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

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CHAPTER I

INTRODUCTION

Calcium and phosphorus have long been recognized as essential elements in skeletal formation and in the maintenance of physiological functions in swine. Dietary levels of calcium and phosphorus for young pigs were reinvestigated because of changes that have taken place or are taking place in which production practices may alter the nutrient needs, particularly where ration formulations are made on a percentage basis. Modern pigs grow faster and consume less feed per unit of gain, therefore, the same percentage in the diet means a lower level of any nutrient is consumed per unit of gain.

These investigations were undertaken to: (1) develop techniques to rear colostrum-free neonatal pigs, obtained by Caesarean section, to three weeks of age utilizing a milk solids diet, (2) evaluate the calcium requirements of young SPF pigs, (3) determine if the pathological condition known as atrophic rhinitis could be initiated on a low calcium diet, (4) evaluate the phosphorus requirements of young SPF pigs and (5) determine the apparent digestibility of calcium and phosphorus and to study the uptake of radioactive ^{45}Ca by specific skeletal bones, utilizing SPF pigs.

Historically growth and feed efficiency have received the major emphasis in the establishment of standard requirements for calcium and phosphorus. In these studies, more emphasis will be placed on the mineral composition of the various parts of the skeleton, blood components and other measures of the level and rate of mineral metabolism in the young pig.

CHAPTER II

LITERATURE REVIEW - GENERAL

Rearing SPF Pigs for Experimental Purposes

Techniques for rearing Specific-Pathogen-Free pigs for experimental purposes must be carried out under specified conditions if uniform animals are to be the end product. The rearing of pigs under carefully controlled laboratory conditions would appear to offer a means of removing many of the environmental influences assumed to be competitively disadvantageous to the smaller pigs.

The development of the pig from birth depends partly on its weight and viability and partly on its intake of nutrients from sows' colostrum and milk and from supplementary feed (Lodge, 1966). The composition of sow colostrum and milk has been studied by Perrin (1954), Morgan and Lecce (1964), Pond et al. (1962) and Blair and Benzie (1964).

Sow's milk is highly nutritious, with a crude protein content increasing from about 25 to 33 percent of the dry matter as lactation advances. Sow's milk is particularly rich in fat, containing approximately 7.0 percent on an as-is basis and 36 percent on a dry matter basis. Fat is the most variable of the major constituents of milk and the most readily influenced by feeding. The fat percentage, although very erratic, tends to reach a peak around the third week of lactation (Lodge, 1959) and has been reported as high as 17.2 percent (Perrin, 1955).

Early attempts to raise baby pigs taken at birth were unsuccessful (Catron et al., 1953; Barrick et al., 1954; Cartwright et al., 1950) and colostrum was considered essential for survival. Most pigs developed diarrhea around the fourth day and if unmedicated, 60 to 100 percent died. Many investigators (Lecce and Matrone, 1960, 1961; Lecce et al., 1961; Konopatkin, 1964; Olsson, 1960) have shown that the baby pig is born with a deficiency in albumin, beta and gamma globulin, and it is the function of colostrum to supply this missing protein to the new born pig.

Rapid maturation of the serum protein profile occurs in nursing pigs (Lecce and Matrone, 1960; Sharpe, 1966) and pigs hand fed colostrum (Hardy, 1965) while pigs fed other diets such as cow's milk experience a delay in their serum protein maturation process and are susceptible to diarrhea, bactereamia and death (Lecce and Reep, 1962; Lecce and Matrone, 1961; Lecce et al., 1961).

Antibody-rich-colostral-globulin is believed to cross unaltered from the gut to the serum of the nursing pig within the first 24 to 36 hours after birth (Kaeberle and Segre, 1964; Wellman and Engel, 1964; Lecce et al., 1964). Thus, the baby pig is given a ready supply of globulin before its own synthesizing mechanisms can fulfill this need.

Colostral globulin, carrying immune bodies, endows the offspring with passive immunity to infectious disease (Konopatkin, 1964) accounting for the vigor in the pigs fed colostrum.

No such resistance to disease is expected in the nonimmune, colostrum-free pigs; hence, the difficulty in raising them (Kenworthy and Crabb, 1963; Owen and Bell, 1964; Owen et al., 1961). However, several workers have demonstrated that pigs deprived of colostrum could be raised if techniques were employed to give the pig a completely sterile environment.

Pigs removed from the mother at birth and placed on a synthetic milk containing all known vitamins and with plasma or serum as colostrum substitutes failed to survive longer than 22 days (Bustad et al., 1948). Pigs fed no colostrum substitute such as serum or plasma died very shortly after birth. Indications were that plasma was superior to serum as a colostrum substitute for the new-born pig. Diet intake was seldom over 400 ml. per pig per day. A severe diarrhea developed with all pigs fed synthetic milk. The pigs gradually became unthrifty in condition and developed a dark exudate around the eyes which often cemented the lids together.

Young and Underdahl (1951) protected the newborn pig from respiratory infection by catching it in a sterilized cloth bag. The buttocks of the sow were first cleansed with a mild antiseptic, and the bag opening was held against the buttocks. After the pig dropped into the bag, the top was immediately closed, and the bag and pig were transferred to an isolated environment kept at a temperature of 26.5 - 32°C. The pigs were fed a modified cow's milk diet four times the first day and three times each successive day. By these techniques, 51 of 62 baby pigs were successfully started with some being maintained from 30 to 90 days of age.

Young and Underdahl (1953) reported further success in developing isolation units for growing baby pigs without colostrum. Pigs were either caught at birth in a sterile canvas bag or removed during hysterectomy and reared in individual units until four weeks of age. Pigs removed by hysterectomy do not traverse the birth canal and are less likely to have infections common to the dam and her environment.

Young _et al._ (1955) anaesthetized sows with carbon dioxide gas and removed the uterus with encased pigs. The sows were bled and salvaged for meat purposes. Pigs were removed from the uterus and reared in individual isolation units. One hundred and fifty-five out of 162 pigs were successfully reared via this technique, indicating a mortality rate of 4.4 percent.

Catron _et al._ (1953) reported that in the apparent absence of disease, it was possible to raise pigs, which had not received colostrum, on a vitamin, mineral and antibiotic fortified dried skim milk ration including lard.

The addition of casein did not improve growth but without lard the pigs died. Pigs were secured through hysterectomy and reared in sterile incubators supplied with heated, filtered air (Betts et al., 1960). Using this technique 16 sows were subjected to hysterectomy, 167 piglets were obtained alive from the sows' uteri and 156 were reared giving a survival rate of 93.4 percent.

Schneider and Sarett (1966) developed facilities and procedures for obtaining SPF pigs by hysterectomy to be used in nutritional studies. Specific-pathogen-free pigs under their program gained weight and grew as well as had been reported for normally born and suckled pigs.

The most recent report (Lecce, 1969) involves catching the pigs on sterile towels at birth and rearing on an automatic feeding machine (AUTOSOW). From zero to 14 days, pigs on this system receiving cow's colostrum or a 24 percent milk solids diet grew faster than the controls reared by the dam.

The literature would indicate that pigs obtained through hysterectomy or by catching on a sterile towel at birth can be successfully reared without colostrum if strict environmental controls are enforced. The highest degree of success was obtained when the diet resembles sow's milk with respect to total solids content.

The literature does not record any reports where colostrum-free SPF pigs were obtained through Caesarean section techniques. By this method sows could be saved and possibly reused for production of additional SPF pigs.

General 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 1.0 percent of 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.

Bone is a highly specialized form of connective tissue, composed of interconnected cells in an intercellular substance and forming the skeleton or framework of the body. Three cellular components are associated with specific functions: osteoblasts with the formation of bone; osteocytes with the maintenance of bone as a living tissue; and osteoclasts with the resorption of bone.

Osteoblasts appear on the surface of a growing or developing bone. During active growth they appear to be in a continuous layer. The osteocyte is an osteoblast that has been surrounded by calcified interstitial substance. The osteoclast is a giant cell with a variable number of nuclei, often as many as fifteen or twenty. They are usually found on the surfaces of bone, in close relationship with areas of resorption.

The hardness of bone is the result of the unique properties of a two-phase system in which a mineral is enmeshed in a fibrous organic matrix. The bone mineral and the organic matrix make up the interstitial substance of bone. The mineral consists mainly of Ca^{2+}, PO_4^{3-}, OH^-, CO_3^{2-} and citrate. Certain statements may be made about the bone mineral: (1) the bone mineral is not a single, homogeneous chemical individual; (2) it includes an amorphous calcium phosphate with a range of Ca/P molar ratios from 1.45 to 1.55; (3) the Ca/P molar ratio of the mineral that is present in calcifying cartilage of growing animals is less than 1.6, while that in bone is 1.6 or more; and (4) the structure and composition of the mineral correspond most closely to those of hydroxyapatite.

Resorption refers to the destruction or solution of the elements of bone. Resorption is in essence, the putting into solution of a complicated structure in such a fashion that it disappears, its end products entering the bloodstream (McLean and Urist, 1968).

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, 1955). 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.

Miller et al. (1962) took pigs from the sow at three days of age, placed them in individual wire-bottom metal cages and fed a synthetic milk diet containing 20 percent of solids consisting of 30 percent vitamin-free casein, 10 percent lard, 55 percent glucose and 5 percent mineral and vitamin mix. The calcium level was varied from zero to 1.6 percent, while phosphorus was held constant at 0.5 percent. Pigs were fed the liquid diet five times daily for four weeks and a dry diet the remaining two weeks of the study. Daily gains on zero to 1.6 percent calcium level ranged from 100 to 170 grams, while the best efficiency resulted on the 0.8 percent calcium diet. Optimal skeletal development, as measured by bone density, ash content, breaking strength and the absence of rachitic symptoms, occurred in pigs receiving 1.0 percent calcium. Maximal calcium retention occurred at this dietary level also. Humeral ash, calcium and phosphorus concentrations were increased by increased dietary calcium levels. However, no significant increases were affected beyond 0.8 percent.

This evidence could suggest a higher calcium requirement than 0.8 percent recommended for the 5-10 kg. pig (N.R.C., 1968).

Using a purified diet, Dudley et al. (1961) fed a total of 96 fourteen day old pigs for 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 data are not consistent with those of Combs and Wallace (1962) who used 202 pigs weaned at 14 days of age to study the influence of increasing increments of calcium with phosphorus held constant at 0.44 percent. When dietary calcium levels of 0.40 and 0.88 percent were compared, the higher level significantly depressed growth rate while the better efficiency occurred on the lower level. When dietary calcium was increased from 0.40 to 0.80 percent by increments of 0.10 percent, daily gains and feed efficiency showed a decreasing linear response.

Zimmerman et al. (1960) investigated the influence of various calcium and phosphorus levels in high milk product rations (25 percent dried skim milk equivalent) on performance of pigs 2 through 6 weeks of age. Rations with more than approximately 0.8 percent calcium caused a growth depression and in some cases adversely affected feed utilization. Bone calcification, estimated by ash content of metatarsal bones, increased with each added increment of calcium up to 1.05 percent. In general, 0.52 percent calcium was inadequate for maximum growth rate and efficiency of utilization, while 0.70 percent calcium was adequate.

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 or 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.

The above review of the literature shows the need for re-evaluation of present calcium requirements for young pigs. Dudley *et al.* (1961), Zimmerman *et al.* (1960), Rutledge *et al.* (1961) and Miller *et al.* (1962) suggested calcium requirements of 0.20, 0.70, 0.80, and 0.80 percent, respectively.

Phosphorus Studies

The phosphorus content of sow's milk, for the first

3 weeks, is consistently near 0.60 percent. This is, perhaps, the principle reason why the requirement for the 5-10 kg. pig is stated as 0.60 percent (N.R.C., 1968).

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 3 to 7 weeks of age), Zimmerman et al. (1961) reported: (1) in regard to gains, the calcium x phosphorus interaction was highly significant; (2) calcium:phosphorus ratios greater than approximately 1.6:1.0 supressed the growth rate, and (3) phosphorus at 0.40 percent of the ration was inadequate for maximum gains regardless of the calcium level. Increasing the phosphorus level significantly improved feed utilization, therefore, the efficiency of feed utilization progressively increased as the calcium:phosphorus ratio narrowed. The treatment effects on percent metatarsal ash were additive and significant. Percent ash was increased by increasing levels of either calcium or phosphorus. Later, Zimmerman et al. (1963) studied the various effects of level of phosphorus in the ration on baby pig performance, while the calcium level was maintained at 0.70 percent. Total body weight gain and metatarsal calcification were maximized when the ration contained 0.57 percent phosphorus, whereas, the efficiency of feed utilization was further improved by increasing the phosphorus level to 0.63 percent.

Evaluations by Combs et al. (1962), utilizing weight gain, feed efficiency, percent ash and radiographs as criteria, indicated that the phosphorus requirements of pigs from 2 to 7 weeks of age was 0.44 percent of the ration and that with this phosphorus level the optimum calcium:phosphorus ratio was 0.9:1.0.

In an extensive study, Miller et al. (1964a) used a synthetic milk diet, maintained the calcium level at 0.80 percent and varied the phosphorus concentration from 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. A level of 0.40 percent of dietary phosphorus was sufficient to support maximal growth rate and economy of food utilization. Serum calcium levels were normal in all pigs, but tended to be higher in animals receiving lower levels of dietary phosphorus. 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 and phosphorus and to provide for an adequate rate of skeletal development. Further calcium and phosphorus balance studies were conducted by Miller et al. (1964b) 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., 1964b). 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 clarify 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 concerning calcium requirements have tended to approximate 1.5 times the value for phosphorus. In fact the N.R.C. requirements for a 5-10 kg. pig represents a 1.33:1 ratio (N.R.C., 1968). 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. The data suggests a ratio of approximately 1.33:1.0 for pigs from 2 through 7 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.

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, lameness and stiffness, and weakened bone structure. The phosphorus deficiency symptoms listed are: slow or interrupted growth, reduced appetite, lameness and stiffness, and weakened bone structure.

Zimmerman et al. (1961) fed 144 baby pigs (from 3 through 7 weeks of age) to study the calcium and phosphorus requirements of pigs fed 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 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 1968 (Table I).

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 10 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.

Bunch _et al._ (1969) used four-week old pigs to study dietary calcium and phosphorus levels from 7.7 to 22.7 kg. body weight. Average daily gain (kg.) and feed gain at lower levels of calcium and phosphorus (0.65 percent calcium - 0.50 percent phosphorus) were 0.35 and 2.07 respectively, and at the higher levels of calcium-phosphorus (1.25 percent calcium - 1.00 percent phosphorus) were 0.36 and 2.04 respectively. These levels of calcium and phosphorus did not influence growth rate or feed conversion.

TABLE I

1968 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.)
5-10	0.80	0.60	1.33:1.0	220
10-20	0.65	0.50	1.30:1.0	200
20-35	0.65	0.50	1.30:1.0	200
35-60	0.50	0.40	1.25:1.0	125
60-100	0.50	0.40	1.25:1.0	125
Breeding stock				
Bred	0.75	0.50	1.50:1.0	275
Lactating	0.60	0.40	1.50:1.0	220

[1]N.R.C. (1968).

