SOME FACTORS AFFECTING MINERAL UTILIZATION BY THE BABY PIG

Thesis for the Degree of Ph. D.
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DELOY G. HENDRICKS
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This is to certify that the

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SOME FACTORS AFFECTING MINERAL UTILIZATION

BY THE BABY PIG

presented by

Deloy G. Hendricks

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E. R. Miller
Major professor

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ABSTRACT

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by Deloy G. Hendricks

Four experiments, involving a total of 52 baby pigs, were conducted to investigate the effects of source and level of protein, and level of ergocalciferol on mineral utilization by the baby pig. Some physiological effects of these various regimens were also studied.

Baby pigs were taken from their dams at 3 days of age and, after a 4 day adaption to the dry diet and environmental conditions, were assigned to the various dietary treatments. Pigs were housed in wire bottomed metal cages in a room where no ultraviolet rays could enter. Serum was obtained from blood drawn from the anterior vena cava initially, at 3 weeks and again at 5 weeks on trial. A 3 day mineral balance study was conducted after which pigs were autopsied and weight of various organs and glands taken. Humeri, femurs and 2 ribs were taken for mineral and strength determinations.

Performance, as measured by growth rate and feed efficiency, was no different when casein or soy was fed at levels providing 32% or 16% crude protein in the diet. Increasing ergocalciferol from 6.25 to 12.50 μg/kg diet did not improve rate of gain or feed efficiency. Food intake was decreased at the higher levels of protein intake irrespective of protein source.

Serum inorganic phosphorus was depressed by high levels of isolated soy protein intake. Increasing the dietary intake of ergocalciferol did not overcome this depression nor the accompanying increase in serum alkaline phosphatase. By contrast, high levels of casein protein in

the diet did not produce these changes in these two serum components.

Three mineral balance studies, conducted after 5 weeks on a given regimen, showed that increasing isolated soy protein in the diet from 20% or 30% up to 40% had no effect on the retention or excretion of Ca, P or magnesium. Increasing ergocalciferol in the diet from 6.25 to 12.50 μg/kg diet did not improve the utilization of these minerals. A balance trial comparing casein with isolated soy protein at levels equal to 16% or 32% crude protein showed that casein enhanced retention of Ca, P and Mg when it comprised 40% of the diet. Isolated soy protein, however, significantly depressed the retention of these minerals when fed at the high level. No consistent dietary treatment effects could be shown on the retention or excretion of Na, K, Fe, Zn, Mn, Cu, Co or chromium.

Bone analyses showed decreasing specific gravity, ash, Ca, P, Mg and breaking strength as dietary protein increased when the protein source was isolated soy. Level of protein supplied by casein had no effect on these bone measurements. Ergocalciferol fed at 12.50 μg/kg diet was no more effective in preventing this depression in bone mineralization than was 6.25 μg ergocalciferol/kg diet.

Physiological responses observed, that were due to dietary treatment, included a mild hypertrophy of kidneys, liver and pancreas as protein level in the diet increased. This was observed whether the protein was provided by casein or isolated soy. Ergocalciferol level in the diet had no effect on the physiological measurements taken.

Influx and efflux of nutrients from various sections of the gastrointestinal tract were studied by the chromic oxide indicator method.

Influx of all nutrients studied was increased in the cranial small intestine when casein was the source of dietary protein as compared to isolated soy. A compensatory efflux of these nutrients was observed to take place from the caudal half of the small intestine. Dietary protein level had no measurable effect on nutrient movement into or out of the gastrointestinal tract.

SOME FACTORS AFFECTING MINERAL UTILIZATION BY THE BABY PIG

by

Deloy G. Hendricks

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURE (Continued)

LIST OF APPENDIX TABLES

I. INTRODUCTION

This laboratory and others have been using purified diets to study various nutrient interactions. Reports by Carlson et al. (1964a, b) that bone ash in turkey poults was depressed by high levels of isolated soy protein in the diet were of interest to us. When soybean oil meal was substituted for the isolated soy preparation, it was discovered that lower levels of vitamin D_3 were adequate for maximal growth. Miller et al. (1965b) showed that purified diets containing casein required lower levels of vitamin D supplementation than similar diets composed of isolated soy protein. They carried out their studies on baby pigs and used serum Ca, P, alkaline phosphatase, and mineral deposition in bones as their criteria of vitamin D adequacy.

This study was undertaken to determine the effects of source and level of protein and level of ergocalciferol on mineral utilization by the baby pig. Attempts were made to determine the digestive, absorptive, assimilative and excretive consequences of these factors.

II. REVIEW OF LITERATURE

A. Protein Level

Economics would generally dictate that diets be utilized which are as low in protein as will keep the organism growing and healthy. A great deal of research has been carried out in all species to determine the minimal levels of protein that will still allow the subject to satisfactorily accomplish its purpose. A review of the literature shows very little work directly involving high levels of dietary protein and its possible metabolic and physiological effects. Manners and McCrea (1962), feeding casein diets to baby pigs, showed that as protein level was increased from 20% to 45% of the diet, average daily gain and feed efficiency were maximum at 25% to 35% crude protein. Serum protein values were shown to respond directly to dietary protein level in the range of 20% to 35% crude protein in the diet. Becker et al. (1954b), working with pigs 1 to 4 weeks of age, showed that performance increased with increasing protein levels up to 22.4% of the diet. Pigs from 5 to 9 weeks of age gave adequate performance when fed a 12% milk protein diet. Peo et al. (1954), feeding a protein mixture of 50% soybean meal and 50% dried skim milk to pigs 1 to 8 weeks of age, showed that better gains were obtained with a high level of fat (10%) and high level of protein (30%). Sewell et al. (1953) reported satisfactory growth from suckling pigs fed simulated milk diets containing 24% and 28% protein. Sewell et al. (1961) reported that the most rapid and efficient gains were obtained with a combination of 20% protein and 8% added fat. There was a progressive decrease in the quantity of feed required to produce a unit of gain as protein level increased. In this latter trial, soy protein was fed to pigs that were 3 to 7 weeks old. Meade, et al. (1965)

found that corn-soybean meal diets containing 18% protein supported as rapid and efficient gains over a 6 week period in pigs weighing 13.4 kg initially as did mixtures of similar composition providing greater amounts of protein. Pigs fed diets containing less than 16% crude protein during one or more of the successive 2 week periods of study were significantly less efficient in feed conversion. Jensen et al. (1957) fed casein-corn protein to pigs from 2 to 8 weeks of age and concluded that 16% to 16.6% protein would produce a rate of gain about equal to that obtained on higher protein levels. Feed required per pound of gain decreased markedly as protein level increased up to 16% to 16.6%. Above these levels, there appeared to be a trend toward further increase in feed efficiency. Reber et al. (1953) studied the influence of level of protein in rations fed to baby pigs from 2 to 7 weeks of age. They concluded that level of protein needed for maximum feed utilization and growth by baby pigs decreases as their body weight increases. A ration containing 41% protein produced maximum weight gains and feed efficiency for the young pigs. As pigs approached 8 weeks of age, a level of 20% protein appeared to be used as efficiently as higher levels.

