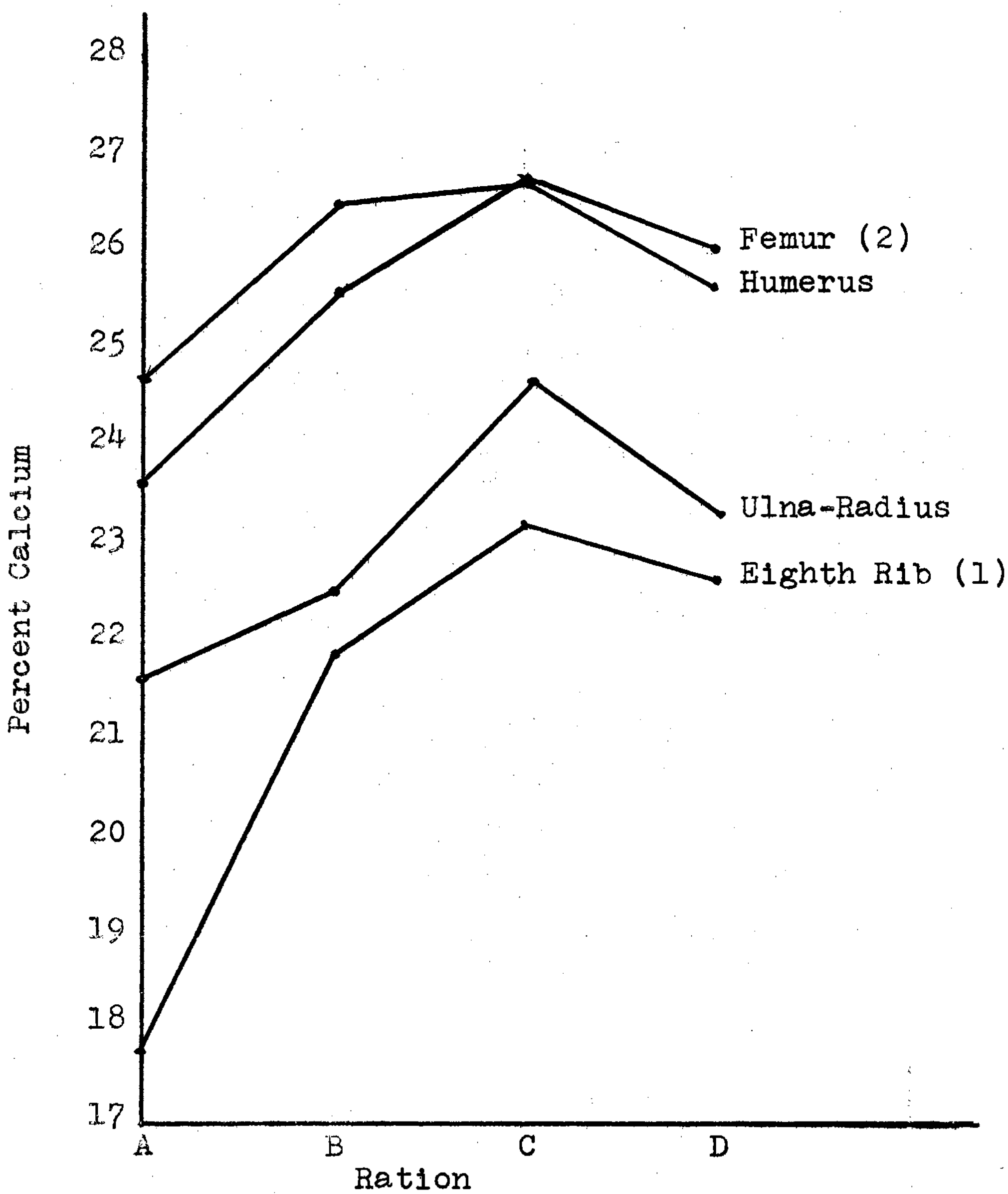
[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

[c]Expressed on a dry, fat-free basis.