Bone ash was significantly ($P<.005$) higher for those pigs receiving the higher calcium and phosphorus levels.

Washam (1968) and Coalson (1969), using laboratory reared SPF pigs from 3 through 9 weeks of age, found that 0.95 percent calcium and 0.73 percent phosphorus produced the heaviest pig weights at 9 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.

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 et al. (1963) recommends 0.80 percent calcium and 0.60 percent phosphorus. The reports of Washam (1968) and Coalson (1969) indicate that 0.95 percent calcium and 0.73 percent phosphorus are needed for maximum performance and skeletal development. These recommendations are lower than the 1.3 percent calcium and 1.0 percent phosphorus suggested by Blair and Benzie (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, Krook and Pond, 1966). The disease prominently features a reduction in volume of the turbinates and inflammatory changes of their mucosa. The approach of

Brown *et al.* (1966) in inducing experimental atrophic rhinitis was based on two facts: (a) the skeletal lesions, including those of the tubular cancellous bone of the turbinates, are generalized osteitis fibrosa and (b) generalized osteitis fibrosa occurs only in hyperparathyroidism, probably being nutritional secondary hyperparathyroidism. Brown *et al.* (1966) fed seven combinations of calcium and phosphorus to 28 pigs, beginning at four weeks of age and continued until reaching 100 kg. weights. The combinations were: (1) 0.80 and 0.60; (2) 1.00 and 0.80; (3) 1.20 and 1.00; (4) 0.80 and 1.60; (5) 0.18 and 0.35; (6) 0.30 and 0.60 and (7) 0.35 and 1.40. Inflammatory nasal discharge was never observed. Mild to moderate deviation of the snout was noted in about half the experimental pigs in groups 4, 6 and 7. More severe and consistent was a retraction of the snout with an accordionlike wrinkling of the skin of the dorsal area. These conditions appeared after 7 to 8 weeks. The retraction was most pronounced in group 5. A slightly less amount of bone existed in turbinates in group 1 than in groups 2 and 3, although group 1 was fed according to N.R.C. (1964) recommendations. Group 4 showed deviation of septum, distortion of ventral turbinates and uneven amounts of bone in turbinates. Groups 5, 6 and 7 showed over-all loss of bone with moderate to severe turbinate distortion. Turbinates and other bones were scored according to normal remodeling, amount of resorption and degrees of gross reduction in volume. Pigs in group 3

(1.2 percent calcium and 1.0 percent phosphorus) all scored highest with normal remodeling while groups 4-7 had the lowest scores indicating severe resorption and gross reduction in volume.

Additional studies on conditions resembling atrophic rhinitis by nutritional techniques should help to resolve the theorical causes of rhinitis.

^{45}Ca Uptake and Digestibility Studies

The most widely used calcium isotope is ^{45}Ca - half life 163.5 days, a pure Beta emitter (0.254 mev). Measurements of ^{45}Ca, particularly in long term studies, have required chemical concentration, e.g., by oxalate precipitation, and suitable preparation for counting, e.g., by dispersing the CaC_2O_4 on filter paper under standardized conditions. The end result of radioassay is usually best expressed in terms of specific element per unit weight of the element present.

Comar <u>et al</u>. (1952) showed by autoradiography that ^{45}Ca appears in the femurs of young pigs by 2.5 minutes after injection into the carotid artery and that longitudinal growth of various parts of a single bone can be estimated by this technique. The redistribution of radioactive calcium in different parts of the skeleton varies with the growth of these parts such that, as time passes after ^{45}Ca administration, the specific activity of the calcium in the most active areas of growth becomes lower than that in areas of less active growth. This has been shown in the rat even within a single bone (Bauer, 1954). Thus, it is possible, by following the movement of injected ^{45}Ca within and among bones, to qualitatively compare rates of bone growth in various parts of the skeleton.

Krusemark et al. (1968) used dietary calcium levels of 0.73 and 0.39 percent, with phosphorus constant at 0.66 percent, to study ^{45}Ca uptake of the turbinate and other bones. The pigs averaged 8 kg. when assigned to treatment, with the experimental period lasting 45 days. At the conclusion of the feeding trial, pigs were injected intravenously with ^{45}Ca (15.4 μc per kg.) and sacrificed at 1.5, 3 and 4.5 hours postinjection. Turbinate ash, calcium and phosphorus were less ($P<0.01$) for pigs receiving 0.39 percent calcium than 0.73 percent calcium. Specific activity was calculated as counts per minute per mg. of calcium. ^{45}Ca uptake by turbinates was greater ($P<0.01$) than for any other bone at each time interval.

Pond et al. (1969) used four-week-old pigs, averaging 8 kg. body weight to compare the uptake of injected ^{45}Ca by the nasal turbinate, humerus, femur and mandible. After a two-day acclimation period in the metabolism cages an isotonic solution of $^{45}CaCl_2$ was given intraperitoneally to each pig (100 μc of ^{45}Ca per kg. of body weight). Pigs were slaughtered in pairs at 1, 2, 4, 8 and 16 days after ^{45}Ca administration. The ^{45}Ca activity of the bone ash was counted using a gas-flow proportional counter and expressed as microcuries of ^{45}Ca per mg. of calcium in the ash.

Comparison of the concentration of ^{45}Ca in the ends of the humerus with that in the shaft reflects the greater metabolic activity of the areas representing the epiphyseal cartilage (growth plate) at each end of the bone than in the diaphysis. The ^{45}Ca activity in the nasal turbinate paralleled that in the proximal and distal ends of humerus, achieving a high level at 24 hours after administration and remaining at that level or slightly higher through day 4 followed by a decline between days 4 and 8 and a further decline from day 8 to day 16. The shaft of the humerus remained at a constant level of ^{45}Ca activity throughout the 16-day period, probably reflecting the early uptake of isotope subperiostially and subsequent trapping of the isotope in the deep layers of bone as bone secretion continued. The failure of the nasal turbinate to show greater ^{45}Ca activity at any time interval than the growth plates of the humerus does not support the concept of greater rate of turnover of bone in the nasal turbinate than in other bones.

The similarity of ^{45}Ca uptake by the nasal turbinate and by the growth plates of long bones suggests that areas of active bone growth in different parts of the skeleton of the pig have similar turnover rates. The nasal turbinate, a tubular cancellous bone, may be more susceptible to excessive resorption than the long bone as an organ even though no more susceptible than the growth plate of long bones as a tissue.

Apparent Digestibility of Calcium and Phosphorus

Apparent digestibility is merely that fraction of the intake that is not recovered in the feces. This presumes that the feces does not contain any portion of a nutrient that has once been digested and absorbed, but this assumption can be made for only a few nutrients, such as sugar. Calcium and phosphorus behave somewhat differently. After ingestion a portion may remain unabsorbed in the digestive system and be passed along with the feces. Concurrently, there is some mobilization from the bone and some released from various soft tissues. A part of this is re-excreted into the intestine and so also finds its way into the feces.

Hansard et al. (1961) reported apparent and true calcium digestibility to be 91 and 99 percent, respectively, for pigs 2 weeks of age. This marked absorption of calcium may be explained by the avidity of skeletal tissue for calcium in young growing animals. Apparent calcium digestibility was shown to decrease from 91 percent in the two-week-old to 42 percent in the five-month old pigs. As age increased, differences between apparent and true digestibility became more marked.

Miller et al. (1962) conducted calcium balance studies using pigs 4 weeks of age. Maximum retention of calcium was obtained in pigs receiving 1.2 percent of dietary calcium but the amount was not significantly greater than that retained by pigs receiving 1.0 percent of dietary calcium. The apparent calcium digestibility of pigs receiving 0.4 percent of calcium was 87 percent, similar to values obtained by Hansard et al. (1961) for two-week-old pigs. Apparent calcium digestibility was inversely related to dietary calcium level. Pigs consuming 0.8 and 1.0 percent dietary calcium retained 82 and 88 percent, respectively. Apparent digestibility is usually higher for phosphorus than for calcium. Miller et al. (1964a) reported phosphorus retention of 84, 88, 75, 63 and 59 percent on dietary phosphorus levels of 0.4, 0.5, 0.6, 0.7 and 0.8 percent, respectively.

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 increased growth rate in pigs which has been made possible by improved amino acid balance, increased feed utilization, improved sanitary conditions, a reduced incidence of infectious diseases and selection for greater genetic growth potential. Current recommendations on dietary levels of calcium and phosphorus require reinvestigation and updating in relation to increased growth rates in young pigs.

CHAPTER III

REARING CAESAREAN DERIVED SPF PIGS FOR EXPERIMENTAL PURPOSES

Introduction

Pigs deprived of colostrum can be reared if techniques are employed to give the pig a sterile environment. The most common practice has been to obtain pigs by hysterectomy (Young and Underdahl, 1953; Young et al., 1955; Schneider and Sarett, 1966), transport them to individual sterile incubators and feed some type of a milk solids diet until at least 3 weeks of age. Generally, weight gains of pigs raised under these conditions appear to be as good as those observed in pigs raised under natural conditions.

When hysterectomy procedures are used, the sow is salvaged for meat purposes but is lost for reproduction purposes. There are no reports concerning the use of Caesarean section techniques in the literature, whereby the sow can be saved and possibly rebred for further production.

Materials and Methods

One hundred and twenty-seven baby pigs (6 groups) were used to develop techniques for successfully rearing Caesarean pigs to 3 weeks of age.

Pigs used in this study were obtained through Caesarean section surgical techniques. Sows were transported to the Clinical Research Laboratory of Veterinary Medicine on the 113th day of gestation and immediately scrubbed with soap and disinfectant. The sow was physically restrained, induced and maintained by halothane anesthesia, using a partial rebreathing system and face mask. Depth of anesthesia was monitored by visual signs as well as anesthetic effect on pulse rate as reflected by veiling heart monitor (NCG).

The sow was positioned in right lateral recumbency. The left flank was closely clipped and scrubbed with pHisoHex[1], alcohol and tincture of iodine. An abdominal incision, 15-20 cm in length, was made through the left flank. One horn of the uterus was exposed, an incision made near the base of the uterus and as many piglets were removed as possible. The second uterine horn was handled in the same manner. Occasionally, piglets were removed from both horns through one incision, or an incision was made at the apex to remove the last one or two piglets. After the blood in the umbilical cord had been milked into the piglet's circulatory system, the cord was clamped near the body with a metal clip. Usually the placenta was left in the uterus and allowed to pass normally after surgical procedures were terminated.

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[1]Winthrop Laboratories, New York, N.Y.

Incisions of the uterus were closed with modified No. 0 medium chromic gut. The abdominal incision was closed using a two layer closure with interrupted sutures. The peritoneum, abdominal muscular and fascia were closed with No. 1 medium chromic gut. The skin was closed with interrupted nylon sutures. Administration of anesthetic gas was stopped during the last few skin sutures. After closure, the skin was cleaned of blood and debris. The animal was ambulatory within 15 minutes of completion of surgery and walked to recovery room. Temperature was recorded for 2 days and routine antibiotics were administered for 2 to 4 days following surgical procedures.

The pig handlers, in addition to surgeons, wore sterile gowns, gloves, caps and masks during the surgery and resuscitation period. The piglets were placed in sterile towels, membranes removed and mucous cleaned from the mouth. Pigs were resuscitated by body massage. An attempt to clear the airway of mucous was made by supporting the head of the piglet and swinging in a downward motion. When the piglets were breathing satisfactorily, 2 to 10 minutes, they were placed directly into an autoclaved pig isolator for transport to the Swine Nutrition Laboratory. Piglets were removed separately, sex and weight recorded, and placed in individual sterile cardboard rearing units.

Incubator Room

The incubator facilities consisted of one room 6.1 X

3.1 X 2.7 m., and an anteroom 3.1 X 3.1 X 2.7 m. for changing clothes and preparing the liquid diet (Table II). Forty individual disposable cardboard incubators[2] were used to hold the pigs during the first 3 weeks of life. The incubators were designed to provide each baby pig with dry, heated, filtered and sterilized air. Inside dimensions were 54.6 X 26.7 X 21.1 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[3] and dehumidified[3], forced over thermostatically controlled heaters, taken through 4 layers of cotton filters which covered four square openings (11.1 X 11.1 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 (17.8 mm. diameter) in a 5 X 5 arrangement and covered with 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 6.4 mm. wire mesh raised 53.3 mm. above the bottom of the incubator kept the piglet free

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

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

of its urine and feces. Each grill was covered with "Neo-tex"[4] diamond shaped pattern rubber matting to protect the piglet's feet and legs from the mesh wire. 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 14.7 X 10.2 X 4.6 cm. The cardboard incubators, including feed trays, wire mesh bottoms and rubber mats were steam sterilized (121°C. for 30 minutes) before being placed in the incubator room.

Each incubator had a 17.8 X 12.7 cm. observation opening in the top which was covered with a plexi-glass material after the pig was placed inside to prevent contamination.

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.

Individual cardboard incubators were used for control

[4]W. H. Curtin & Company, Tulsa, Oklahoma.

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. Upon entering the anteroom, the personnel who cared for the pigs put on a clean lab coat, rubber boots and rubber gloves. They also stepped into a disinfectant[5] pan when entering and leaving the incubator room. The floors of the anteroom and incubator room were cleaned with disinfectant[5] immediately after each feeding period.

The air conditioning 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 was maintained at 26°C. and 35 percent relative humidity. To avoid contamination, the pressure in all rooms was maintained slightly higher than the environmental pressure.

The room was prepared a few days before the pigs were to enter the facilities. The room was aseptically cleaned with a detergent and disinfectant[5] and thoroughly steamed. The autoclaved incubators were put in place and connected to the sterile air flow.

Ultraviolet germicidal lamps were used as aids in

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

cleaning the air in the incubator room. 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.

Liquid Milk Diet

The pigs did not receive colostrum at any time. The liquid diet fed the pigs from birth to 3 weeks of age consisted of pasteurized, homogenized cows' milk fortified with dried whole milk, minerals and vitamins (Table II). The dried whole milk was 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. The analyzed chemical composition of the diet is presented in Table III. On a dry matter basis, calcium and phosphorus content was 0.96 and 0.80 percent, respectively.