Noland and Scott (1960) showed that protein level in the diet linearly affected rate of gain of pigs from weaning to 75 pounds, but had little effect from 75 pounds to market weight. Levels tested were 12, 16 and 20% crude protein. Becker et al. (1954a) showed that as crude protein in the diet increased, from 10% to 16%, average daily gain increased as did feed efficiency. However, when the pigs were over 100 pounds, maximum performance could be obtained when lower levels of protein were fed. Pond et al. (1960) showed that high protein rations

(20% to 18%), when fed to growing and finishing swine, promoted significantly greater average daily gains than low protein rations (12% to 10%). The addition of 10% stabilized beef tallow increased rate of gain significantly with the higher protein rations but not with the low protein ration. Serum cholesterol was reduced and serum albumin and total serum protein were increased by feeding a high protein ration to pigs previously consuming the low protein ration. Kropf et al. (1959) and Hale and Southwell (1967) reported that market pigs fed protein levels of 12% to 16% were more efficient in utilizing feed and had a higher yield of lean cuts than pigs fed lower levels of protein. Greeley et al. (1964) noted linear decreases in daily dry matter consumption and in dry matter consumed per unit of gain with increasing protein content from 13% to 19% in the diets of market hogs. As dietary protein level increased, there was a highly significant linear trend toward reduced efficiency of utilization of digestible protein. Wagner et al. (1963) observed in trials with growing swine that, as dietary protein level increased from 13% to 25%, average daily gain and feed required per pound of gain decreased.

Woodman et al. (1937), by means of balance trials, studied the effects of adding 12% of soybean meal at the expense of barley meal to a normal bacon pig diet. Nitrogen retention from the high-protein diet was no higher than from the normal-protein diet. No evidence was obtained to indicate that mineral metabolism (Ca, P, Cl) was affected by the higher level of protein. Armstrong and Mitchell (1955), after a series of nitrogen balance studies with growing swine concluded that fecal nitrogen output is linearly related to the protein level of the diet fed. Crampton and Rutherford (1954) had earlier shown this to be

true for rats at dietary protein levels from 5% to 50% of the diet. Lloyd and Crampton (1955) and Whiting and Bezeau (1957) observed that the level of crude fiber in the diet as well as the level of crude protein would affect the apparent digestibility of protein. Rippel et al. (1965) found that in gravid gilts percent nitrogen retained plateaued at approximately 12.5% of dietary protein. They also found that serum protein was closely related to the level of dietary protein. Of the individual serum components, albumin concentration closely resembled the changes in total protein. Forbes et al. (1958) found that the biological value of a protein depended on the protein concentration in the diet of the growing rat. As the dietary protein level increased, the biological value dropped. Sibbald et al. (1957) and Meyer (1956), using the rat as the experimental animal, made observations similar to those made with swine in that the level of crude fiber in the diet limited the retention of the nitrogenous portion of the diet. Sibbald et al. have shown that, at least in the rat, this is due to nitrogen intake being limited by energy concentration in the diet. Nitrogen content of the ration appeared to exert a negligible influence upon food consumption. Garcia and Roderuck (1964) showed that during ad libitum feeding, rats that consumed 464 mg N/5 days had a significantly higher intake of food than rats fed 1159 mg N/5 days. Nitrogen retention was greater at the higher level of N intake. There were no significant differences observed in liver weights.

Klavins et al. (1962) found that approximately 15% to 18% protein was necessary to meet the normal needs of the rat as far as iron absorption was concerned. When smaller amounts of protein were fed, the absorption of iron was impaired. Abernathy et al. (1965) reported that

7 to 9 year old girls absorbed 2.5% to 25% of their dietary iron. Contrary to the report of Klavins et al., these studies failed to demonstrate any consistent effect of level of protein in the diet on iron absorption. Protein intakes varied from 30% to 150% of the recommended allowances. Hegsted et al. (1948) reported that low protein diets (8% casein) with 2.2% ferric citrate added were toxic to rats. However, the addition of protein at higher levels prevented all evidences of iron toxicity.

McCall and Davis (1961) presented data which indicates that the dietary level of protein has a significant effect on the accumulation of copper in the livers of rats. An adequate or more than adequate level of dietary protein inhibited the accumulation of toxic levels of copper in the liver when large amounts of copper were ingested. No indication of copper deficiency was noted, however, when normal levels of copper were fed with high levels of protein.

McCance et al. (1942) in metabolic studies on adults, have shown that increasing the protein intake increased the amount of Ca and Mg absorbed from the gut and subsequently excreted in the urine. Colby and Frye (1951) increased the severity of magnesium deficiency syndrome in rats by feeding high levels of protein (50% casein). Bunce et al. (1963) observed similar results with chicks as well as rats. A balance study in the rat failed to produce evidence of an effect of dietary protein on apparent absorption although urinary excretion of magnesium was increased with the higher protein diet (36% casein).

Tepperman et al. (1943) and Leathem (1951) reported that high dietary protein levels increased rat adrenal weights. Ingle et al. (1943), however, did not observe significantly higher adrenal gland

weights in rats fed high amounts of protein, and in one experiment by Benua and Howard (1945) mice fed high levels of protein did not have hypertrophied adrenals. Stoewsand and Scott (1964a) fed high protein diets to chicks and produced a stress as evidenced by hypertrophy, hyperactivity and depletion of phospholipid content of the adrenal cortex. No adrenal hypertrophy nor hyperactivity occurred when corticosterone was injected daily in chicks fed high protein diets.

Addis et al. (1951) showed that high levels of dietary protein (60%) caused renal hypertrophy. Differences were observed between sources of protein in the magnitude of the effect on the kidney. From calculated urea work load of the kidney, these workers concluded that the differential effect of dietary protein upon kidney size is not due to any differences in the inherent nutritive value for the kidney, but is secondary to the work load imposed upon the kidney by urea excretion.

Imondi and Bird (1967) observed that the size of the pancreas of chicks, when expressed as a percentage of the body weight, generally increased with increasing level of protein from 15% to 70% of the diet. When comparing casein and isolated soybean protein diets they concluded that the rapid growth of pancreatic tissue during protein repletion was not caused by any unique property of the soy protein.