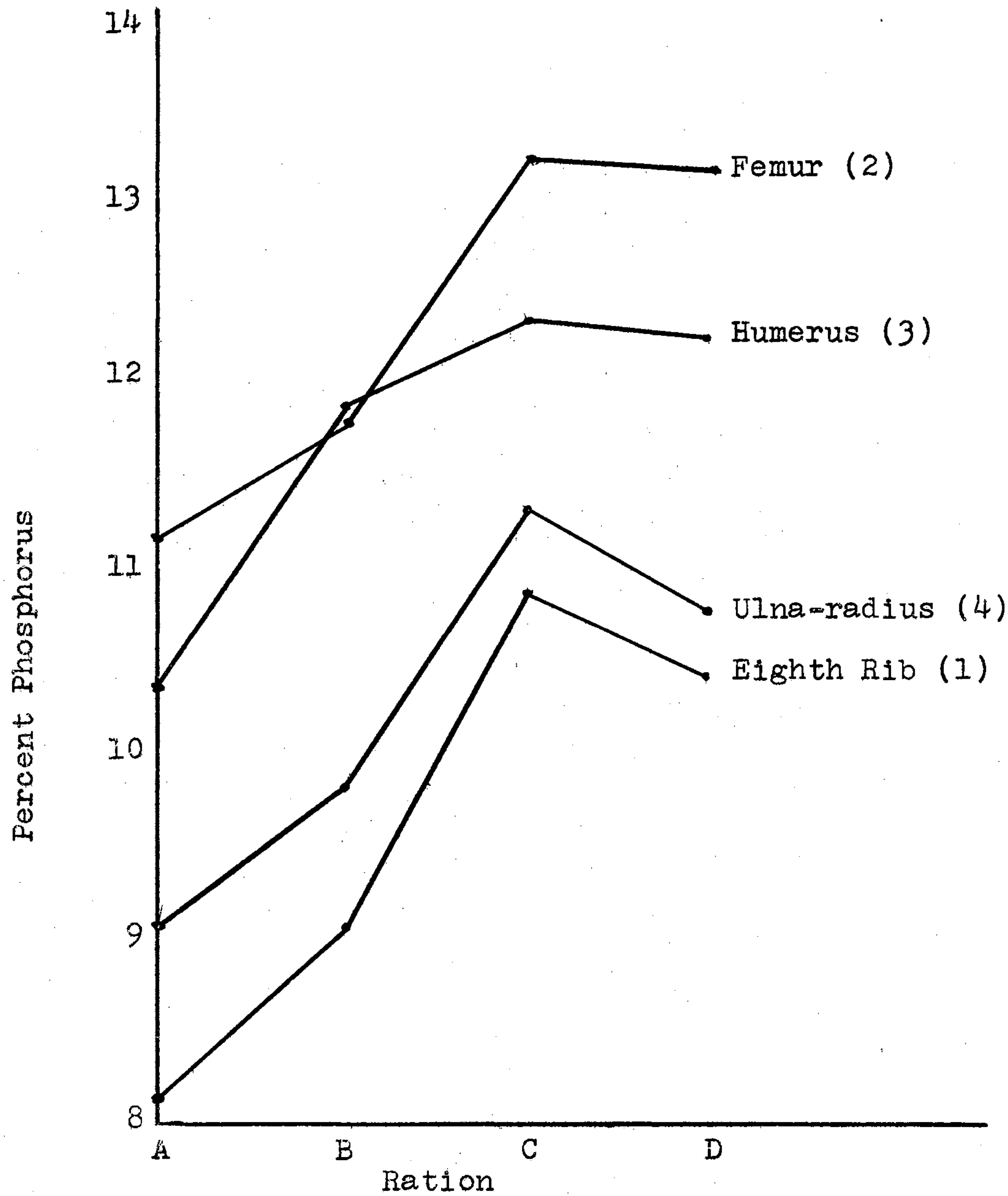
Test for Significant Linear and Quadratic Effects.
(1) Linear ($P<.01$)
(2) Linear and Quadratic ($P<.005$)
(3) Linear ($P<.01$)

Figure 8. Influence of Ration Calcium and Phosphorus Levels on Percent Ash of Bones from Pigs Slaughtered at Nine Weeks of Age



Test for Significant Linear and Quadratic Effects.
(1) Linear ($P<.005$)
(2) Linear ($P.01$), Quadratic ($P<.05$)

Figure 9. Percent Calcium in Bone Ash as Affected by Ration Calcium and Phosphorus Levels