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

TABLE II

COMPOSITION OF THE FORTIFIED
COWS' MILK DIET

Ingredient	Composition
Homogenized cow milk	3,785 ml.
Dried whole milk (28% fat)	636.00 g
Citric acid	5.00 g
Niacin	16.67 mg
Vitamin K	3.039 mg
Vitamin D_2	100 I.U.
Vitamin E	10.7 mg
$ZnSO_4 \cdot 7H_2O$	320.0 mg
$FeSO_4 \cdot 7H_2O$	520.0 mg
$MnCl_2 \cdot 4H_2O$	120.0 mg
$CuSO_4 \cdot 4H_2O$	40.0 mg
$MgSO_4 \cdot 7H_2O$	2.0 g
KI	0.4 mg

TABLE III

ANALYZED CHEMICAL COMPOSITION OF THE FORTIFIED COWS' MILK DIET

Nutrient	Liquid[a] Basis	Dry Matter Basis
	%	%
Total milk solids	21.59	- -
Crude protein (N X 6.38)	6.40	30.08
Fat	5.85	27.09
Solids-not-fat	15.74	74.45
Nitrogen-free extract	9.29	43.93
Ash	1.35	6.24
Calcium	0.21	0.96
Phosphorus	0.17	0.80

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

The remaining 2.3 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. Diet prepared by this method was fed to groups 1 and 2.

The liquid diet was placed in a pasteurization unit[6] 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.

This system for preparing the milk diet was satisfactory when less than 10 pigs were being fed, but it required entirely too much labor when a large number of pigs were on the milk diet at one time. Subsequently, another system was devised whereby the milk diet was mixed, homogenized

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

and pasteurized in the milk plant of the Department of Dairying. The diet was prepared in quantities of 40 to 80 gallons and filled into one quart cartons. Groups 3, 4, 5 and 6 received the milk diet prepared by this method. This procedure worked very well when large volumes of the diet were used daily.

Feedings were at intervals of four hours, 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. 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 with neomycin sulfate[7] gave excellent results.

Tray feeding was initiated approximately 3 hours after the surgical delivery was completed. The fortified milk diet was heated to approximately 37°C. by placing it in a water bath maintained at 70°C. Feeding was accomplished with the use of a 50 cc. plastic syringe and a 12 gauge needle. The needle was inserted through a rubber stopper to place the milk diet in the metal feeding tray. All syringes and needles were steam sterilized prior to being

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

used. The pigs started with a quantity of 30 to 35 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 each feeding except for individual pigs that did not consume the previous feeding. Dietary consumption by individual pigs was accurately measured and recorded. Normally, the pigs were consuming approximately 220 ml. per day at one day of age and this was gradually increased to a maximum of approximately 1200-1500 ml. per day at 21 days of age. On the 21st day, the pigs were fasted four hours, removed from the sealed incubators and weighed.

Results and Discussion

Six groups of neonatal pigs, obtained by Caesarean section, were fed a fortified cow's milk diet (21.59 percent solids) until 3 weeks of age.

The overall survival of the 6 groups was considered good (Table IV). Group 2 had the lowest survival rate, partially due to bacterial infections resulting from unsanitary conditions with respect to the needles, syringes and glassware used in the feeding procedure and some ruptured

TABLE IV

SURVIVAL, WEIGHTS AND DRY MATTER CONVERSIONS OF CAESAREAN SPF PIGS REARED TO THREE WEEKS OF AGE

Group No.	No. Pigs	% Survival	Initial wt. (Kg)	Final wt. (Kg)	Gain (Kg)	gm. D.M. / gm. gain
1; June '69	24	91.6	1.305	5.262	3.957	0.76
2; July '69	36	58.3	1.313	4.637	3.324	0.73
3; Sept. '69	29	82.8	1.208	5.203	3.995	0.77
4; Feb. '70	13	100.0	1.184	6.098	4.914	1.00
5; Mar. '70	11	90.0	1.239	5.636	4.397	0.94
6; June '70	14	85.7	1.342	6.212	4.870	1.15

spleens and livers incurred during the manual resuscitation period. *E. coli* was the most prominent diagnosis when necropsy was performed. Survival rate was 80 percent for all 6 groups and 89 percent when group 2 was not considered, since it was the only group to have severe bacterial infections.

Groups 1, 2 and 3 had the best feed efficiency but they exhibited the lowest weight gain (3.957, 3.324 and 3.995 kg.) and feed intake. Maximum average daily intakes for groups 1, 2 and 3 were 1200, 1120 and 1200 ml., respectively (Figures 1, 2 and 3). Group 5 was slightly better in body weight gains and intake (Figure 5) but poorer in rate of feed conversion. Groups 4 and 6 gained the most weight (4.914 and 4.87 kg.), consumed the most diet (Figures 4 and 6) and had the least desirable feed conversion rate.

Schneider and Sarett (1966) reported 3 week gains of 4.4 kg. for hysterectomy-obtained SPF pigs fed a liquid diet containing skim milk, casein, vegetable fats and lactose with the caloric density closely resembling sow's milk. Gains of 3.7 kg. were obtained using a commercial formula. The higher weight gain obtained with the laboratory preparation as compared with that found with the commercial formula was probably due mainly to the higher caloric density of the laboratory preparation, since the volumes of formula consumed by the two groups were approximately the same. The average gain of 4.4 kg. was greater

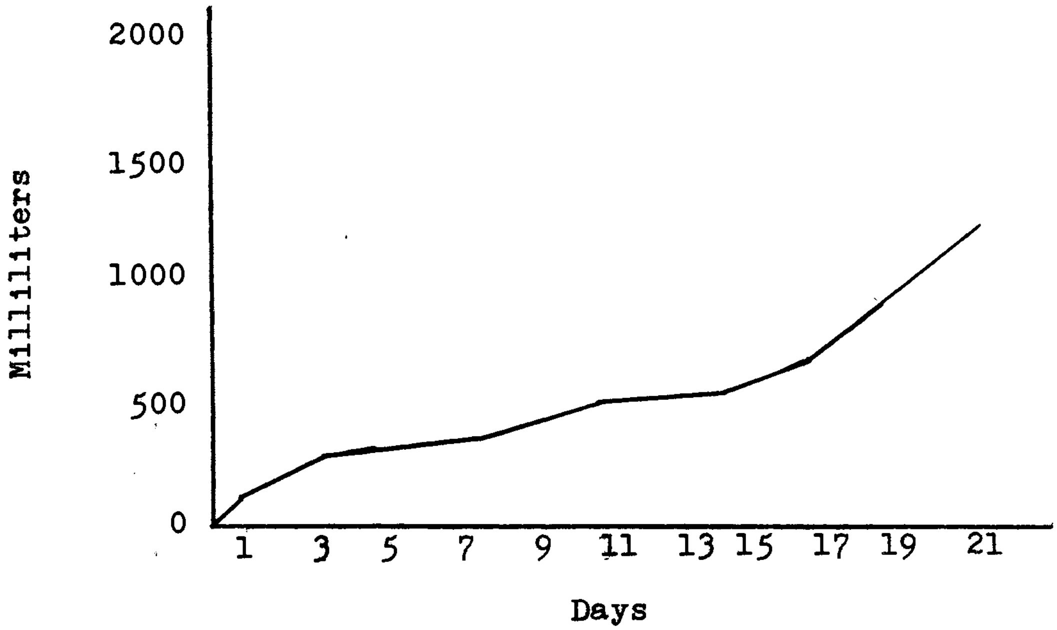


Figure 1. Average Daily Intake of Group I.

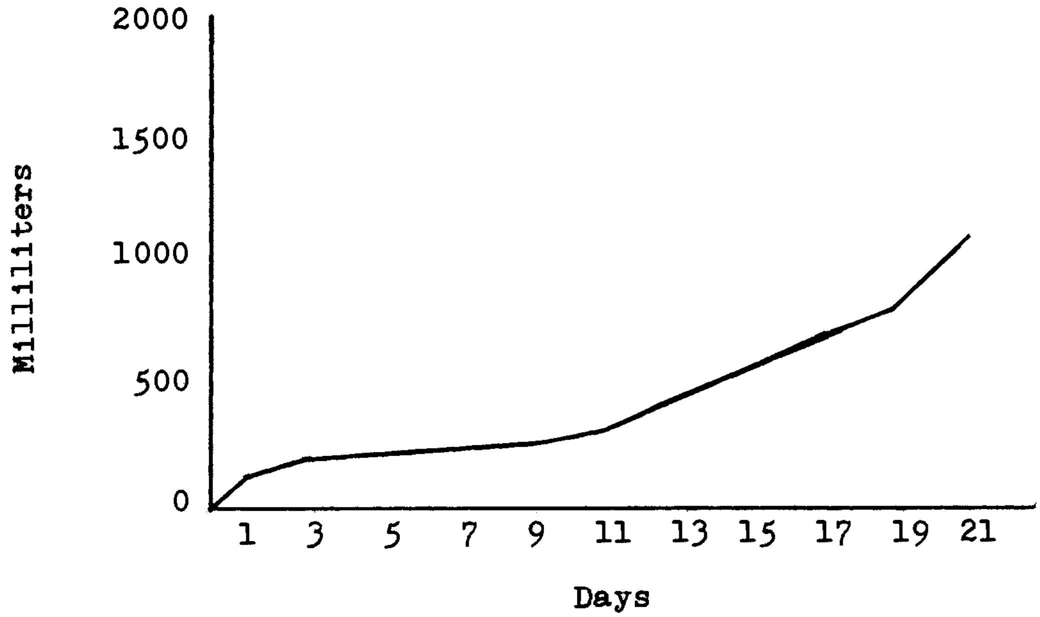


Figure 2. Average Daily Intake of Group II.

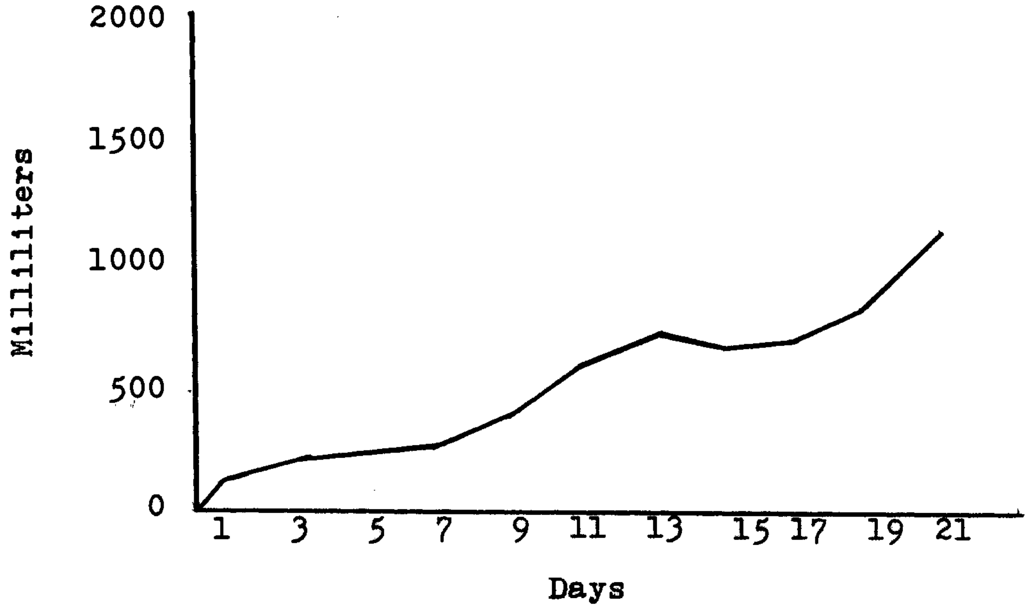


Figure 3. Average Daily Intake of Group III.

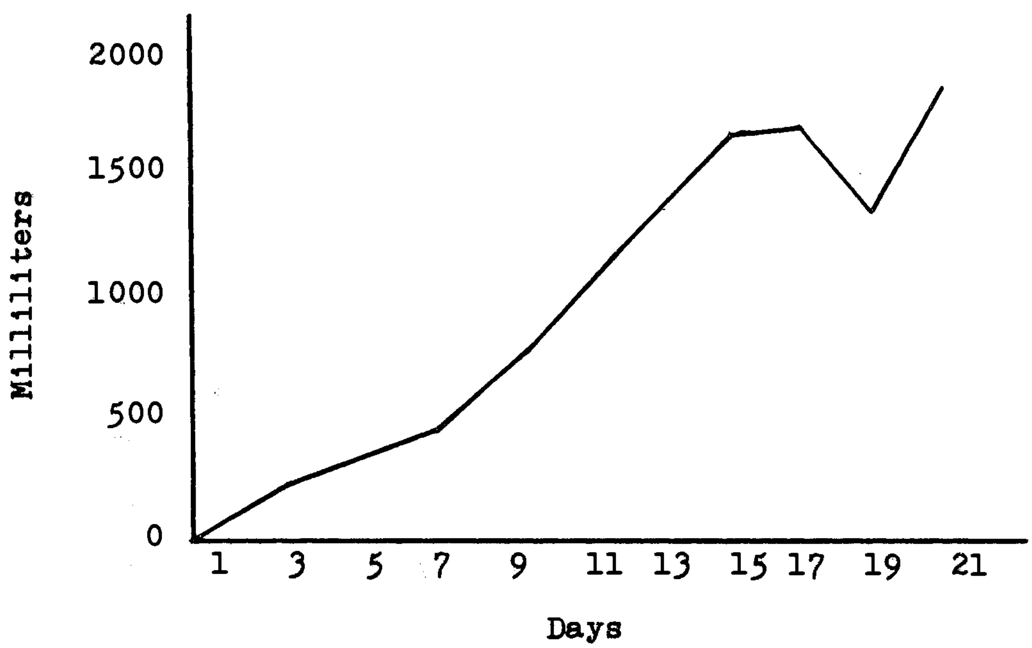


Figure 4. Average Daily Intake of Group IV.

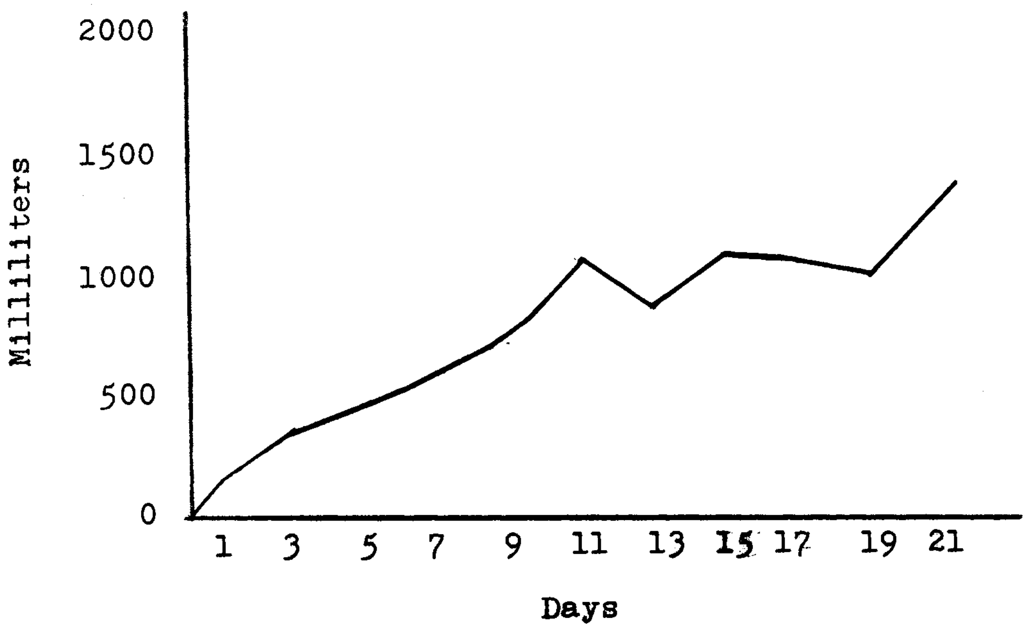


Figure 5. Average Daily Intake of Group V.