Tumbleson and Meade (1966) have shown that liver weight and percent nitrogen in the liver of young pigs increased significantly with increasing levels of dietary protein (8% to 20%).

Tuba et al. (1952), in a series of studies with the rat, found that serum alkaline phosphatase was not affected by protein level (0, 5, 10, 30, or 91% casein) nor protein source (casein, dried brewer's yeast or wheat gluten) in the diet. There was, however, a significant correlation

between serum alkaline phosphatase activity and daily consumption of fat.

Thomas and Combs (1967), in studies with young chicks, showed that, when the dietary protein level was reduced without changing the energy level, both total serum protein and albumin levels were reduced. However, when the daily energy allowance was reduced without changing the protein intake, there was a rise in both serum protein and albumin levels.

Stoewsand and Scott (1961) showed that the tibia bone ash of chicks fed high protein diets (71.5%) decreased as compared to that of chicks receiving a moderate level of protein (21.9%) in the diet. El-Maraghi et al. (1965) found that when the protein level in the diet was high, a low calcium diet led to severe mineral-osteoporosis in the bones of young rats, but had only slight effect in the bones of older rats. Young rats given diets of high protein value had larger bones containing more mineral than litter-mates maintained on a low-protein regimen although both groups received the same amount of food and calcium. Improving the protein value of diets fed to adult rats whose bones had become rarefied led to a remineralization of their bones. Even in aged rats, matrix-osteoporosis brought about by diets of low protein value could be corrected by increasing the intake of protein. Silberberg and Silberberg (1952) observed that skeletal development was accelerated in growing mice fed diets containing 53% casein or 46% fish-protein as compared with the conditions seen in mice fed a 26% protein stock diet. Articular aging was retarded and the onset of degenerative joint disease was delayed in the mice fed the protein-enriched diets.

B. Protein Source

Different sources of dietary protein vary quantitatively and qualitatively in their composition. Varying composition of amino acids,

carbohydrates, fats, minerals and other constituents in different protein sources can affect their usefulness in the diet.

Jones and Pond (1964) found that, during the first 42 days after weaning, pigs fed diets containing dried whole milk gained significantly faster than pigs fed soybean meal rations. Pigs fed dried skim milk rations also gained faster than the pigs fed soybean meal rations, but the difference was not significant. Witczak et al. (1963) showed that pigs fed milk powder rations gained more rapidly than pigs fed either fishmeal or soya meal rations. Lewis et al. (1955) observed that skim milk and casein diets produced significantly ($P < 0.05$) heavier pigs at 5 weeks of age on significantly ($P < 0.05$) less feed than Drackett soy diets. In an effort to improve the utilization of the soy diets, they added digestive enzymes to the basal soy diet. The average effect of all enzyme additions (1% pancreatin, 1% pepsin, .5% Star-zyme P, 1% papain, or 1% Mycozyme) was to significantly improve feed efficiency of the soy diets. The results of Maner et al.(1961), Cunningham and Brisson (1957), Alsmeyer et al. (1957), Hudman and Peo (1957) and Calder et al. (1959), however, did not show that the addition of proteolytic enzymes to either liquid or solid diets containing soybean protein has a measurable effect on growth or protein digestibility. Barnes et al. (1966) found that, on the basis of rate of return of serum protein values to normal and the rate of weight gain, casein was definitely superior to a fishmeal, a heat treated soybean meal and three different cottonseed meal preparations for the malnourished baby pig. Geurin et al. (1951) reported that, for growing swine, dried whole egg and dried skim milk rations supported faster growth than rations with corn oil meal, soybean meal, tankage, corn gluten meal, or corn solubles plus soybean meal as

the primary protein sources. The quality of protein as measured by protein efficiency was highest for skim milk, dried whole egg and corn oil meal. Hays et al. (1959) and Combs et al. (1963) found that young pigs were better able to utilize soybean protein at 5 to 8 weeks of age than at 2 to 3 weeks of age. These findings are in agreement with those of Lloyd et al. (1957) when rations containing a mixture of milk, soybean and fish protein were studied with pigs at 3 and at 7 weeks of age. In contrast, there was very little improvement in the animals' ability to utilize the milk protein with increasing age. Sewell and West (1965), using pigs weaned at 21 days of age, saw no significant improvement in protein digestibility with age. These authors concluded that at least a part of the difference in response to protein from a skim milk source as compared to a soybean protein was due to the lactose content of the milk protein diets. Henry and Kon (1957) compared the casein and soy protein utilization by the rat at 2 different levels. They observed that the true digestibility of soy protein was about 10% less than that of casein, and protein level had no effect on the digestibility. Higher levels of protein resulted in lower biological values.

Miller et al. (1965b), in work with the baby pig fed purified diets, did not note any differences in rate of gain or feed efficiency that was due to protein source. They did show that diets containing casein required less vitamin D_2 to support maximal bone mineralization in the baby pig than diets containing soy protein. Mineral balance studies carried out in this series of experiments showed that a greater percent of Ca and Mg were retained when casein was the protein source than when soy was fed.

Morris, et al. (1967) reported Cu deficiency in rats fed Cu deficient

diets containing egg albumin or dried skim milk as the source of protein. Animals fed a similar soybean protein diet did not become deficient in copper.

Lengemann et al. (1957), in a series of nutrient utilization studies showed that ^{45}Ca was absorbed one and one-half times as readily from milk diets as from $CaCl_2$ solutions or $CaCl_2$ plus grain by rats. Rabbits, however, did not show this response to the milk diet. Calcium retention was improved in an 11-year-old cow by the addition of dry skim milk to the ration.

Forbes and Yohe (1960) found that the requirement of Zn by the rat depended on the protein source used in the diet. When 7 ppm Zn was provided by soy protein, the rat required 18 ppm in the total diet. When casein provided 7 ppm Zn to the diet the total Zn requirement was only 12 ppm. If egg white provided 2 to 4 ppm of Zn, then the total Zn requirement was still 12 ppm. Apparent absorption values were 44%, 84%, and 51% for Zn from isolated soy, casein and $ZnCo_3$, respectively. Smith et al. (1962) observed Zn deficiency symptoms in pigs receiving soy protein rations (16 to 22 ppm Zn) but not in pigs fed milk protein rations (6 to 18 ppm Zn). Forbes (1964) found that substitution of soy protein for whole egg white protein in the diets of rats increased weight gain of animals fed Zn deficient diets. It decreased concentration of femur ash, Zn, Ca and P absorption and balance, and Mg, Zn and Fe absorption. Fitch et al. (1964) reported that, compared to casein, soybean protein in the diet caused a significant reduction in gastrointestinal absorption of ^{59}Fe.