Test for Significant Linear and Quadratic Effects.
(1) Linear ($P<.005$), Quadratic ($P<.10$)
(2) Linear ($P<.005$)
(3) Linear ($P<.005$), Quadratic ($P<.005$)
(4) Linear ($P<.005$), Quadratic ($P<.10$)

Figure 10. Percent Phosphorus in Bone Ash as Affected by Ration Calcium and Phosphorus Levels

TABLE XXII

INFLUENCE OF DIETARY CALCIUM (Ca) AND PHOSPHORUS (P) LEVELS ON ABSOLUTE ORGAN AND GLAND WEIGHTS OF PIGS SACRIFICED AT NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
			Absolute weights, gm.			
Kidneys	89.8	110.3	126.3	135.8	15.9	NS
Spleen	18.8	34.5	38.3	27.0	9.9	NS
Stomach	81.8	115.3	130.3	126.5	14.6	NS
Liver	336.2	477.0	540.0	544.0	65.7	NS
Heart	58.3	81.8	101.3	90.0	10.4	NS
Thymus	52.5	76.3	98.3	75.8	20.7	NS
Lungs	111.3	133.5	174.3	164.5	14.4	*

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

TABLE XXIII

INFLUENCE OF DIETARY CALCIUM (Ca) AND PHOSPHORUS (P) LEVELS ON RELATIVE ORGAN AND GLAND WEIGHTS OF PIGS SACRIFICED AT NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs.	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Relative weights, units[c]/Kg. live body weight						
Kidney	6.4	5.9	5.7	6.4	0.4	NS
Spleen	1.3	1.8	1.7	1.2	0.4	NS
Stomach	5.7	6.6	6.0	6.1	0.7	NS
Liver	24.3	26.6	24.6	25.4	1.8	NS
Heart	4.1	4.4	4.6	4.2	0.2	NS
Thymus	3.5	3.7	4.4	3.6	0.7	NS
Lungs	7.9	7.3	8.1	7.9	0.5	NS

[a]Standard error of treatment means.

[b]NS = non-significant (P>.05).

[c]Units are gms. per Kg. live body weight

weights was also reported by Washam (1968). This significant difference in lung weights disappeared when organ and gland weights were expressed on a relative basis (units per kg. of live slaughter weight).

In the work of Miller et al. (1967), a calcium deficiency in young pigs resulted in significantly reduced liver weights and significantly increased relative weights of kidneys and heart. A phosphorus deficiency in baby pigs resulted in significantly reduced actual weights of liver, kidneys, heart, spleen and thyroid. The same general trends were noticed in this study, but were not significant, probably because of the small numbers per treatment (four) and the large standard errors.

Ration C produced the heaviest actual weights of the spleen, stomach, heart, thymus and lungs. This would be expected in that pigs on Ration C had the heaviest actual slaughter weights.

The calcium and phosphorus content of the left kidney is presented in Table XXIV. All differences with regard to calcium content were not significant, although the kidneys from pigs on Ration C did have the largest calcium content in mg. per 100 gm. tissue. All differences with regard to phosphorus content were not significant. The pigs on ration A had an elevated phosphorus concentration. This is in line with the work of Miller et al. (1964b) who observed an elevated kidney phosphorus concentration in phosphorus deficient pigs receiving a ration containing 0.80 percent

TABLE XXIV

CALCIUM (Ca) AND PHOSPHORUS (P) CONTENT OF THE LEFT KIDNEY OF NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM AND PHOSPHORUS FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 4 0.27 0.14	B 4 0.57 0.44	C 4 0.95 0.73	D 4 1.25 1.05	SE[a]	SD[b]
Weight, fresh gm.	45.75	56.50	65.75	67.75	8.23	NS
Ash, dry basis %	5.88	5.84	7.28	9.60	1.45	NS
Ca, dry basis,% mg./100 gm.	29.61	31.29	40.42	40.02	7.38	NS
Total Ca, mg. left kidney	2.99	4.08	5.64	6.21	1.05	NS
P, dry basis mg./100 gm.	1599.87	1274.50	1311.92	2128.18	242.50	NS
Total P, mg. left kidney	152.15	163.73	197.32	282.24	28.15	*
P/Ca, dry basis	58.45	40.81	37.11	70.36	10.87	NS

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

calcium and 0.20 percent phosphorus.