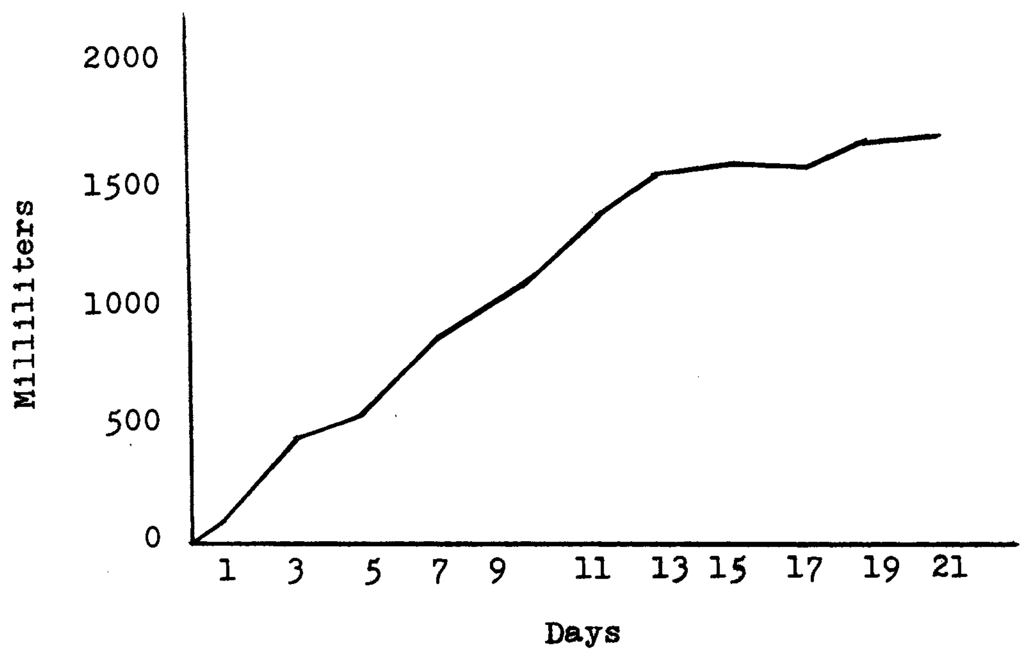


Figure 6. Average Daily Intake of Group VI.

than the 3.6 kg. and 4.0 kg. reported for naturally born and suckled Durocs and Hampshire-Yorkshire crosses.

At 14 days of age, groups 4 and 6 were consuming 1500 and 1600 ml. per pig per day, respectively. This compares very favorably with the work of Lecce (1969) where pigs on the "AUTOSOW", 14 days of age, were consuming 1200 ml. per day of a 24 percent milk solids diet. These pigs were not obtained by hysterectomy but were caught in sterile towels as they emerged from the birth canal. On Lecce's "AUTOSOW", by two weeks of age, pigs consuming the 24 percent milk solids diet had gained about twice as much weight as the naturally suckled control pigs.

Daily diet intakes for groups 1, 2 and 3 were not rapidly increased. Therefore, Figures 1, 2 and 3 show a general linear increase in daily intake. Daily diet intakes for groups 4, 5 and 6 were increased as rapidly as possible. Figures 4, 5 and 6 show a characteristic leveling off in daily diet consumption at 14 days of age.

Groups 1, 2 and 3 were given neomycin sulfate (11 mg. per kg. of body weight per day) in their diet when any signs of diarrhea were noticed. Groups 4, 5 and 6 did not receive any antibiotics, even though an occasional case of diarrhea was evident.

Summary

One hundred and twenty-seven neonatal pigs, making up 6 groups, were obtained by Caesarean section, reared in

individual incubators and fed a fortified cow's milk diet until three weeks of age. Group survival rates ranged from 58 to 100 percent. It is felt that survival rates of 90 percent or better can routinely be obtained by trained personnel, and that the 3 week weights will be as good or slightly better than those of the naturally born and suckled pigs.

The ability to rear colostrum deprived pigs to 3 weeks of age without antibiotics presents experimental models that can be extremely useful in studying intermediate metabolism of the neonate.

CHAPTER IV

EVALUATION OF CALCIUM REQUIREMENTS FOR YOUNG PRIMARY SPF PIGS

Introduction

The calcium requirement for young Specific-Pathogen-Free pigs has not been satisfactorily resolved. Recommendations since 1960 have varied, depending on whether growth and performance or skeletal development served as criteria. Dudley et al. (1960), Zimmerman et al. (1960), Rutledge et al. (1961) and Miller et al. (1962) suggest dietary calcium requirements of 0.20, 0.70, 0.80 and 0.80 percent, respectively, for pigs from 3 through 9 weeks of age. Washam (1968) and Coalson (1969) have shown that young SPF pigs of this age may require a dietary calcium level of 0.95 percent for maximum growth, feed efficiency and skeletal development. The experiments in these studies were designed to further elucidate the calcium requirement for young pigs.

Materials and Methods

Primary SPF pigs, three weeks of age (Chapter III), were moved to individual, open-topped, solid-sided metal pens and assigned to a purified diet containing one of five calcium levels. The experimental period covered 42 days

(21 to 63 days of age). The pigs were housed in the nursery for the first 21 days and in the grower room for the last 21 days.

Nursery Room

The nursery room was 9.4 X 3.1 X 3.1 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 waterer. The feeder was fitted with a heavy lip around the inside to minimize feed loss. A waste feed tray (34.9 X 25.4 X 14.0 cm.) was located under the self feeder. The nursery room contained no windows, thus eliminating the entrance of sunlight. Also, ultraviolet germicidal lamps were used as aids in sterilizing the air.

Grower Room

The grower room contained 25 pens similar to those described for the nursery. Each pen was 11.8 X 59.7 X 59.7 cm. in dimensions and provided adequate space for each pig through 9 weeks of age.

Calcium Purified Diets

The percentage composition of the diets used in the calcium study is presented in Table V. The calcium levels by analysis were: Diet A, 0.41; Diet B, 0.58; Diet C,

TABLE V

PERCENTAGE COMPOSITION OF EXPERIMENTAL PURIFIED DIETS FOR CALCIUM STUDY

Ingredient[a]	Diet A	Diet B	Diet C	Diet D	Diet E
	%	%	%	%	%
Casein[b]	25.58	25.58	25.58	25.58	25.58
Corn starch [c]	44.90	44.50	44.10	43.75	43.30
Glucose monohydrate[d]	12.00	12.00	12.00	12.00	12.00
α-Cellulose[e]	6.00	6.00	6.00	6.00	6.00
Corn oil[f]	5.30	5.30	5.30	5.30	5.30
Mineral mixtures[g]	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.22	1.22	1.22	1.22	1.22
Calcium carbonate	0.00	0.39	0.79	1.18	1.58
Sodium phosphate	0.83	0.83	0.83	0.83	0.83
Water soluble vitamins[h]	1.00	1.00	1.00	1.00	1.00
Fat soluble vitamins[i]	0.0041	0.0041	0.0041	0.0041	0.0041
Antioxidant[j]	0.0125	0.0125	0.0125	0.0125	0.0125
Sulfamethazine-antiobiotic[k]	0.125	0.125	0.125	0.125	0.125

TABLE V 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 VI.

[h]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.

[i]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.

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

[k]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.

0.74; Diet D, 0.90; Diet E, 1.06 percent with phosphorus constant at 0.75 percent.

The protein, cellulose, fat, calcium and phosphorus free basal mineral mixture (Table VI) and vitamin portions of the diet were held constant in all rations. The quantities of cornstarch and calcium carbonate were varied in Diets A through E.

The rations were formulated to be adequate in all other nutrients and contained 125 grams of antibiotic activity per ton. The vitamin levels used in making the diets are shown in Table V. 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.

Assignment to Treatment

At 21 days of age, the pigs were removed from their individual sealed incubator boxes and transported to the nursery where they remained for 3 weeks. The pigs were weighed, individually identified (ear notched) and placed in individual metal pens. Pigs were randomly assigned to each treatment diet and started on the dry meal diets immediately. The pigs were consuming the diet within 2 to 4 days. The animals were fed *ad libitum*, with the feeders refilled as necessary.

TABLE VI

PERCENTAGE 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 g)
$FeSO_4 \cdot 2H_2O$[b]		1.40 (635.6 g)
$ZnSO_4 \cdot H_2O$[b]		0.80 (363.2 g)
$MnSO_4 \cdot H_2O$[b]		0.30 (136.2 g)
$CuSO_4$[b]		0.20 (90.8 g)
$CoCO_3$[b]		0.20 (90.8 g)
KI		0.004 (1.816 g)
Cerelose[c]		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]Cerelose 2001, Corn Products Company, Argo, Illinois.

Chemical and Statistical Analyses

All pigs were weighed at 3 weeks of age and weekly thereafter through 9 weeks of age. Weekly feed consumption was recorded. Blood samples for the calcium study were collected by anterior vena cava puncture as described by Carle and Dewhirst (1942) at 3, 6 and 9 weeks of age. A 10 cc. plastic disposable syringe fitted with a 38.1 mm. 20-guage needle was used to withdraw approximately 6 milliliters of blood.

The blood sample was placed in a plastic centrifuge tube and 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 and inorganic phosphorus.

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).

At the conclusion of the calcium experiment, 20 pigs (four from each diet - two per replicate) were selected at random and sacrificed by exsanguination. Feed and water were withdrawn approximately 12 hours prior to slaughter.

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 (paralleled 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).

A cross-section of the turbinate behind the first maxillary premolar teeth was removed for chemical analysis. The four bones and turbinate section 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 dried fat-free bones were ashed at 550°C. for 16 hours, with the ashes being dissolved in 4 N HCL to a known volume.

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

The left humerus, eighth rib, small section of mandible and a cross-section of the turbinate through the first maxillary premolar teeth were removed for histological study. Bone samples were immediately fixed in 10 percent buffered neutral formalin. Bone specimens were placed in large quantities of formic acid-sodium citrate solution until decalcification was completed. Solutions were changed daily to obtain the best results. After decalcification, bone tissues were stained with Harris hematoxylin for five minutes and eosin for two minutes. Upon completion of numerous washings with alcohol specimens were impregnated with paraffin and sectioned at a thickness of 6 microns for further study.

Analyses of varience, 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 when analysis of varience for various criteria were significant ($P<0.10$).

Results and Discussion

Forty-three primary SPF pigs, 3 weeks of age, were used in two replicates to evaluate dietary calcium levels of 0.41, 0.58, 0.74, 0.90 and 1.06 percent while dietary phosphorus was held constant at 0.75 percent. Total gains, feed intakes and feed efficiencies of the two replicates were not pooled for statistical analysis due to a highly significant (P<.005) replicate effect, i.e., replicates were not estimating the same population parameter because of bacterial infections present in varying degrees in the second replicate.

Total gain, total feed intake and feed efficiency for Replicate 1 are presented in Table VII. There was a significant linear effect (P<.005) for total kg. gain with Diet E (1.06 percent calcium) being the best. Pigs on Diet E consumed the most feed and pigs on Diets C, D and E were more efficient than those on Diets A and B. The feed consumption for Diet E would indicate that the 1.06 percent dietary calcium did not severely limit feed intake.

Total gain, feed intake and feed efficiency for Replicate 2 are presented in Table VIII. There were no significant linear or quadratic effects, although the pigs on Diet D (0.90 percent calcium) gained the most weight and had the best feed efficiency. Pigs on Diets D and E gained more weight and exhibited better feed efficiencies than those on Diets A, B and C. The data for total kg. gain and feed efficiency for both replicates are presented in Figures 7 and 8, respectively.

The data for body weights, weekly weight gain and feed efficiencies are presented in Tables IX and X. There were no significant differences in any of the variables measured at weekly intervals. Similar growth rates occurred across all five dietary calcium levels. There was a significant linear effect in weight gain in Replicate 1 with a similar

TABLE VII

TOTAL GAIN, FEED INTAKE AND FEED EFFICIENCY OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 1)

Diet No. of Pigs Dietary Ca, %	A 5 0.41	B 5 0.58	C 4 0.74	D 4 0.90	E 4 1.06	
Gain, kg.	22.94	23.12	25.46	25.29	26.28	0.99[a,b]
Feed, kg.	51.81	49.83	51.35	51.99	54.48	2.06[a]
Gain/feed, kg.	2.26	2.22	2.05	2.07	2.09	0.88[a]

[a]Standard error of treatment means.

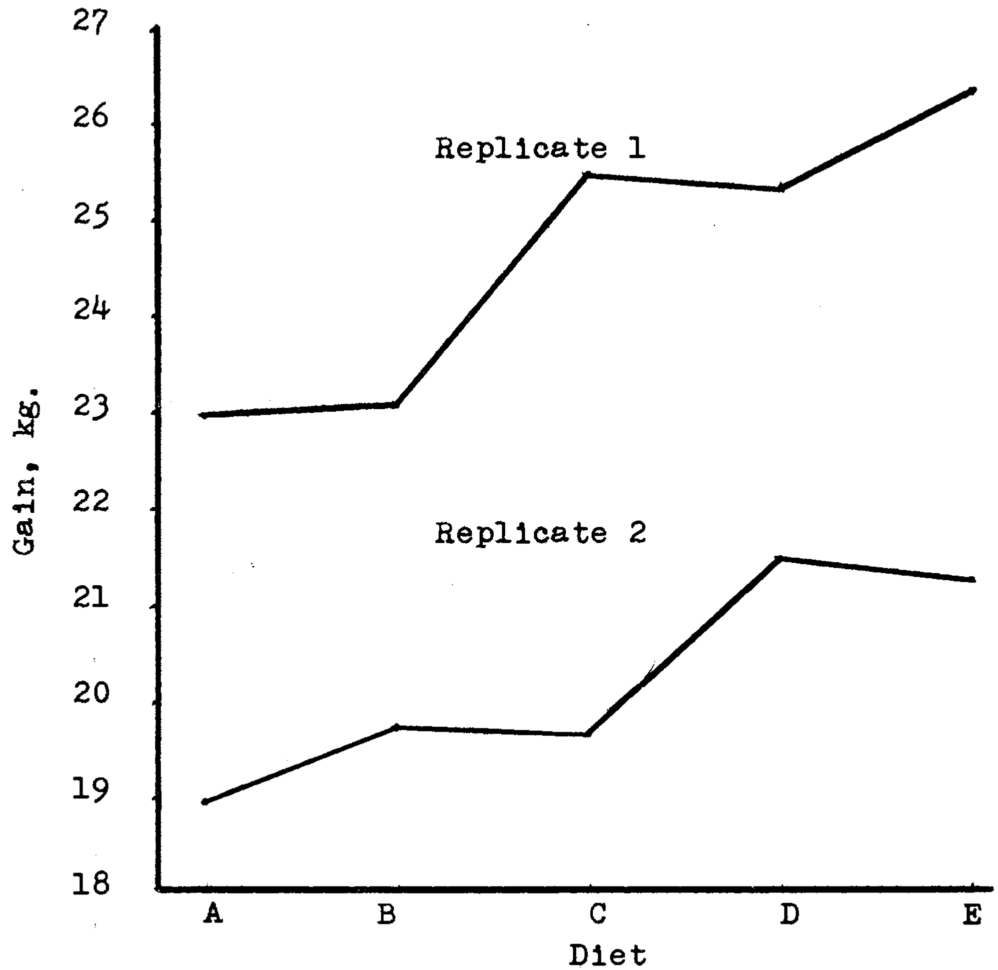
[b]Significant Linear Effect, ($P<.005$).