Abernathy et al. (1965) reported that Fe absorption was reduced when diets containing no animal products were fed to preadolescent

children. Martin (1965), however, did not find any effect on Fe absorption when the amount of plant protein in the diet was increased. She also reported no differences in Zn absorption due to the relative proportions of plant and animal protein in the diet.

Jensen and Mraz (1966b) reported that tibia ash of chicks fed levels of 2.68% Ca and 1.45% P was significantly less than that of chicks fed a diet with casein-gelatin substituted for isolated soybean protein.

Tuba and Dickie (1955) showed that casein and vitellin elevated the levels of intestinal alkaline phosphatase significantly above fasting levels in rats. Neither lactalbumin, egg albumin, zein, gelatin nor wheat gluten resulted in any intestinal enzyme response when fed six hours before sacrifice of the animal.

Chachutowa et al. (1963) reported that pH values taken from caecal fistulae of pigs fed different sources of protein showed no differences due to protein source. Caecal ammonia values were lowest when skim milk was fed followed by soybean meal and fishmeal. Wilbur et al. (1960) reported the following pH values for pigs fed different diets.

	Starch diet	Lactose diet
Stomach	4.1	3.7
Duodenum	5.4	5.1
Ileum	6.3	6.8
Cecum	6.1	5.6
Rectum	7.0	6.8

C. Vitamin D Level

Vitamin D level in the diet can affect rate of growth, serum Ca, P and alkaline phosphatase values, bone deposition and the absorption

and excretion of minerals as well as the levels of enzymes within the body. Researchers working with vitamin D have used one or more of these criteria as measures of vitamin D adequacy in their diets.

Miller et al. (1964a, 1965a, b), in studies aimed at studying the vitamin D requirement of the baby pig and the effects of dietary vitamin D on the utilization of nutrients by the pig, used many of these parameters. They found that 100 IU of vitamin D_2/kg of a diet, which was adequate in Ca and P, would support normal growth, feed efficiency, serum Ca, P, Mg and alkaline phosphatase, and adequate skeletal development with the absence of rachitic pathology. However, when the dietary protein was changed from casein to isolated soy protein it was found that 100 IU vitamin D_2/kg of diet were not adequate to keep these criteria in the normal ranges.

Wahlstrom and Stolte (1958) found that the addition of 90 USP units of vitamin D/lb to a mixed complete ration resulted in no differences in rate of gain, serum Ca and P, nor Ca, P or ash in femurs of pigs. They pointed out that all pigs had prior access to sunlight and that natural diets were fed; therefore, the controls which received no supplementary vitamin D could have stored adequate amounts of vitamin D. Johnson and Palmer (1939) showed that white pigs stored more vitamin D than colored pigs. Pigs fed low levels of vitamin D showed a reduced level of Ca in the serum. Baintner et al. (1963) showed that growth rate affects the vitamin D requirement of chicks. A deficiency of vitamin D_3 caused poor feed conversion and low values for Ca, Mg and P in bone and body tissues in chicks used in these studies. Migicovsky (1957) observed that serum alkaline phosphatase values in vitamin D supplemented chicks were less than one-half that of non-treated chicks. Vitamin D supplementation

(5000 IU) resulted in double the Ca in the bones as compared to the same chicks raised on a rachitogenic diet but not given the vitamin D supplement. Howland and Kramer (1921) observed that in children during the period of active rickets the serum Ca concentration may be normal or slightly reduced. The serum inorganic phosphorus, however, is regularly reduced sometimes to an extreme degree in active rickets. Pincus et al. (1954) found that vitamin D, when administered during the first weeks of life to infants fed a diet containing 3 to 4 times the amount of P found in human milk, tended to lower serum Ca levels. A trend toward lower serum Ca and higher serum P levels was noted in breast-fed infants receiving breast milk only.

The effects of vitamin D on mineral absorption and utilization have been studied both in vivo and in vitro. Nicolaysen (1951), Schachter and Rosen (1959), and Hurwitz et al. (1967) have all shown that vitamin D treatment enhanced the transport of Ca in vitro from the mucosal to serosal surface of intestinal segments. Harrison and Harrison (1961) have shown this to be true with P as well. Nicolaysen reported that the upper part of the small intestine absorbed Ca at a higher rate than did the lower part. Schachter and Rosen concluded that the active transport mechanism is rather specific for Ca^{++} and Mg^{++}. Harrison and Harrison found that the concentrative transport of P requires the presence of calcium. The complete removal of Ca from their everted loop system inhibited transfer of P against a concentration difference and also eliminated the vitamin D effect. Hurwitz et al. found that, in the in vitro preparations, Ca was not transferred across the wall of the duodenum. In situ, however, the most rapid efflux of Ca occurred in the duodenal loop. Vitamin D treatment greatly enhanced

the efflux of calcium. Wasserman et al. (1966) and Taylor and Wasserman (1966) have reported a supernatant factor in homogenates of intestinal mucosa of the rachitic chick after vitamin D administration that has Ca binding activity in vitro. They propose that this factor may be an intracellular Ca carrier, the synthesis of which is promoted by vitamin D.

Armstrong and Varnum (1942) were unable to show any response of vitamin D treatment on P absorption. They concluded, however, that vitamin D may facilitate Ca absorption when a low Ca, high P diet was fed. Wasserman (1962) showed that vitamin D dosing of rachitic chicks did not alter the absorption of Na, K, Cu or Zn. It did, however increase the intestinal absorption of cesium and cobalt. Phosphate and phytate depressed ^{47}Ca absorption in chicks with or without vitamin D supplementation. Arensmeyer and Stroder (1963) showed that feeding irradiated milk to infants increased Ca retention by 33% and P retention by 48%. Nicolaysen (1937) stated that the action of vitamin D in the gut is confined to a direct action on the absorption of calcium. The well known reduced absorption of P in vitamin D deficiency is due to a precipitation by the increased amount of Ca in the bowel. Cohn and Greenberg (1939) found that the addition of vitamin D to the diet of rachitic rats resulted in an increase of only 10% to 15% in the net absorption of phosphorus. Whiting and Bezeau (1958) were not able to show a response of Ca absorption and retention to vitamin D treatment. The apparent absorption of P was decreased by the addition of both Ca or vitamin D to the diets. Zinc absorption was decreased by vitamin D but unaffected by dietary Ca level. Worker and Migicovsky (1961a, b) reported that vitamin D may exert an influence on the metabolism of Zn and cadmium. They showed that there was an increase in bone deposition

of Ca, Be, Mg, Sr and Ba from an oral dose of vitamin D treated chicks. Becker and Hoekstra (1966) concluded that the increased absorption of dietary Zn attributed to vitamin D probably results not from a direct effect of the vitamin, but from a homeostatic response to the increased need for Zn which accompanies enhanced skeletal growth and calcification. Masuhara and Migicovsky (1963) increased Fe and Co absorption in chicks by feeding vitamin D when there were low levels of Ca in the diet but were not able to show any response to vitamin D treatment when Ca levels were high.