The calcium and phosphorus analyses of the liver are given in Table XXV. Differences were not significant, but livers from pigs fed Ration C had the largest concentration of calcium per 100 gm. tissue.

The calcium and phosphorus content of the heart is shown in Table XXVI. The only significant difference ($P<.05$) was in calcium concentration per 100 gm. tissue with Ration B having the largest value. This difference has not appeared in previous reviews.

The calcium content of the hair (Table XXVII) did not differ significantly. Great variation within treatment groups was observed. There was a significant difference ($P<.05$) among treatment means with regard to dry matter content. Ration C produced the largest value and Ration A produced the smallest value. Ration C produced the largest concentration of calcium per 100 gm. of hair, as it should, since Ration C had the greatest percent dry matter.

TABLE XXV

CALCIUM (Ca) AND PHOSPHORUS (P) CONTENT OF THE LIVER OF NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM AND PHOSPHORUS FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 4 0.27 0.14	B 4 0.57 0.44	C 4 0.95 0.73	D 4 1.25 1.05	SE[a]	SD[b]
Weight, fresh gm.	336.3	477.0	540.0	544.0	65.7	NS
Ash, dry basis %	5.12	4.93	5.29	5.25	0.17	NS
Ca, fresh tissue mg/100 gm.	6.14	7.39	8.69	8.14	0.95	NS
Ca, dry basis mg/100 gm.	22.24	26.09	31.09	28.59	3.42	NS
Total liver Ca, mg.	20.31	36.69	47.77	44.95	8.29	NS
P, mg/100 gm. flesh tissue	360.30	362.44	373.04	402.35	13.97	NS
P, dry basis mg/100 gm.	1297.49	1272.49	1324.99	1409.99	62.00	NS
Total liver P, mg.	1231.26	1761.22	2016.89	2170.52	263.0	NS
P/Ca, dry basis	60.58	49.77	46.11	50.60	5.87	NS

[a]Standard error of treatment means.

[b]NS = non-significant (P>.05)

TABLE XXVI

CALCIUM (Ca) AND PHOSPHORUS (P) CONTENT OF THE HEART OF NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM AND PHOSPHORUS FROM THREE TO NINE WEEKS OF AGE

Ration No. of Pigs Dietary Ca, % Dietary P, %	A 4 0.27 0.14	B 4 0.57 0.44	C 4 0.95 0.73	D 4 1.25 1.05	SE[a]	SD[b]
Weight, fresh gm.	58.3	81.8	101.3	90.0	10.4	NS
Ash, dry basis %	4.50	4.81	4.66	4.38	0.15	NS
Ca, dry basis mg/100 gm.	22.73	28.32	23.98	23.35	1.01	*
Total heart Ca, mg.	3.08	4.99	5.50	4.71	0.58	NS
P, dry basis mg/100 gm.	1193.55	1163.98	1075.62	1119.79	76.30	NS
Total heart P, mg.	160.0	208.58	249.05	222.81	31.50	NS
P/Ca, dry basis	51.52	41.09	44.75	48.04	2.56	NS

[a]Standard error of treatment means.

[b]Significant differences, *($P<.05$). NS = non-significant ($P>.05$).

TABLE XXVII

CALCIUM (Ca) CONTENT OF THE HAIR OF NINE WEEK OLD PIGS FED FOUR LEVELS OF CALCIUM AND PHOSPHORUS FROM THREE TO NINE WEEKS OF AGE

Ration	A	B	C	D		
No. of Pigs	4	4	4	4		
Dietary Ca, %	0.27	0.57	0.95	1.25		
Dietary P, %	0.14	0.44	0.73	1.05	SE[a]	SD[b]
Dry Matter, %	82.71	87.90	88.63	88.15	1.49	*
Ca, dry basis mg./100 gm.	22.72	32.34	45.92	21.91	11.83	NS

[a]Standard error of treatment means.

[b]Significant differences, *(P<.05). NS = non-significant (P>.05).