TABLE VIII

TOTAL GAIN, FEED INTAKE AND FEED EFFICIENCY OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 2)

Diet No. of Pigs Dietary Ca, %	A 4 0.41	B 4 0.58	C 5 0.74	D 4 0.90	E 4 1.06	
Gain, kg.	18.95	19.71	19.66	21.47	21.26	1.09[a]
Feed, kg.	44.39	45.94	48.05	47.06	50.78	2.19[a]
Gain/feed, kg.	2.39	2.33	2.45	2.22	2.28	0.19[a]

[a]Standard error of treatment means.



Replicate 1. Significant linear effect (P<.005)

Figure 7. Total gain (kg.) of pigs fed five levels of calcium.

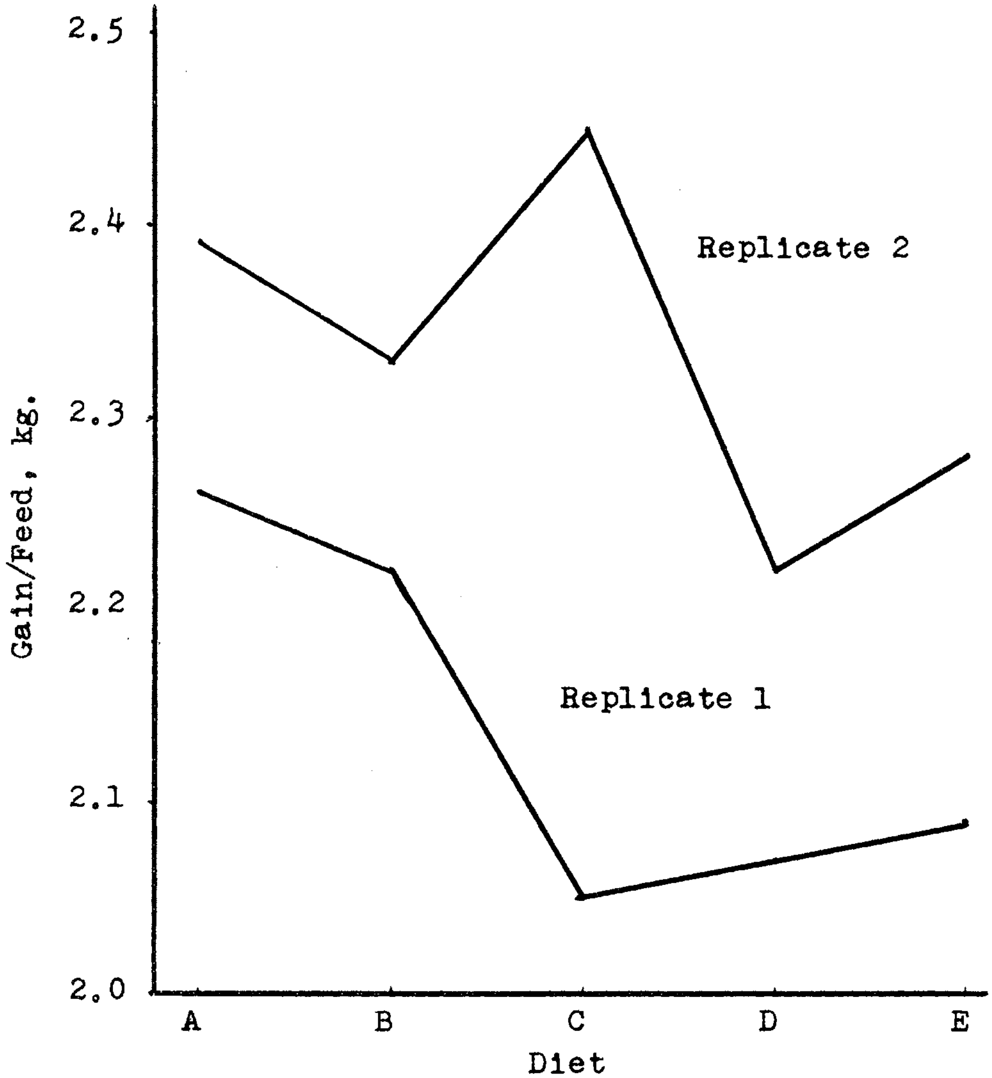


Figure 8. Feed efficiency of pigs fed five levels of calcium.

TABLE IX

BODY WEIGHT, WEEKLY WEIGHT GAIN AND FEED EFFICIENCY OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 1)

Diet No. of Pigs Dietary Ca, %	A 5 0.41	B 5 0.58	C 4 0.74	D 4 0.90	E 4 1.06	SE[a]
Initial wt.	5.32	5.20	5.27	5.12	5.38	0.16
21 to 28 days						
28 day wt., kg.	6.53	6.72	6.94	6.49	6.57	0.34
Gain, kg.	1.21	1.46	1.67	1.37	1.19	0.28
Gain/Feed, kg.	2.54	2.53	1.99	2.84	3.07	0.50
28 to 35 days						
35 day wt., kg.	9.72	10.11	10.61	10.15	10.07	0.44
Gain, kg.	3.19	3.20	3.67	3.66	3.50	0.66
Gain/Feed, kg.	1.95	2.13	1.91	1.83	1.91	0.10
35 to 42 days						
42 day wt., kg.	14.12	14.29	15.06	14.97	14.94	0.55
Gain, kg.	4.40	3.99	4.45	4.82	4.87	0.28
Gain/Feed, kg.	2.03	2.01	1.90	1.83	2.01	0.10
42 to 49 days						
49 day wt., kg.	18.29	18.64	19.69	19.59	20.00	0.66
Gain, kg.	4.18	4.13	4.63	4.62	5.07	0.34
Gain/Feed, kg.	2.30	2.42	2.20	2.09	2.21	0.19

TABLE IX CONTINUED

49 to 56 days						
56 day wt., kg.	23.27	23.54	25.21	25.12	25.55	0.78
Gain, kg.	4.97	4.80	5.52	5.54	5.54	0.27
Gain/Feed, kg.	2.52	2.02	1.91	2.04	2.12	0.16
56 to 63 days						
63 day wt., kg.	28.26	29.36	30.74	30.41	31.66	0.90
Gain, kg.	5.00	5.02	5.15	5.13	6.11	0.39
Gain/Feed, kg.	2.36	2.44	2.39	2.46	2.21	0.20

[a]Standard error of treatment means.

TABLE X

BODY WEIGHT, WEEKLY WEIGHT GAIN AND FEED EFFICIENCY OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 2)

Diet No. of Pigs Dietary Ca, %	A 4 0.41	B 4 0.58	C 5 0.74	D 4 0.90	E 4 1.06	SE[a]
Initial Wt.	5.02	4.38	4.20	4.98	4.73	0.33
21 to 28 days						
28 day wt., kg.	5.87	5.55	5.38	6.40	5.51	1.38
Gain, kg.	1.09	1.17	1.18	1.41	0.93	0.29
Gain/Feed, kg.	3.95	4.14	3.72	2.83	3.02	0.80
28 to 35 days						
35 day wt., kg.	8.92	8.80	8.19	9.59	8.91	0.66
Gain, kg.	3.19	3.25	2.82	3.19	3.40	0.26
Gain/Feed, kg.	2.28	2.44	2.75	2.27	2.31	0.37
35 to 42 days						
42 day wt., kg.	11.87	11.95	10.67	12.40	12.37	0.71
Gain, kg.	2.95	3.16	2.48	2.81	3.46	0.24
Gain/Feed, kg.	2.51	2.38	3.05	2.45	2.54	0.53
42 to 49 days						
49 day wt., kg.	14.83	14.72	13.62	16.40	15.20	1.26
Gain, kg.	2.96	2.77	2.94	4.00	2.82	0.74
Gain/Feed, kg.	3.46	2.88	3.23	2.40	3.17	0.59

TABLE X CONTINUED

49 to 56 days						
56 day wt., kg.	19.27	19.81	18.77	21.28	20.77	1.22
Gain, kg.	4.44	5.09	5.15	4.88	5.58	0.29
Gain/Feed, kg.	2.25	1.99	2.12	2.07	2.11	0.82
56 to 63 days						
63 day wt., kg.	24.10	24.09	23.86	26.45	25.99	1.51
Gain, kg.	4.83	4.29	5.09	5.17	5.22	0.43
Gain/Feed, kg.	1.98	2.05	1.94	2.12	2.27	0.14

[a]Standard error of treatment means.

trend in Replicate 2.

The dietary calcium level needed in this study for maximum gain and efficiency was quite different compared to previous literature reports. Zimmerman et al. (1960) reported that rations with more than approximately 0.8 percent calcium caused a growth depression and in some cases adversely affected the utilization of feed of pigs from 2 through 6 weeks of age. Reports by Miller et al. (1960) indicated that 0.6 percent calcium will support maximum growth and feed efficiency in baby pigs. Rutledge et al. (1961), using pigs weaned at 3 weeks of age with calcium levels of 0.4, 0.6, 0.8 and 1.0 percent, indicated that rate of gain and efficiency of feed utilization were not affected significantly by calcium content of the diet, although maximum daily gain and efficiency occurred on 0.4 and 1.0 percent calcium, respectively.

When ration calcium was increased by increments of 0.10 from 0.40 to 0.80 percent for pigs 2 through 7 weeks (Combs and Wallace, 1962), daily gains tended to decrease linearly with increasing calcium. The most efficient utilization of feed occurred with the low calcium rations.

Serum calcium and inorganic phosphorus concentrations for Replicates 1 and 2 are presented in Tables XI and XII, respectively. Mean serum calcium levels ranged from 11.37 to 13.44 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,

TABLE XI

SERUM CALCIUM AND PHOSPHORUS LEVELS OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 1)

Diet	A	B	C	D	E	
No. of Pigs	5	5	4	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
Weeks on feed	Serum calcium, mg./100 ml.					
0	12.08	11.78	11.37	11.46	11.55	0.23[a]
3	12.43	12.91	12.53	13.31	13.44	0.25[a,b]
6	11.37	11.83	12.31	12.32	12.30	0.29[a]
	Serum inorganic phosphorus, mg./100 ml.					
0	9.84	9.69	10.12	10.30	9.55	0.49[a]
3	10.61	10.45	9.77	10.15	10.14	0.45[a]
6	10.35	10.35	9.93	10.21	10.67	0.32[a]

[a]Standard error of treatment means.

[b]Significant treatment difference, ($P<0.05$).

TABLE XII

SERUM CALCIUM AND PHOSPHORUS LEVELS OF SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE (REPLICATE 2)

Diet	A	B	C	D	E	
No. of Pigs	4	4	5	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
Weeks on feed	Serum calcium, mg./100 ml.					
0	11.97	11.78	12.10	12.07	12.20	0.41[a]
3	12.99	12.34	12.16	13.30	12.99	0.38[a]
6	12.29	12.09	12.01	12.81	13.17	0.37[a]
	Serum inorganic phosphorus, mg./100 ml.					
0	9.72	9.87	9.85	9.32	9.85	0.42[a]
3	9.78	10.34	9.53	9.19	10.29	0.40[a]
6	10.26	10.31	10.30	9.71	9.97	0.58[a]

[a]Standard error of treatment means.

1965). After six weeks pigs on Diet D of Replicate 1 and Diet E of Replicate 2 had the highest serum calcium levels. These pigs would be expected to maintain normal serum calcium levels over a wide range in dietary levels of calcium since one of the functions of parathyroid hormone is the maintenance of serum calcium homeostasis (Harrison, 1966). Serum inorganic phosphorus values (Tables XI and XII) ranged from 9.55 to 10.67 mg. per 100 ml. These values are higher than those reported by Dukes, 1955, but no noticable trends were observed. Miller et al. (1962), reported significant differences among serum phosphorus values and indicated a possible inverse relationship between calcium intake and serum inorganic phosphorus.

Absolute weights of the eighth rib, humerus, femur and ulna-radius from the right half of the carcass are presented in Table XIII. Although there were no significant linear or quadratic effects, Diets D and E (0.90 and 1.06 percent calcium) tended to support heavier weights of each of the four bones.

There were no differences or apparent trends in absolute bone lengths (Table XIV). Absolute bone diameters tended to increase with increasing levels of calcium and a significant linear response ($P<.005$) was noted with the humerus.

The specific gravities for each bone studied were found to be significantly different (Table XV). The femur showed both a significant linear ($P<.005$) and quadratic

TABLE XIII

INFLUENCE OF DIETARY CALCIUM (Ca) ON ABSOLUTE BONE WEIGHTS OF SPF PIGS SACRIFICED AT NINE WEEKS OF AGE

Diet	A	B	C	D	E	
No. of Pigs	4	4	4	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
			Absolute weights, g			
Femur	83.38	81.60	91.45	101.65	100.68	6.01[a]
Humerus	71.80	69.83	76.90	88.00	84.28	6.39[a]
Eighth rib	6.90	9.20	9.10	9.90	9.90	0.73[a]
Ulna-radius	53.45	54.30	58.00	65.53	65.88	4.67[a]

[a]Standard error of treatment means.

TABLE XIV

INFLUENCE OF DIETARY CALCIUM (Ca) ON ABSOLUTE BONE LENGTH AND DIAMETER OF SPF PIGS SACRIFICED AT NINE WEEKS OF AGE

Diet	A	B	C	D	E	
No. of Pigs	4	4	4	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
			Absolute Length, mm.			
Femur	129.2	120.8	122.4	127.6	126.8	3.11[a]
Humerus	113.0	106.5	108.9	111.6	113.0	2.27[a]
Eighth rib	119.0	118.4	116.9	120.3	119.7	2.97[a]
Ulna-radius	127.6	127.9	125.8	128.5	129.8	2.31[a]
			Absolute Diameter, mm.			
Femur	15.2	16.6	17.0	17.4	17.5	0.75[a]
Humerus	12.5	13.3	14.1	14.9	15.1	0.49[a,b,c]
Eighth rib	5.4	6.6	6.0	6.3	5.9	0.47[a]
Ulna-radius	14.8	15.5	15.9	16.8	16.9	0.67[a]

[a]Standard error of treatment means.