Krieger and Steenbock (1940) and Krieger et al. (1940) showed that vitamin D will enhance the utilization of phytic acid phosphorus. The former report indicates that Ca-P ratio, however, was a more prevalent factor in determining the utilization of the organic phosphorus. Spitzer et al. (1948) observed that adding vitamin D to a ration containing nearly optimum inorganic phosphorus had little or no effect on P utilization as indicated by bone ash. However, the addition of vitamin D to a ration containing calcium phytate greatly enhanced P utilization. There were no differences in intestinal phytase activity attributable to vitamin D level, in rats receiving calcium phytate and varying amounts of vitamin D in their diet. Jensen and Mraz (1966a) concluded that isolated soy protein interferes with the absorption of Ca or P or both of these elements in the chick. Their studies, however, showed that the interference was probably not in the absorption or metabolism of vitamin D because chicks previously fed an isolated soy protein diet could absorb radioactive Ca as readily as similar chicks previously fed casein-gelatin diets.

Cheesman et al. (1964, 1966) observed that phosphatase activity

was decreased in the jejunum and kidneys of rats deprived of vitamin D.

D. Nutrient Absorption and Secretion

Many researchers have endeavored to explain the interactions of nutrients and how, where and under what conditions elements are absorbed from or secreted into the gastrointestinal tract. At a symposium on the interaction of mineral elements in nutrition and metabolism (1960) sponsored by the American Institute of Nutrition, some of the leading scientists gave reviews of work in their respective fields. Wasserman concluded from the numerous studies which he cited that there is no apparent simple uncomplicated relationship between the metabolism of Ca and phosphorus. Forbes attempted to bring together the research relating to Zn and Ca interactions. He concluded that interference in Zn function by Ca occurs at the cellular level. The exact mechanisms causing the deficiency lesion(s) could not be described. O'Dell pointed out that when the diet is low in P, excess dietary Mg causes loss of Ca from the body. Also, consumption of excess Ca accentuates the symptoms of Mg deficiency in an animal consuming a deficient diet. Cotzias summarized the research dealing with Mn by pointing out that interactions do exist between Mn and other important mineral nutrients, however, these interactions probably occur outside the body proper. Matrone concluded from his survey of research involving Fe and Cu that these elements interact only at the level of hemoglobin formation. He pointed out that any interaction of these elements is probably at the cellular level. Miller and Engel pointed out that there is evidence that Zn, Mn, and Ca as well as Mo and SO_4 may be concerned with Cu metabolism.

Manners and McCrea (1964) very thoroughly reviewed studies involved

in establishing the mineral requirements of early weaned pigs. They calculated from data on body composition and mineral retention by sow-reared piglets the following dietary mineral requirements of pigs 2 to 18 days old:

	Good utilization[1]	Poor utilization[2]
Ca	1.24%	1.73%
P	0.66%	0.92%
K	0.28%	0.39%
Na	0.14%	0.19%
Mg	375 ppm	525 ppm
Fe	152 ppm	304 ppm
Zn	102 ppm	204 ppm
Cu	16.5 ppm	33.0 ppm
Mn	1.76 ppm	3.51 ppm

[1]Assuming 70% utilization of major minerals and 20% utilization of trace minerals.

[2]Assuming 50% utilization of major minerals and 10% utilization of trace minerals.

These figures are generally higher than requirement figures set forth by the National Research Council (1964).

Moore and Tyler (1955a) critically reviewed methods of measuring Ca and P absorption by means of reference substances. They concluded that Bergeim's method (1926a) of studying the absorption and excretion of Ca and P is seriously limiting because (1) an entirely reliable reference substance is not known and (2) there is no satisfactory method known to distinguish between endogenous and exogenous Ca and P. Moore and Tyler (1955b), using ^{45}Ca and ^{32}P to mark Ca and P absorption or

secretion, concluded that Ca was absorbed most actively from the proximal fourth of the small intestine and P from the proximal half. Both Ca and P were secreted into the lumen of the upper small intestine but were reabsorbed from the lower segments of the small intestine. Neither Ca nor P appeared to be secreted through the wall of the large intestine. Yang and Thomas (1965), using both lignin and chromic oxide as reference substances reported that dry matter and organic matter were secreted into the omasum, abomasum and upper section of the small intestine of calves. Absorption of these materials occurred in the remainder of the tract. Efflux of P, Ca and ash exceeded influx in the rumen and lower GI tract but was variable in omasum and abomasum and influx exceeded efflux in the upper section of the small intestine. Sodium net absorption occurred in the rumen, lower section of the small intestine and rectum, whereas net secretion occurred in the abomasum, upper section of the small intestine, large intestine and cecum. Ali (1967), using chromic oxide as a reference material in Ca studies with the rat, found that absorption occurred in all sections of the tract except the duodenum and stomach. Kvasnitskij (1951), using reentrant preparations on pigs in a very thorough series of studies, was able to determine digestion and absorption in various segments of the GI tract of the pig. He observed that 35% to 54% of the minerals taken in were absorbed in the stomach and small intestine and 15% to 23% of the mineral intake was excreted back into the large intestine.

Kramer and Ingelfinger (1961) compared the characteristics of ileal effluence of ileostomized human subjects with those of normal feces. They concluded that the colon appears to absorb Na and water and excrete potassium. Herndon and Hove (1955) found that surgical removal of the

cecum of rabbits resulted in lowered Na and K utilization. Protein and fat digestibility were somewhat lower while ash and Ca were unchanged. Lloyd et al. (1958) concluded that caecectomy had no deleterious effect on the growth rate of pigs fed either experimental or practical type rations. Differences in the ability of normal and caecectomized animals to digest various ration constituents were of small magnitude. Karel (1948) reviewed the research that had been done in the area of gastric absorption. He concluded that the stomach is definitely not an absorptive organ in the same sense as the intestine and cannot be of especial importance in supplying the nutritional needs of the normal organism. Its absorptive ability, however, has been grossly underestimated, particularly as regards substances physiologically active in minute amounts.