CHAPTER V

SUMMARY AND CONCLUSIONS

Forty-five pigs were used in a study to determine the calcium and phosphorus requirements of baby pigs reared on purified diets from three to nine weeks of age. Observations were made on the condition of the nasal turbinates in an effort to determine the relationship between dietary calcium and phosphorus levels and the condition referred to as atrophic rhinitis.

The pigs were caught in sterile plastic bags as they emerged from the birth canal at normal farrowing. They were transported to the laboratory under sterile conditions and placed in individual disposable boxes, each having a separate supply of sterile air. After two weeks they were transferred from the incubator room to individual, open-topped metal cages in the nursery.

Each pig received by stomach tube, an initial supply of pasteurized sow colostrum before being placed in an incubator box. A fortified and pasteurized cows' milk diet, fed during the first two weeks, contained 1.32 percent calcium and 0.98 percent phosphorus.

During the third week of life, the pigs were gradually changed from the liquid diet to a dry, purified, test diet, on which they remained until they were nine weeks of age. During the test period, three to nine weeks of age, Rations A through D contained 0.27, 0.57, 0.95 and 1.25 percent calcium and 0.14, 0.44, 0.73 and 1.05 percent phosphorus, respectively. Body weight and feed intake for each pig were recorded weekly. Blood samples were drawn at the end of the third, sixth and ninth weeks. Four pigs from each treatment group were slaughtered at the conclusion of the trial and various bones and organs were removed for observation and chemical analyses.

Ration A appeared to support normal feed efficiency but the total gain was not comparable to the other rations. Ration A produced normal values for serum calcium, serum inorganic phosphorus, hemoglobin concentrations and hematocrit. However, Ration A produced the least desirable serum alkaline phosphatase values while Ration C produced the most desirable values. The serum alkaline phosphatase values indicate that pigs on Ration A had the greatest bone resorption rate and pigs on Ration C had the least amount of resorption. Ration C produced the heaviest pigs at nine weeks, the best efficiency, heaviest absolute bone weight, diameter and length, highest percent bone ash and the highest concentrations of calcium and phosphorus in bone ash.

The nasal turbinates were grossly examined for symptoms of atrophic rhinitis but none were positively diagnosed.

On the basis of this study, a level of 0.95 percent calcium and 0.73 percent phosphorus is recommended in complete rations from three to nine weeks of age when soundest skeletal development is desired. This level of calcium and phosphorus would probably result in increased daily gains and improved feed efficiency, but the added phosphate would increase the cost of the ration.

Apparently the pig, on the basis of this study and recent literature, can tolerate wide ranges of ration calcium and phosphorus. On low levels (0.57 percent calcium and 0.44 percent phosphorus) the blood readings were normal for all components. High levels (1.25 percent calcium and 1.05 percent phosphorus) also produced normal blood values with the additional asset of a stronger skeleton. There does not seem to be a precise level of calcium or phosphorus required to maintain normal blood values or acceptable rate of growth, rather the pig is able to gain relatively well on levels varying from 0.57 to 1.25 percent calcium when these levels are accompanied by phosphorus levels of 0.44 and 1.05 percent, respectively. Data presented in this research indicates the higher levels of calcium and phosphorus result in greater mineral deposition in the skeleton and presumably a sounder structure. Phosphorus is a rather expensive nutrient, thus, the economics of feeding the higher mineral levels would be of concern to commercial producers interested particularly in feed costs per unit gain to market weights. The producer of breeding animals could probably produce a sounder skeleton by using the higher mineral levels

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VITA

James Arthur Coalson

Candidate for the Degree of

Master of Science

Thesis: A STUDY OF THE CALCIUM AND PHOSPHORUS REQUIREMENTS OF YOUNG PIGS REARED UNDER ARTIFICIAL ENVIRONMENTAL CONDITIONS

Major Field: Animal Science

Biographical:

Personal Data: Born in Winters, Texas, September 6, 1942, the son of John and Nettie Coalson. Married Clara Duncan, October 27, 1961, and have one child, Michelle.

Education: Graduated from Meadow High School in 1961. Received the Bachelor of Science degree from Abilene Christian College, with a major in Animal Science, May, 1966.

Professional Experience: Raised on a farm in west Texas; Graduate Assistant at Oklahoma State University from 1966-1968; Swine Production instructor 1968.

Professional Organizations: Member of American Society of Animal Science.