[b]Significant treatment difference, ($P<0.05$).

[c]Significant Linear Effect, ($P<.005$).

TABLE XV

SPECIFIC GRAVITY OF BONES TAKEN FROM NINE WEEK OLD SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE

Diet	A	B	C	D	E	
No. of Pigs	4	4	4	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
			Specific Gravity			
Femur	1.04	1.07	1.14	1.14	1.14	.01[a,b,c,d]
Humerus	1.04	1.08	1.11	1.17	1.15	.02[a,b,c]
Eighth rib	1.04	1.07	1.07	1.12	1.14	.01[a,b,c]
Ulna-radius	1.04	1.09	1.12	1.15	1.15	.02[a,b,c]

[a]Standard error of treatment means.

[b]Significant treatment difference, ($P<0.01$).

[c]Significant Linear Effect, ($P<.005$).

[d]Significant Quadratic Effect, ($P<.005$).

($P<.005$) effect. The humerus, eighth rib and ulna-radius revealed significant linear ($P<.005$) effects. Miller et al. (1962) reported that specific gravities of the humerus, femur and eighth rib were positively related to dietary calcium levels, but were not significantly increased by increasing dietary calcium level beyond 0.8 percent. In the present study, maximum specific gravity of the femur was obtained on dietary calcium levels of 0.74, 0.90 and 1.06 percent. These calcium levels are higher than those of the report of Newman et al. (1967) where maximum femur specific gravity was obtained in young pigs receiving 0.60 percent dietary calcium.

Mean values of bone ash, calcium, phosphorus and bone calcium:phosphorus ratio are presented in Table XVI. The turbinate appears to be the most sensitive to dietary calcium levels of all bones examined and chemically analyzed in this study. Percent ash, calcium, phosphorus and calcium:phosphorus ratio exhibited significant linear effects ($P<0.01$). These data would indicate that phosphorus, although fed at a constant dietary level of 0.75 percent, was not a limiting factor for turbinate calcification for any of the dietary calcium levels used.

The femur showed significant linear ($P<.01$) and quadratic ($P<.01$) responses for percent ash with Diet D (0.90 percent calcium) showing the highest value. The percentages of calcium and phosphorus were greatest on Diet C (0.74 percent calcium), but no trends were evident. The

TABLE XVI

ASH, CALCIUM AND PHOSPHORUS CONTENT OF BONES TAKEN FROM NINE WEEK OLD SPF PIGS FED FIVE LEVELS OF CALCIUM (Ca) FROM THREE THROUGH NINE WEEKS OF AGE

Diet	A	B	C	D	E	
No. of Pigs	4	4	4	4	4	
Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
			Turbinate[f]			
Ash, %	46.74	51.07	52.89	53.00	55.45	1.19[a,c,d]
Calcium, %	13.56	15.79	15.82	16.71	18.37	0.63[a,c,d]
Phosphorus, %	9.32	10.27	10.12	10.47	11.05	0.31[a,b,d]
Ca/P	1.45	1.54	1.57	1.60	1.66	0.03[a,c,d]
			Femur[f]			
Ash, %	62.21	64.52	66.46	66.93	65.96	0.78[a,c,e]
Calcium, %	19.47	20.29	20.89	20.20	20.33	0.47[a]
Phosphorus, %	12.22	12.26	13.12	12.83	12.85	0.34[a]
Ca/P	1.59	1.66	1.59	1.58	1.59	0.03[a]
			Humerus[f]			
Ash, %	62.25	65.41	64.87	67.07	66.79	0.93[a,b,d]
Calcium, %	20.18	21.15	20.15	20.64	21.35	0.27[a,b,d]
Phosphorus, %	12.54	12.33	12.24	12.92	13.15	0.32[a]
Ca/P	1.61	1.72	1.65	1.60	1.63	0.04[a]

TABLE XVI CONTINUED

			Eighth Rib[f]			
Ash, %	53.10	53.31	56.03	56.58	57.93	0.66[a,c,d]
Calcium, %	16.90	16.08	16.84	16.80	18.60	0.46[a,b,d,e]
Phosphorus, %	10.47	9.90	10.76	10.66	11.66	0.26[a,c,d,e]
Ca/P	1.62	1.63	1.56	1.58	1.59	0.03[a]
			Ulna-radius[f]			
Ash, %	57.94	59.45	61.15	61.92	60.83	0.54[a,c,d,e]
Calcium, %	19.02	18.90	19.28	17.69	16.84	0.76[a]
Phosphorus, %	11.51	11.10	11.58	11.28	10.96	0.48[a]
Ca/P	1.65	1.71	1.67	1.57	1.53	0.04[a]

[a]Standard error of treatment means.

[b]Significant treatment difference, ($P<0.05$).

[c]Significant treatment difference, ($P<0.01$).

[d]Significant Linear Effect, ($P<0.01$).

[e]Significant Quadratic Effect, ($P<0.01$).

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

calcium:phosphorus ratios were approximately the same across all dietary calcium levels. These data are not consistent with the data reported by Rutledge et al. (1961) where they showed no marked differences in ash, calcium and phosphorus contents among femur samples from nine-week-old pigs fed 0.4, 0.6, 0.8 and 1.0 percent dietary calcium from 3 through 9 weeks of age. However, small differences were consistent and showed a highly significant ($P<.01$) trend toward increased ash and calcium content of the femurs as dietary calcium increased to 1.0 percent.

Significant linear effects were noted in the ash and calcium contents ($P<.01$) of the humerus. Diets D and E supported the greatest ash values. No trends were noticed with respect to phosphorus content and calcium:phosphorus ratios. Miller et al. (1962) reported humeral ash, calcium and phosphorus concentrations were increased by increased dietary calcium levels, but no significant increases were affected beyond 0.8 percent.

Analysis of the eighth rib revealed a significant linear effect ($P<.01$) for ash content. Linear and quadratic effects were significant ($P<.01$) for calcium and phosphorus concentrations. Diet E (1.06 percent calcium) supported the greatest ash, calcium and phosphorus concentrations. The eighth rib would appear to be a sensitive measure of skeletal development, similar in many respects to the turbinate.

Significant linear and quadratic effects were found for ash content in the ulna-radius. No trends were noticed for calcium, phosphorus or calcium:phosphorus ratio, therefore, the ulna-radius does not appear to be a very sensitive bone for evaluation of dietary calcium requirements in relationship to skeletal development.

The histological slides made from the nasal turbinate slices were scored and are presented in Table XVII. According to the scoring system (Table XVII), none of the turbinates were considered to be normal. A significant linear effect was found with respect to the treatment means. In general, as the dietary calcium level increased, a lower score resulted. All turbinates exhibited varying degrees of resorption and confluence of tubules. Prominent features of the higher scored turbinates were shrinkage of dorsal and ventral turbinate and distortion of ventral turbinate. These data are very similar to some of the atrophic rhinitis cases presented by Brown et al. (1966). However, they fed one higher dietary calcium and phosphorus combination and continued until 100 kg. body weight, whereas, pigs in this study were sacrificed at approximately 25-30 kg. body weight.

Histologically, similar differences were found among the samples of the humerus, rib and mandible, but these were not as pronounced as those of the turbinate.

TABLE XVII

SUMMARY OF HISTOLOGIC CHANGES OF TURBINATES TAKEN FROM PIGS FED FIVE LEVELS OF CALCIUM FROM THREE THROUGH NINE WEEKS OF AGE

Dietary Ca, %	0.41	0.58	0.74	0.90	1.06	
Individual Scores[b]	5 5 5 3 4	4 5 5 3	2 5 3 4 4	4 3 4 2	1 3 4 3	
Means	4.40	4.25	3.60	3.25	2.75	0.52[a,c]

[a]Standard error of treatment means.

[b]Scoring System: 0 = normal remodeling, 1 = slightly to moderately increased resorption, and 5 = very severe resorption, fibrous replacement, various degrees of gross reduction in volume.

[c]Significant Linear Effect, ($P<.025$).

Summary

Data from this study indicate that young SPF pigs will perform reasonably well over a wide range of calcium levels from 3 through 9 weeks of age. A dietary calcium level of 0.41 percent did not adversely affect gain and efficiency, but maximum gain and efficiency occurred on the diet containing 1.06 percent. There were no significant differences in bone weights, length or diameters although the largest values usually occurred on 0.90 or 1.06 percent calcium. Maximum specific gravities occurred on 0.90 or 1.06 percent calcium. As a measure of maximum skeletal development, the largest ash, calcium and phosphorus values occurred on diets containing 0.90 or 1.06 percent calcium.

Histological slides indicate varying degrees of turbinate bone resorption on all calcium levels. After 6 weeks on treatment, none of the turbinates were diagnosed as coming from pigs with atrophic rhinitis.

CHAPTER V

EVALUATION OF PHOSPHORUS REQUIREMENTS FOR YOUNG PRIMARY SPF PIGS

Introduction

As early as 1933, Bethke et al. asserted that the minimal phosphorus requirement for swine was 0.60 percent. Combs et al. (1962) utilizing weight gains, efficiency and percent ash as criteria, indicated the phosphorus requirement of young pigs was 0.44 percent of the diet. Miller et al. (1964) indicated that 0.50 percent dietary phosphorus was adequate for weight gain and efficiency but that 0.60 percent was necessary to obtain maximal strength of bone and to insure the absence of rachitic lesions. The studies reported in this chapter were designed to further elucidate the phosphorus requirements for young pigs.

Materials and Methods

Primary SPF pigs, 3 weeks of age, were handled in the same way as reported in Chapter IV and experiments were conducted using the same facilities. Likewise, the experimental period covered 42 days (21 to 63 days of age).

Phosphorus Purified Diets

The physical composition of the diet used in the phosphorus study is presented in Table XVIII. The phosphorus levels as determined by analysis were: Diet I, 0.53; Diet II, 0.63; Diet III, 0.73 and Diet IV, 0.83 percent with calcium constant at 0.90 percent.

The protein, cellulose, fat, calcium-phosphorus free basal mineral mixture (Table VI) and vitamin portions of the diet were held constant in all diets. The cornstarch and sodium phosphate were varied in Diets I through IV to form the various dietary treatments studied. The rations were formulated to be adequate in all other nutrients and contained 125 grams of antibiotic activity per ton.

Assignment to Treatment

At 21 days of age, the pigs were removed from their individual sealed incubator boxes and transported to the nursery room where they remained for 3 weeks. The pigs were weighed, individually identified (ear notched) and placed in individual metal pens. Pigs were randomly assigned to each treatment and started on the dry meals immediately. The pigs were fed *ad libitum*.

Chemical and Statistical Analyses

Weekly weights and feed consumption were recorded. Blood samples were collected, prepared and analyzed by the methods outlined in Chapter IV.

TABLE XVIII

PERCENTAGE COMPOSITION OF EXPERIMENTAL PURIFIED DIETS
FOR PHOSPHORUS STUDY

Ingredient[a]	Diet I	Diet II	Diet III	Diet IV
	%	%	%	%
Casein	25.58	25.58	25.58	25.58
Corn starch	44.60	44.20	43.75	43.30
Glucose monohydrate	12.00	12.00	12.00	12.00
α-cellulose	6.00	6.00	6.00	6.00
Corn Oil	5.30	5.30	5.30	5.30
Mineral mixture	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.22	1.22	1.22	1.22
Calcium carbonate	1.18	1.18	1.18	1.18
Sodium phosphate	0.00	0.37	0.82	1.26
Water soluble vitamin	1.00	1.00	1.00	1.00
Fat soluble vitamin	0.0041	0.0041	0.0041	0.0041
Antioxidant	0.0125	0.0125	0.0125	0.0125
Sulfamethazine-antibiotic	0.125	0.125	0.125	0.125

[a]Sources of ingredients same as for Table V.

When the phosphorus experiment was completed, 16 pigs (four from each diet) were randomly selected and sacrificed by exsanguination. The right humerus, femur, ulna-radius and eighth rib were removed, weighed, and measured as designated in Chapter IV. Bone samples, plus a cross-section of the nasal turbinates were extracted with ethanol and petroleum ether, ashed and analyzed as previously outlined.

The left humerus, eighth rib, mandible and a section of the nasal turbinate were removed, stored in 10 percent formalin, decalcified and cut into six micron thicknesses for histological slides.

Analyses of variance, calculation of standard errors and orthogonal comparisons among treatment totals were conducted according to methods outlined by Steele and Torrie (1960).

Results and Discussion

Twenty-one primary SPF pigs, 3 weeks of age, were used to evaluate dietary phosphorus levels of 0.53, 0.63, 0.73 and 0.83 while dietary calcium was held constant at 0.90 percent. This represented calcium:phosphorus ratios of 1.70:1, 1.43:1, 1.23:1 and 1.08:1.

Total gains (Table XIX) were fairly constant across all levels of phosphorus. Although not statistically significant, feed efficiency improved linearly with each successive increment of dietary phosphorus. Miller et al. (1964) reported that growth rate and feed consumption were

TABLE XIX

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

Diet No. of Pigs Dietary P, %	I 6 0.53	II 5 0.63	III 5 0.73	IV 5 0.83	
Weight gain, kg.	22.36	22.90	22.28	22.20	1.07[a]
Feed intake, kg.	51.40	51.90	48.17	46.87	2.32[a]
Gain/Feed, kg.	2.33	2.27	2.14	2.12	0.10[a]

[a]Standard error of treatment means.

depressed only in those pigs receiving less than 0.4 percent of phosphorus. The 0.4 percent dietary phosphorus level was sufficient to support maximal growth rate and economy of food utilization. When studying dietary phosphorus levels of 0.24, 0.36, 0.48, 0.60 and 0.72 percent (Combs *et al.*, 1962), neither weight gain nor feed efficiency were significantly affected by phosphorus level. In a later study, Combs and Wallace (1962) reported 0.44 percent phosphorus fed with a calcium:phosphorus ratio of 0.9:1 was sufficient to meet the phosphorus requirements of pigs two to seven weeks of age.

There were no significant differences with respect to weekly body weights, gains or efficiencies (Table XX).

There were no significant differences or trends among treatment means for serum calcium values (Table XXI). Miller *et al.* (1964) reported normal serum calcium levels in all pigs, but they tended to be higher in pigs receiving lower levels of dietary phosphorus. Serum phosphorus (Table XXI) concentrations tended to increase with increased phosphorus levels. Serum phosphorus is apparently quite sensitive to dietary levels of phosphorus. Reports by Miller *et al.* (1964) revealed significant increases in serum phosphorus for each increment increase of dietary phosphorus.