Calcium

Balance trials have been used a great deal in research to evaluate the utilization of various nutrients. De and Basu (1949) showed in human balance trials that the administration of a large dose of Ca, Mg, P, Fe, Cu or Mn with a basal diet increased the elimination of all other minerals to a considerable extent. The increased excretion of minerals was found to occur mainly through the feces. Widdowson et al. (1963) noted that P supplementation of breast-fed babies promoted, rather than hindered, the absorption of Ca and Mg. The urinary excretion of Ca, Mg and Sr was reduced when supplementary P was given. Miller et al. (1962) established the minimal requirement of Ca to be between 0.8 and 1.0% for the baby pig. They showed that, when growth and bone development were optimum, 77% to 82% of the dietary Ca was retained. Hansard et al. (1961) showed that the amount of Ca normally

excreted in the urine is small with a fecal-urinary excretion ratio between 22:1 and 50:1. As the pigs used in these studies became older, less and less of the Ca intake was absorbed. As age increased, the amount of endogenous Ca in the feces increased.

Bergeim (1926b) studied rachitic and normal rats in an effort to determine at what level within the gastrointestinal tract Ca and P absorption was impaired. Iron oxide was used as a reference material to determine absorption or secretion within the tract. Both normal and rachitic animals showed a considerable excretion of P into the intestines. Calcium absorption appeared to take place most rapidly where the excretion of P was most marked. In the lower bowel there was an approximate balance between excretion and absorption of Ca. Phosphorus was reabsorbed at this level. Harrison and Harrison (1951) found that the most rapid rate of Ca absorption occurred within the first two to four hours of its administration. This absorption was from the proximal portion of the small intestine, and the amount of ^{45}Ca absorbed during this interval was not influenced by vitamin D. Absorption of Ca from the distal portion of the intestine was found in rats receiving vitamin D, but not in untreated rachitic rats, except in animals in which the intestinal tract had previously been emptied of Ca by feeding a Ca-free diet. Marcus and Lengemann (1962) showed that Ca absorption was greatly affected by the physical state of the dose. Calcium from a liquid dose was 45% absorbed and only 19% from a solid dose. The total relative percent of ^{85}Sr and ^{45}Ca absorbed in each of the gut components was estimated after absorption had ceased. The values obtained were as follows:

	Solid dose	Liquid dose
Stomach	0%	0%
Duodenum	8%	15%
Jejunum	4%	23%
Ileum	88%	62%

Hurwitz and Bar (1965) reported experimental results which appear to indicate that the major portion of Ca and P absorption occurred in the anterior parts of the intestine. From P/^{91}Y ratios in the duodenum, it was apparent that large quantities of endogenous P were emptied into this segment. The pattern of dry matter/^{91}Y indicated absorption of dry matter along the entire intestine with a reduced rate at the posterios segments. Wallace et al. (1951) used radioactive Ca to study Ca excretion into the GI tract. They conclude that all segments of the tract participate in the excretion of calcium. The small intestine plays a major role in the excretion of ^{45}Ca after an intramuscular injection. After a two-week period, 15% and 41% of the injected dose had been recovered in the feces of young and mature rats, respectively. Cramer (1964), however, after a series of studies using healed gut loops in dogs concluded that under physiological conditions, net Ca transfer is in one direction -- from gut lumen to blood. McCance and Widdowson (1939) had earlier concluded this in work with humans.

Bronner and Harris (1956) suggested that bone salt formation and resorption are rate-limiting processes in a scheme of Ca metabolism that involves absorption, transport, bone salt formation, bone salt resorption and excretion.

Wasserman et al. (1957) showed that singly administered L-lysine

and vitamin D promoted ^{45}Ca absorption in the vitamin D-deficient rat. The combined effect of L-lysine and vitamin D was about the sum of the effects of the individual components. Wasserman and Taylor (1962) showed that the percent absorbed ^{47}Ca deposited in the tibia varied with intraduodenal pH and vitamin D status of the chick. At low pH values (1.9, 2.0) there were no differences in the percent of duodenally absorbed ^{47}Ca accumulated by tibia in rachitic or vitamin D-treated chicks. However, at high pH values, proportionally less of the absorbed ^{47}Ca was deposited in rachitic tibia; pH was without effect on uptake of ^{47}Ca by tibia in the vitamin D-treated birds.

Phosphorus

Ammerman et al. (1963) studied the absorption and retention of various phosphates by the use of radio active phosphorus. From an oral dose of P they found that 27% to 41% was absorbed by swine and 8% to 11% was excreted in the urine. Net retention of P ranged from 20% to 30%. Cramer (1961) found that all parts of the intestinal tract of adult rats were able to absorb ^{32}P. The rate of absorption was greatest in the duodenum, followed by the jejunum, ileum, colon and stomach in decreasing order. However, since ^{32}P passed rapidly through the duodenum and jejunum, less material was available to be absorbed, with the result that absorption was less effective in these segments than it was in the ileum. When the progress and rate of absorption was combined quantitatively, the greatest effective absorption was found to occur in the ileum (which absorbed 38% of the total), followed by the duodenum (29%), jejunum (25%) and colon (8%). McHardy and Parsons (1956) had earlier reported that P was absorbed more rapidly from the jejunum than

from the ileum. They also reported that the net absorption rate of inorganic phosphates increased with decreasing H^+ concentration.

Sodium and Potassium

Meyer et al. (1950) established the Na and K requirements of swine by using a combination of plasma studies and mineral balance trials. Growing pigs retained 80% to 90% of their dietary Na intake when Na levels in the diet did not exceed 0.09%. Potassium retention ranged from 40% to 50% of the dietary intake.

Hamilton (1938) detected radioactive Na, Cl, Br and I in the hand 3 to 6 minutes after ingestion. Potassium was absorbed more slowly, requiring 6 to 15 minutes after ingestion to appear in the hand. The author concluded that these data indicated that these elements were absorbed from the stomach or certainly in the upper small intestine. Reitemeier et al. (1957a) and Code et al. (1955) failed to observe any absorption of radioactive Na from the stomach and concluded that there was a barrier to Na absorption in normal gastric mucosa. Reitemeier et al. found that when the radioactive Na did pass into the small intestine it was readily absorbed. McHardy and Parsons (1957) showed that alkaline conditions in the jejunum and ileum of the rat favored more rapid absorption of water and Na. Reitemeier et al. (1957b) observed that labeled water (D_2O) uniformly passed more rapidly than ^{22}Na from the contents of the small intestine into the blood stream. The mean initial rate of absorption of D_2O was about 20%/minute of that administered and the mean rate of absorption of radio sodium was about 10%/minute. Visscher et al. (1944) showed that Na^+ moves in both directions across the intestinal epithelium. These authors reported that the rate of Na ion movement both out of and into the gut are positively correlated

with Na ion concentration in the small gut. The jejunum and ileum show greater variability in measured rates of Na movement than does the colon. Levitan et al. (1962), using a constant perfusion fluid of 0.85% NaCl found that the entire colon absorbs water and Na and at the same time secretes potassium.

Magnesium

A review by Hathaway (1962) summarizes the research carried out prior to that time involving Mg in human nutrition. Magnesium requirements appear to be related to protein intake; i.e., higher Mg intakes are required for equivalent retentions when protein intakes are high. Its relation to Ca and P intakes and metabolism seems to be more complicated. O'Dell (1959) reviewed research involving the effect of other nutrients on the dietary requirement of Mg by non-ruminants. He reported that high levels of Ca, P, K and protein will all hasten the onset and severity of Mg deficiency. Dempsey et al. (1958) reported that fecal Mg is unrelated to Mg intake but was highly correlated with fecal nitrogen. Bartley et al. (1961) showed that retention of Mg, over a 7 day balance trial, increased linearly with increasing Mg intake. These researchers recommended 40 mg of Mg/100 g of ration as adequate to maintain health of pigs 3 to 6 weeks of age.

Chutkow (1964), using ^{28}Mg found that Mg is absorbed throughout the intestinal tract of rats and that probably more is absorbed in the ileum than in the jejunum. Over 70% of the total absorption of Mg occurs in the colon. The pattern of the intestinal excretion of endogenous Mg appeared to be the reverse of absorption, with most of the loss occurring in the proximal gut. Aikawa (1959) suggested that poor gastrointes-

tinal absorption of Mg accounts for its low renal excretion. He also commented that absorption does not appear to occur from the large intestine. Aikawa carried out his studies with rabbits.

Iron

Gubler (1956) reviewed the absorption and metabolism of iron. He emphasized that: (1) ferrous iron is generally absorbed to a greater extent than ferric iron, (2) there is usually an inverse correlation between the size of dose and the percentage of the dose that is absorbed, (3) in an acid medium (pH below 5), the Fe in foods and in ferric hydroxide is converted to the soluble ionic form, and the formation of insoluble and undissociated complexes is inhibited, (4) since ferric iron readily forms insoluble and undissociable complexes with phosphate ions, the presence of much phosphate in the diet can materially reduce the absorption of Fe, (5) phytic acid, by virtue of its ability to form insoluble Fe complexes, also inhibits Fe absorption.

Barer and Fowler (1937) found that the amount of Fe excreted by men and women in the urine was fairly constant in the same individual regardless of the Fe intake. Schulz and Smith (1958), in balance studies with children, determined that normal children absorbed about 10% of the Fe from milk, eggs, chicken liver and Fe supplements added to commercially prepared infant's cereal. Iron deficient children absorbed 2 to 3 times as much food Fe as normal children. Normal children absorbed more Fe from milk than normal male adults. Bannerman et al. (1962) observed that rats receiving low levels of Fe (2 mg Fe/kg diet) absorbed 83% of a 50 μg ferrous iron dose. Rats supplemented with 240 mg Fe/kg diet absorbed only 8% of the test dose.

Copp and Greenberg (1946) used radioactive Fe to study the absorption and excretion of iron. They concluded that Fe absorption took place in both the small and large intestine. Austoni and Greenberg (1940) had earlier shown that Fe moved through the GI tract of anemic rats more slowly than through normal rats. They suggested that the delay in the passage rate may be a factor in the increased absorption. McCance (1937) and Widdowson and McCance (1937) concluded that the intestine does not excrete iron. Hahn et al. (1939) concurred that the excretion of Fe was negligible. Hahn et al. (1943) showed that Fe absorption takes place largely in the stomach and duodenum. MaCallum (1894) had earlier observed that, when the dose was small, absorption occurred only in the part of the intestine adjacent to the pylorus. Arrowsmith and Minnich (1941), using an inflated balloon and a Miller-Abbott double lumen tube, were able to study Fe absorption at various levels within the human GI tract. Their data indicated that absorption occurred most readily in the stomach and duodenum, to a smaller degree in the jejunum and to an even lesser extent in the ileum. No evidence of absorption was noted when Fe salts were given rectally. Pearson et al. (1967) observed that the bulk of the Fe absorbed from a stock diet was absorbed by the first 30 cm of the intestine, with little absorption in the more distal portion of the gut. Whitehead and Bannerman (1964) concluded that the anemia of totally gastrectomized rats was caused by a quantitative defect in absorption while excretion of Fe continued at a normal or possibly increased rate. Koepke and Stewart (1964) presented evidence for the existence of a substance in gastric juice from anemic dogs which facilitated Fe absorption from the GI tract of normal dogs. Duthie (1964) observed no significant decrease in Fe absorption

in dogs after removal of the duodenum. He was able to show absorptive superiority of the duodenum in rats only with large doses of Fe. Ohkawara et al. (1963), studying Fe absorption from the human large intestine, presented evidence that the human subject can absorb small quantities of soluble ferrous Fe from the large intestine.

VanCampen and Mitchell (1965) used ligated sections of the rat GI tract to study the absorption of Fe and other trace minerals. They reported that ^{65}Zn and ^{59}Fe were taken up most rapidly from the duodenum, somewhat more slowly from the ileum and the mid-section and the least absorption occurred from the stomach. Copper^{64} absorption was greatest from the stomach and declined as the isotope was placed further away from the pylorus.

Zinc

McCance and Widdowson (1942) reported that urinary excretion of Zn by adults did not vary with dietary intake nor was it raised by intravenous injections. They also reported that some adults excreted additional Zn in the feces when injected daily with 6 mg of Zinc. Sheline et al. (1943) obtained similar results with mice in that 50% of ^{65}Zn administered IV was recovered in the feces. Two percent of the administered dose was recovered in the urine of the mouse. Tribble and Scoular (1954) reported that human subjects excreted 8% of their dietary Zn intake in the urine and 42% in the feces. Feaster et al. (1955) reported that only 5% of an oral tracer dose of ^{65}Zn was retained by the adult rat.

Newland, et al. (1958) and Heth and Hoekstra (1963, 1965) have studied the antagonistic effect of Ca on Zn absorption and utilization.

All authors concluded that their results indicate a decreased absorption of Zn with increased dietary calcium.

Sahagian et al. (1966), using intact strips of rat intestine, studied the absorption and interactions of Zn, Mn, Cd and Hg. The regional uptakes of Zn, Cd and Hg were least by the jejunum. Jejunum and ileum preparations took up Zn<Hg<Cd<Mn. Zinc and Mn uptakes were diphasic, with initial rapid uptakes followed by slower continued uptakes for one hour. Manganese had no effect on Zn or Cd uptake.

Birnstingle et al. (1956) observed that zinc is excreted in greater concentration and in greater quantity in pancreatic juice than in bile or duodenal sections. Ligation of the pancreatic ducts materially decreased the ^{65}Zn activity of otherwise intact duodenal aspirate.