Absolute weights of the femur, humerus, rib and ulna-radius (Table XXII) did not differ significantly. Miller *et al.* (1964) reported heavier femur and rib weights as

TABLE XX

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

Diet No. of Pigs Dietary P, %	I 6 0.53	II 5 0.63	III 5 0.73	IV 5 0.83	SE[a]
Initial Weights	4.77	4.89	4.76	4.54	0.17
21 to 28 days					
28 day wt., kg.	6.14	6.13	6.10	5.74	0.35
Gain, kg.	1.37	1.24	1.34	1.19	0.31
Gain/Feed, kg.	3.57	5.06	4.66	4.74	2.50
28 to 35 days					
35 day wt., kg.	9.02	9.25	9.16	8.53	0.42
Gain, kg.	2.89	3.12	3.06	2.80	0.15
Gain/Feed, kg.	1.98	1.92	1.86	2.22	0.16
35 to 42 days					
42 day wt., kg.	12.45	12.74	12.67	11.92	0.50
Gain, kg.	3.43	3.49	3.51	3.39	0.25
Gain/Feed, kg.	1.97	2.09	1.99	2.00	0.18
42 to 49 days					
49 day wt., kg.	16.93	17.59	16.91	16.21	0.67
Gain, kg.	4.48	4.85	4.25	4.29	0.28
Gain/Feed, kg.	2.26	2.18	2.14	1.95	0.13

TABLE XX CONTINUED

49 to 56 days					
56 day wt., kg.	21.85	22.84	22.34	21.63	0.88
Gain, kg.	4.92	5.24	5.43	5.42	0.34
Gain/Feed, kg.	2.38	2.28	2.01	2.10	0.18
56 to 63 days					
63 day wt., kg.	27.12	27.78	27.04	26.74	1.01
Gain, kg.	5.28	4.95	4.70	5.11	0.30
Gain/Feed, kg.	2.60	2.57	2.67	2.34	0.15

[a]Standard error of treatment means.

TABLE XXI

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

Diet	I	II	III	IV	
No. of Pigs	6	5	5	5	
Dietary P, %	0.53	0.63	0.73	0.83	
Weeks on feed	Serum Calcium mg./100 ml.				
3	11.86	11.92	11.28	11.66	0.28[a]
6	13.09	13.08	12.22	12.55	0.25[a]
9	12.57	12.52	12.30	12.81	0.47[a]
	Serum Phosphorus mg./100 ml.				
3	9.70	9.61	9.88	9.64	0.50[a]
6	9.71	11.13	11.91	11.43	0.35[a,b]
9	10.87	11.27	11.60	11.89	0.32[a]

[a]Standard error of treatment means.

[b]Significant treatment difference, ($P<0.01$).

TABLE XXII

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

Diet No. of Pigs Dietary P, %	I 4 0.53	II 4 0.63	III 4 0.73	IV 4 0.83	
Femur, g	88.93	94.30	90.00	92.00	5.89[a]
Humerus, g	76.80	80.00	78.00	79.73	5.14[a]
Eighth rib, g	8.03	8.40	8.35	8.78	0.69[a]
Ulna-radius, g	60.20	61.10	60.55	64.40	3.10[a]

[a]Standard error of treatment means.

dietary phosphorus levels increased, but each successive increment was twice that used in this study.

Absolute bone lengths and diameters (Table XXIII) showed no significant differences or trends across the four phosphorus levels.

There were no differences in specific gravities of the four bones (Table XXIV), although the trend was for the highest values to be from pigs consuming 0.63 or 0.73 percent dietary phosphorus. The rib was the only bone to show a linear trend from the lowest to the highest phosphorus diet.

Ash, calcium, phosphorus and calcium:phosphorus ratio values for the turbinate, femur, humerus, rib and ulna-radius are shown in Table XXV.

Turbinate values for ash, calcium, phosphorus and calcium:phosphorus ratio were not significant. This would indicate that the turbinate is less sensitive to dietary levels of phosphorus than to dietary levels of calcium. The greatest ash and calcium concentrations were from those pigs receiving 0.63 and 0.73 percent phosphorus.

The ash content of the femur was not significant nor was any trend noticable. A significant quadratic effect was found for calcium concentration, with the largest value occurring on 0.63 percent phosphorus. The lowest value occurred on 0.53 percent phosphorus. The phosphorus concentration of the femur was relatively constant across all diets.

TABLE XXIII

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

Diet	I	II	III	IV	
No. of Pigs	4	4	4	4	
Dietary P, %	0.53	0.63	0.73	0.83	SE[a]
		Absolute length, mm.			
Eighth rib	120.6	115.8	113.3	113.6	2.98
Humerus	107.7	112.9	110.9	111.8	2.04
Femur	120.0	128.4	129.6	127.0	3.26
Ulna-radius	132.9	128.4	128.5	131.1	2.96
		Absolute diameter, mm.			
Eighth rib	6.4	6.4	6.0	6.1	0.22
Humerus	14.5	14.2	13.9	13.6	0.26
Femur	17.5	18.0	17.1	16.8	1.73
Ulna-radius	16.7	16.4	16.1	16.5	0.32

[a]Standard error of treatment means.

TABLE XXIV

SPECIFIC GRAVITY OF BONES TAKEN FROM NINE WEEK OLD SPF PIGS FED FOUR LEVELS OF PHOSPHORUS (P) FROM THREE THROUGH NINE WEEKS OF AGE

Diet No. of Pigs Dietary P, %	I 4 0.53	II 4 0.63	III 4 0.73	IV 4 0.83	SE[a]
		Specific Gravity			
Femur	1.03	1.08	1.08	1.05	.02
Humerus	1.03	1.08	1.09	1.07	.02
Eighth rib	1.03	1.04	1.06	1.09	.02
Ulna-radius	1.05	1.12	1.10	1.09	.02

[a]Standard error of treatment means.

TABLE XXV

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

Diet	I	II	III	IV	
No. of Pigs	4	4	4	4	
Dietary P, %	0.53	0.63	0.73	0.83	
			Turbinate[f]		
Ash, %	57.32	58.23	58.12	54.30	1.07[a]
Calcium, %	18.42	18.91	18.42	17.09	0.56[a]
Phosphorus, %	11.35	11.53	11.23	10.49	0.38[a]
Ca/P	1.63	1.64	1.64	1.63	0.04[a]
			Femur[f]		
Ash, %	60.37	64.03	61.87	64.38	1.48[a]
Calcium, %	18.44	22.01	20.56	21.02	0.70[a,b]
Phosphorus, %	11.41	11.76	11.37	12.47	0.28[a]
Ca/P	1.62	1.87	1.81	1.69	0.06[a]
			Humerus[f]		
Ash, %	60.84	65.79	67.80	65.37	1.01[a,c,d,e]
Calcium, %	19.77	22.94	22.16	21.33	0.65[a,b]
Phosphorus, %	11.36	12.36	12.51	12.85	0.22[a,b]
Ca/P	1.74	1.86	1.77	1.66	0.04[a]

TABLE XXV CONTINUED

			Eighth Rib[f]		
Ash, %	53.09	56.02	56.18	54.03	1.33[a]
Calcium, %	16.97	19.62	18.52	17.50	0.57[a,b,d]
Phosphorus, %	10.18	10.49	10.62	10.79	0.23[a]
Ca/P	1.67	1.87	1.74	1.62	0.03[a,d]
			Ulna-radius[f]		
Ash, %	55.77	59.46	60.05	56.63	1.05[a]
Calcium, %	17.12	21.28	19.27	18.56	1.08[a]
Phosphorus, %	10.50	10.95	11.08	11.02	0.24[a]
Ca/P	1.63	1.94	1.74	1.69	0.03[a,c,d]

[a] Standard error of treatment means.

[b] Significant treatment difference, ($P<0.05$).

[c] Significant treatment difference, ($P<0.01$).

[d] Significant Quadratic Effect, ($P<.01$).

[e] Significant Linear Effect, ($P<.01$).

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

The humerus showed significant linear and quadratic effects for percent ash, with the largest values occurring on 0.63 and 0.73 percent phosphorus. Again, calcium concentration showed a significant quadratic effect with the largest value on 0.63 and the lowest on 0.53 percent phosphorus, respectively. Miller et al. (1964) reported that humeral ash, calcium and phosphorus concentrations were maximal in pigs receiving 0.6 percent of dietary phosphorus.

Ash content was not significant for the rib but a significant quadratic effect was observed for calcium. The greatest ash concentrations occurred on phosphorus levels of 0.63 and 0.73 percent. The largest calcium value occurred on 0.63 percent phosphorus, similar to that of the humerus. Phosphorus concentrations were similar for all diets. There were no significant difference for ash, calcium and phosphorus concentrations for the ulna-radius. The same general trends appeared in that the largest ash values occurred on 0.63 and 0.73 percent phosphorus and the largest calcium concentration occurred on 0.63 percent phosphorus. Phosphorus concentrations were similar for all four phosphorus diets.

A summary of the scores of the histologic changes of the nasal turbinates are presented in Table XXVI. There were no significant differences in the turbinate scores. The best single score occurred on 0.83 percent phosphorus and 0.90 percent calcium. This is very close to the ratio recommended by Krook et al. (1966) for maximum calcifica-

TABLE XXVI

SUMMARY OF HISTOLOGIC CHANGES OF TURBINATES TAKEN FROM PIGS FED FOUR LEVELS OF PHOSPHORUS FROM THREE THROUGH NINE WEEKS OF AGE

Dietary P, %	0.53	0.63	0.73	0.83	
Individual Scores[b]	3	3	2	3	
	4	3	4	4	
	3	3	4	3	
	3	3	2	1	
Mean Scores	3.25	3.00	3.00	2.75	0.44[a]

[a]Standard error of treatment means.

[b]Scoring System: 0 = normal remodeling, 1 = slightly to moderately increased resorption, and 5 = very severe resorption, fibrous replacement, various degrees of gross reduction in volume.

tion of the turbinate. The variation found in each treatment group is evident from the individual scores.

Summary

Twenty-one primary SPF pigs were used to evaluate four levels of dietary phosphorus, 0.53, 0.63, 0.73 and 0.83 percent, with the dietary calcium held constant at 0.90 percent. Total gains were the same for all four diets. Phosphorus levels of 0.73 and 0.83 percent appeared to promote slightly better feed efficiency than 0.53 or 0.63 percent phosphorus. No differences or trends were found for bone weights, lengths or diameters. There were no differences in specific gravities, although the highest values occurred on 0.63 or 0.73 percent phosphorus level. The highest percent of ash, calcium or phosphorus usually occurred on 0.63 or 0.73 percent phosphorus levels. The major significant difference to occur in the bone data was a quadratic effect with respect to calcium concentration, with the lowest values occurring on 0.53 percent phosphorus. Although there were no significant differences in bone weights, there were significant differences in bone mineralization.

CHAPTER VI

^{45}Ca UPTAKE IN BONES AND APPARENT CALCIUM AND PHOSPHORUS DIGESTIBILITY IN YOUNG PRIMARY SPF PIGS

Introduction

Comar et al. (1952) showed by autoradiography that ^{45}Ca appears in the femur of young pigs by 25 minutes after injection into the carotid artery. It is possible, by following the movement of injected ^{45}Ca within and among bones, to compare rates of bone growth in various parts of the skeleton. Krusemark et al. (1968) showed that ^{45}Ca uptake by turbinates was greater ($P<.01$) than for any other bone at specified time intervals. Pond et al. (1969) reported that ^{45}Ca activity in the nasal turbinate paralleled that in the proximal and distal ends of the humerus.

Hansard et al. (1961) reported apparent calcium digestibility to be 91 percent for pigs 2 weeks of age. Miller et al. (1962) reported apparent digestibility of pigs receiving 0.4 percent calcium to be 87 percent. Apparent calcium digestibility was inversely related to dietary calcium level. Apparent digestibility is usually higher for phosphorus than for calcium. Miller et al. (1964) reported apparent phosphorus digestibility of 84, 88, 75, 63 and 59 percent on dietary phosphorus levels of 0.4, 0.5, 0.6, 0.7 and 0.8 percent, respectively.

Materials and Methods

^{45}Ca Study

Twelve pigs were used, six in each time period, to investigate the uptake of ^{45}Ca in specific bones. At 3 weeks of age, pigs were randomly assigned to diets C, D and E containing 0.74, 0.90 and 1.06 percent calcium, respectively. Six pigs, two per treatment, were injected intraperitoneally with 20 µc ^{45}Ca per kg. body weight after 3 weeks on treatment. Three pigs, one from each treatment, were sacrificed 4 days after injection with the remaining three being sacrificed after 8 days. The remaining six pigs were injected with 20 µc ^{45}Ca per kg. body weight at 9 weeks of age and sacrificed in the manner previously discussed.

Immediately after injection, pigs were placed in digestion pens where they remained until sacrificed. A piece of 6 millimeter plastic (442 X 208 cm.) was placed under the six digestion pens to minimize contamination of the concrete floor. Urine was collected in one-gallon containers, diluted and flushed into the sewer utilizing large volumes of water. Feces and waste feed were collected and frozen until proper burial procedures were arranged. After all 12 pigs had been sacrificed, the remains of the carcasses, plastic covering, fecal material and waste feed were buried in accordance with procedures set forth for isotope handling. When the areas subject to contamination had been thoroughly cleaned, seven swipe tests were taken and counted to determine amount of contamination.

Digestibility Study

Six males (two per treatment) were randomly assigned to Diets C, D and E containing 0.74, 0.90 and 1.06 percent calcium, respectively, at 3 weeks of age and continued throughout the 9th week. Chromic oxide was added to the diets at the level of 112 grams per 100 pounds to serve as an indicator for calculations of apparent digestibility. The digestition stalls used measured 91 X 52 X 70 cm. and were equipped with automatic waterers and self-feeders. Each waterer and feeder was placed over a section of expanded metal measuring 25 X 25 cm. to assure the absence of feed and water in the fecal collections. Fecal material was allowed to fall through the expanded metal floor and was collected on fine mesh wire covered with nylon.

The pigs were put in the digestion stalls during the 8th week and allowed a 5 day adjustment period before collections were made during the 9th week. Feces and feed samples were collected daily at six p.m. for 5 days and stored at -17°C. until analyzed for chromium, calcium and phosphorus.

Data Obtained and Chemical Analysis

^{45}Ca Study

Seven bone samples were selected for determination of specific activity. These were the mandible, turbinate, right eighth rib, humerus, distal end of humerus, femur and distal end of femur. Bone samples were extracted for 16 hours with ethyl ether and an additional three hours with petroleum ether. Fat-free, dried samples were ashed for 12 hours at 550°C. and dissolved in 4 N HCl to a 25 ml. volume. Calcium was determined in duplicate using a Perkin-Elmer Atomic Absorption Spectrophotometer. Total counts in a 0.1 ml. sample were determined using a Packard Tri-Carb Model 3003 Liquid Scintillation Spectrometer equipped with an IBM Selectric Typewriter. Scintillation fluid used was Bray's[8] solution and was mixed as follows:

Naphthalene	120 g
2,5-Diphenyloxazole (scintillation grade)	8 g
1,4-bis-2-(5-phenyloxazolyl)-Benzene (scintillation grade)	0.4 g
Methanol	200 ml.
Ethylene glycol	20 ml.
Diluted to two liters with p-Dioxane (spectroquality)	

Counts per minute (cpm) were converted to disintegrations per minute (DPM) through the use of an external standard. Radioactive calcium uptake was expressed as μc ^{45}Ca per g calcium.

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[8]Analytical Biochemistry, Vol. I, p. 279-285, 1960.

Calcium and Phosphorus Digestibility Study

For calcium and phosphorus analysis, feed and feces samples were dried for 10 hours at 100°C., ashed for 12 hours at 550°C. and dissolved in a known volume of 4 N HCl. Phosphorus was analyzed in duplicate by the method of Fiske and SubbaRow (1925). Calcium analysis was performed in duplicate using a Perkin-Elmer Atomic Absorption Spectrophotometer.

Feed and feces samples for chromium determination were dried for 10 hours at 100°C., ashed for 8 hours at 600°C., dissolved in phosphoric acid and determined in duplicate using a Perkin-Elmer Atomic Absorption Spectrophotometer. Apparent digestibility was calculated using the following formula:

$$\text{Digestibility} = 100 - \left[100 \times \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}} \times \frac{\% \text{ nutr. in feces}}{\% \text{ nutr. in feed}}\right]$$

Results and Discussion

^{45}Ca Study

Twelve pigs, six in each of two time periods, were used to investigate the uptake of ^{45}Ca in specific bones.

The ash, calcium and ^{45}Ca content of bones taken from 6 week old pigs injected with ^{45}Ca and sacrificed after either 4 or 8 days post injection are presented in Tables XXVII, XXVIII and XXIX, respectively. Since only one pig was sacrificed per time period, any statistical analysis

TABLE XXVII

ASH CONTENT OF BONES TAKEN FROM SIX WEEK OLD PIGS
INJECTED WITH ^{45}Ca AND SACRIFICED
AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	16.12	12.94	14.30	12.94	12.94	14.76
Pig No.	1	2	3	4	5	6
<u>Bones - % Ash</u>						
Mandible	60.68	59.04	61.06	59.93	61.16	62.16
Turbinate	51.62	52.12	54.04	50.72	49.70	53.18
Eighth Rib	52.69	52.20	53.91	54.16	57.21	58.87
Humerus - Shaft	59.24	63.94	64.55	67.01	62.66	68.28
Humerus - Distal	14.18	12.14	11.23	11.73	14.11	12.74
Femur - Shaft	63.58	64.12	65.49	62.83	63.73	67.39
Femur - Distal	17.75	13.63	16.31	14.20	22.69	18.96

TABLE XXVIII

CALCIUM CONTENT OF BONES TAKEN FROM SIX WEEK OLD PIGS INJECTED WITH ^{45}Ca AND SACRIFICED AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	16.12	12.94	14.30	12.94	12.94	14.76
Pig No.	1	2	3	4	5	6
<u>Bones - % Ca</u>						
Mandible	23.16	21.65	22.25	21.91	26.05	23.36
Turbinate	19.18	18.45	19.63	16.21	18.22	18.73
Eighth Rib	18.50	18.72	20.21	19.95	21.68	21.33
Humerus - Shaft	21.97	23.62	23.26	24.92	23.39	29.96
Humerus - Distal	2.82	1.80	1.41	1.87	2.26	2.58
Femur - Shaft	23.95	23.31	24.09	23.63	25.12	25.47
Femur - Distal	4.44	2.89	3.71	2.97	2.20	5.16

TABLE XXIX

^{45}Ca CONTENT OF BONES TAKEN FROM SIX WEEK OLD PIGS INJECTED WITH ^{45}Ca AND SACRIFICED AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	16.12	12.94	14.30	12.94	12.94	14.76
Pig No.	1	2	3	4	5	6
Bones - μc^{45}Ca/g Ca						
Mandible	4.25	4.93	2.55	4.14	2.68	3.72
Turbinate	4.57	3.85	2.70	3.32	3.26	3.39
Eighth Rib	4.50	4.46	3.01	3.13	3.34	3.60
Humerus - Shaft	3.42	3.24	2.12	2.26	2.25	2.41
Humerus - Distal	7.77	5.04	4.17	3.37	5.72	3.50
Femur - Shaft	2.97	3.36	1.88	2.40	2.34	1.99
Femur - Distal	8.17	5.21	4.39	3.29	18.85	3.65

would be meaningless because there would be no way to estimate experimental error. Therefore, these data can only be discussed with respect to general trends. Considering ash concentration, the largest difference between two pigs on the same diet was 6 percent. This may have been a true difference or due to experimental error. Percent calcium varied as much as 3 percent for one bone for the two pigs on one diet.

In general, Radioactive ^{45}Ca uptake data indicated that the distal ends of the humerus and femur had the highest uptake, shafts the lowest and the mandible, turbinate and eighth rib somewhere in the middle (Table XXIX). However, assuming these values to be correct, data for pigs one and two would indicate that the turbinate has a faster calcium turnover rate than the mandible.

Ash, calcium and ^{45}Ca contents of bone taken from nine week old pigs are shown in Tables XXX, XXXI and XXXII, respectively. The ash and calcium concentrations showed the same variations as did the younger pigs. The same general trends were evident within each pig, i.e., the distal ends had the highest uptake, the shafts the lowest with the mandible, turbinate and eighth rib in the middle. Those indications would tend to agree with the report by Krusemark et al. (1968) and disagree with the work of Pond et al. (1969). Krusemark et al. (1968) maintained that the uptake of ^{45}Ca by the nasal turbinate was greater than for any other bone (shaft), while Pond et al. (1969) reported

TABLE XXX

ASH CONTENT OF BONES TAKEN FROM NINE WEEK OLD PIGS
INJECTED WITH ^{45}Ca AND SACRIFICED
AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	26.33	31.33	29.51	30.65	25.88	28.15
Pig No.	7	8	9	10	11	12
<u>Bones - % Ash</u>						
Mandible	61.70	61.21	61.29	62.78	61.85	62.11
Turbinate	51.72	54.05	54.43	51.13	54.26	52.51
Eighth Rib	53.64	53.67	53.04	55.05	57.26	54.32
Humerus - Shaft	65.76	66.82	65.02	67.54	65.32	66.67
Humerus - Distal	12.07	15.10	13.24	13.01	13.28	14.02
Femur - Shaft	62.38	67.63	64.86	66.43	66.04	67.06
Femur - Distal	14.80	15.29	17.37	19.85	13.12	18.48

TABLE XXXI

CALCIUM CONTENT OF BONES TAKEN FROM NINE WEEK OLD PIGS INJECTED WITH ^{45}Ca AND SACRIFICED AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	26.33	31.33	29.51	30.65	25.88	28.15
Pig No.	1	2	3	4	5	6
Bones - % Ca						
Mandible	23.80	18.69	22.19	22.66	23.66	23.58
Turbinate	18.62	20.12	19.11	18.79	21.60	17.69
Eighth Rib	19.98	20.84	19.09	20.61	21.86	20.96
Humerus - Shaft	24.77	25.34	23.76	25.97	25.16	25.21
Humerus - Distal	2.23	3.50	2.59	2.61	3.03	2.92
Femur - Shaft	24.03	25.02	23.77	24.75	25.83	24.97
Femur - Distal	3.51	3.73	4.19	5.02	2.89	5.05

TABLE XXXII

^{45}Ca CONTENT OF BONES TAKEN FROM NINE WEEK OLD PIGS INJECTED WITH ^{45}Ca AND SACRIFICED AFTER EITHER 4 OR 8 DAYS

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Days post injection	4	8	4	8	4	8
Body wt., kg.	26.33	31.33	29.51	30.65	25.88	28.15
Pig No.	1	2	3	4	5	6
Bones - $\mu c\ ^{45}Ca/g$ Ca						
Mandible	3.08	8.02	5.27	5.75	3.80	4.96
Turbinate	3.29	4.71	3.15	2.97	2.12	4.04
Eighth Rib	3.30	4.22	3.44	3.48	2.91	4.05
Humerus - Shaft	2.04	3.22	2.29	2.66	2.61	2.68
Humerus - Distal	7.64	5.40	6.77	4.90	6.19	5.34
Femur - Shaft	2.32	3.27	2.63	2.73	2.17	3.12
Femur - Distal	7.85	6.33	7.47	4.06	7.04	4.98

that ^{45}Ca activity in the nasal turbinate paralleled that in the distal and proximal ends of the humerus.

Apparent Digestibility Study

Six male pigs (two per treatment) were maintained on dietary calcium levels of 0.74, 0.90 and 1.06 percent, with phosphorus held constant at 0.75 percent, from 3 to 9 weeks of age. Apparent digestibility of calcium and phosphorus was determined during the 9th week using chromic oxide as an internal indicator.

Apparent digestibilities of calcium and phosphorus are shown in Table XXXIII. Apparent calcium digestibility was highest on the diet containing 0.74 percent calcium and lowest on the diet containing 0.90 percent calcium. Miller et al. (1962) reported that apparent calcium digestibility was inversely related to dietary intake. Dietary phosphorus was constant at 0.75 percent and apparent digestibility averaged approximately 80 percent. This is somewhat higher than Miller et al. (1964) who reported apparent phosphorus digestibilities of 63 and 59 percent on diets containing 0.7 and 0.8 percent phosphorus, respectively.

TABLE XXXIII

APPARENT DIGESTIBILITIES OF DIETARY CALCIUM AND PHOSPHORUS IN NINE WEEK OLD SPF PIGS USING Cr_2O_3 AS INTERNAL INDICATOR

Diet	C		D		E	
Dietary Ca, %	0.74		0.90		1.06	
Dietary P, %	0.75		0.75		0.75	
Pig No.	1	2	3	4	5	6
	Apparent Calcium Digestibility, %					
	56.30	64.45	52.61	55.66	61.69	55.91
Mean, %	60.38		54.14		58.80	
	Apparent Phosphorus Digestibility, %					
	78.59	81.75	78.35	79.75	84.66	80.16
Mean, %	80.17		79.05		82.41	

CHAPTER VII

SUMMARY AND CONCLUSIONS

One hundred and twenty-seven neonatal pigs (6 groups) were used to develop techniques for successfully rearing Caesarean pigs to 3 weeks of age. Utilizing Caesarean section techniques, neonatal pigs were taken from the sow on the 113th day of gestation, resuscitated by body massage, placed directly into an autoclaved isolator and transported to the Swine Nutrition Laboratory. After surgery and subsequent recovery, each sow was transported to the swine barn and rebred. A few of the sows were used a second time for Caesarean surgery. Each pig was weighed, placed in an individual sterile, heated incubator and fed a fortified milk diet containing 21.59 percent solids until 3 weeks of age. Survival for each of the 6 groups ranged from 58 to 100 percent. Group 2 had the lowest survival rate because it consistently contained bacterial infections of varying degrees. Survival rate was 80 percent for 6 groups and 89 percent if group 2 was not considered. Average gains per group for the 3 week period ranged from 3.324 kg. to 4.914 kg., while efficiency (g dry matter per g gain) ranged from 0.73 to 1.15. There was an inverse relationship between gains and efficiencies, i.e., the greater the gains, the lower the efficiencies. It is felt that survival rates of 90 percent or better can routinely be obtained by trained personnel, and that the 3 week weights will be as good or slightly better than those of the naturally born and suckled pigs. The ability to rear colostrum deprived pigs to 3 weeks of age without antibiotics presents experimental models that can be extremely useful in studying intermediate metabolism of the neonate.

Forty-three primary SPF pigs, 3 weeks of age, were used in two replicates to evaluate dietary calcium levels of 0.41, 0.58, 0.74, 0.90 and 1.06 percent while dietary phosphorus was held constant at 0.75 percent. Growth was considered adequate on all diets. Total gains in Replicate 1 showed a significant linear effect with the largest gains occurring on 1.06 percent dietary calcium. Feed efficiencies were best on dietary calcium levels of 0.90 and 1.06 percent. There were no significant differences for gains and efficiencies for Replicate 2 although the best gains and efficiencies occurred on the higher calcium levels. Four pigs from each treatment group were slaughtered at the end of the 9th week and various bones removed for chemical analysis and histological staining. No significant differences with respect to absolute weight, length or diameter were found for the right femur, humerus, eighth rib and ulna-radius. Specific gravities exhibited significant linear effects and to an extent were positively related to dietary calcium level. Chemical analysis of the turbinate, femur, humerus, eighth rib and ulna-radius indicated the turbinate was the most sensitive of all bones studied to dietary calcium levels. Percent ash, calcium, phosphorus and calcium:phosphorus ratios for the turbinate were positively related to levels of dietary calcium. Histological studies of the turbinate indicate perfect bone formation was not achieved on any of the levels of dietary calcium, although differences were noted. When scores were assigned to the turbinates, a significant linear effect was found for increased bone formation going from the lowest to the highest calcium diets.

Twenty-one primary SPF pigs, 3 weeks of age, were used to evaluate dietary phosphorus levels of 0.53, 0.63, 0.73 and 0.83 percent while dietary calcium was held constant at 0.90 percent. Total gains were relatively constant across all diets. Feed efficiency tended to increase as dietary phosphorus increased. The same bones were removed for analysis as in the calcium study. There were no significant differences with respect to absolute bone weight, length, diameter or specific gravity. Bones from pigs receiving 0.63 percent phosphorus tended to have the highest calcium concentrations. There were no significant differences with respect to turbinates and turbinate scores.

Twelve pigs on calcium levels of 0.74, 0.90 and 1.06 percent were used to study the uptake of ^{45}Ca by the turbinate, mandible, eighth rib, femur distal, femur shaft, humerus distal and humerus shaft. The distal end of the femur and humerus had the greatest uptake, while the shafts of the femur and humerus had the lowest uptake. The uptakes of the mandible, turbinate and rib were usually between the high and low values of the shafts and distals, respectively.

Apparently the pig, on the basis of these studies and recent literature, can tolerate wide ranges of dietary calcium and phosphorus. Growth was adequate on 0.41 percent calcium but maximum gain and efficiency were achieved on 1.06 percent dietary calcium. The high calcium diet did not decrease feed intake. Growth on 0.53 percent phosphorus was as good as on 0.83 percent, although, feed efficiency was the best on 0.83 percent phosphorus. Over the dietary ranges of calcium and phosphorus used on these studies, apparently calcium level exerts a more drastic effect than phosphorus. This applies equally to growth and performance as well as skeletal development, the latter measured by mineral content and histological studies of various skeletal bones. These data could be applied to a commercial operation. Calcium is the cheapest element used in a computer formulation, therefore, increased levels of calcium could be fed without an increase in feed cost. Phosphorus is one of the most expensive dietary ingredients, therefore, a decision would have to be made as to whether the increase in efficiency would pay for the added cost of additional phosphorus.

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