Manganese

Vorab'eva (1965) confirmed the earlier observations of Everson and Daniels (1934) that (1) Mn retention varies inversely with age of children, (2) total urinary excretion is virtually constant irrespective of age, and (3) fecal Mn excretion varies directly with age, and therefore with total dietary intake. North et al. (1960) in a study of Mn metabolism in women found that over 8 periods of 5 days each 9 college women retained 41% of their dietary Mn intake. Fecal Mn accounted for 91% of the total Mn excretion. Lang et al. (1965) did not show any effect on Mn retention when skim milk was added to the diet of young men fed all vegetable diets. Greenberg and Campbell (1940) and Greenberg et al. (1943) had earlier reported that very little of the Mn absorbed by rats was excreted in the urine.

Liebholz et al. (1962) showed that Mn intake was reflected by the

Mn content of bone, liver and hair of the baby pig. Tal and Guggenheim (1965) reared mice on a basal diet of meat which is poor in both Mn and Ca. They found that the addition of small amounts (2.5 to 5.0 mg/kg of meat) of Mn improved weight gain and calcification of bone and decreased incorporation of injected radio Ca into bone.

Copper

Buescher et al. (1961) showed that 60% of an oral dose of ^{64}Cu was recovered in the feces of swine. Only 1% to 3% was found to be excreted in the urine. Mahoney et al. (1955) had earlier found similar results in dogs. They concluded from their work that the major pathway of excretion of Cu is through the biliary system. Bowland et al. (1961) concluded that Cu transfer across the gut wall apparently occurred mainly in the small intestine and the colon. They, too, found the feces to be the major route of Cu excretion with the bile accounting for up to 40% of the total excretion. VanRavesteyn (1944) stated that, in his studies with man, the excretion of Cu via the bile and the feces does not appear to run parallel. He drew the conclusion that the Cu found in the stools, at least in part, is excreted through the intestinal wall.

VanCampen (1966) and VanCampen and Scaife (1967) observed that Zn affected Cu uptake from the stomach and from the duodenum in the same manner and to about the same extent. In both cases high levels of Zn depressed ^{64}Cu uptake, but did not produce any change in the tissue distribution pattern. Their data indicated that the depression of Cu absorption by high levels of Zn is mediated either in or on the intestine.

Cobalt

Kent and McCance (1941) suggested that the gut is the main channel

of excretion of Co in foods, probably because relatively little is absorbed. Once the Co had reached the tissues, however, the process of elimination was mainly via urine. Greenberg et al. (1943) concluded that the urine is the chief pathway of excretion of absorbed Co. They pointed out, however, that orally administered Co is only partially absorbed and thus, a large proportion is eliminated in the feces. Harp and Scoular (1952) reported that young women absorb 73% to 97% of their dietary Co (5 to 8 μg/day). An average of 67% of the total daily intake of Co appeared in the urine in these studies. Paley and Sussman (1963) found that the absorption of Co was generally diminished when the Co was administered after a meal pretagged to protein or administered carrier free, wherein binding to residual protein in the digestive tract may be presumed to have occurred. They concluded from this that the absorption of Co takes place in the GI tract above the region of protein digestion. In contrast to the previously cited paper, only 16% of the cobalt load was recovered in the urine in these studies. Henderickx (1964) found evidence that ^{57}Co was absorbed from the large intestine of swine.

III. EXPERIMENTAL PROCEDURE

A. Introduction

Four experiments were carried out to study the effects of level and source of protein and level of vitamin D on mineral utilization by the baby pig. These experiments were:

Experiment I. Effect of protein level on mineral utilization by the baby pig.

Experiment II. Effect of level of protein and vitamin D on mineral utilization by the baby pig.

Experiment III. Effect of level of protein and vitamin D on mineral utilization by the baby pig.

Experiment IV. Effect of source and level of protein on mineral utilization by the baby pig.

Experiment I was carried out in an effort to determine if higher levels of protein in the diet were more rachitogenic than lower levels. The subsequent two experiments were conducted to determine if additional vitamin D would overcome the effects seen in Experiment I. Experiment IV was conducted to determine if those differences observed in the previous experiments were due to source of protein as well as to the level of protein in the diet.

B. General Conduct of Experiments

Baby pigs were taken from their dams at 3 days of age and bottle-fed homogenized non-fortified cows milk 4 times daily during the first few days. A purified diet in the form of a dry meal (table 1) was also placed in small feeders and intake was encouraged by placing small amounts in the animal's mouth after liquid consumption. The pigs were

Table 1. Composition of purified diets.

Protein level	16% crude protein		24% crude protein		32% crude protein	
Protein source	soy protein	casein	soy protein	casein	soy protein	casein
	%	%	%	%	%	%
Soy[1]	19.7		29.7		39.7	
Casein[2]		20		30		40
DL-Methionine	0.3		0.3		0.3	
Lard	5	5	5	5	5	5
α- Cellulose[3]	5	5	5	5	5	5
Glucose[4]	63	63	53	53	43	43
Mineral mixture[5]	6	6	6	6	6	6
Corn oil[6]	1	1	1	1	1	1
Vitamin mixture[7]	+	+	+	+	+	+

[1] ADM C-1 Assay Protein, Archer-Daniels-Midland Company, Minneapolis.
[2] Vitamin-free casein, Nutritional Biochemicals Corporation, Cleveland.
[3] Solka Floc, Brown Company, Chicago.
[4] Cerelose, Corn Products Company, Argo, Illinois.
[5] See Appendix 18.
[6] Mazola, Corn Products Company, Argo, Illinois.
[7] See Appendix 19.

consuming the dry diet very well by one week of age. Liquid milk feeding was discontinued as soon as the dry diet was being consumed voluntarily. At one week of age pigs were randomly allotted to treatment after equalizing for sex, litter and weight. Pigs were housed in wire bottomed metal cages in a room in which all windows were painted to prevent the entrance of ultraviolet rays. Room temperature was held constant at 20° C and infrared heat lamps were utilized to maintain a cage temperature of 30° C during the first 2 weeks of the trial. Pigs were ad-libitum fed and had free access to fresh water during the experimental period. All feed was weighed into the feeders and pigs were individually weighed weekly.

Blood was withdrawn from the anterior vena cava on 3 occasions (initial, 3 weeks, and final) during each experiment for determination of serum constituents.

Mineral balance studies were conducted following the fourth or fifth week of each experiment. Pigs were individually fed 3 times daily an amount of food and water which they would consume within a 5 to 10 minute period. After a 3 day adjustment period, they were placed in individual metabolism cages for urine and fecal collection. Pigs were removed 3 times daily for feeding after which their mouths were wiped clean and then returned to their cages. Constant daily feed intakes were maintained throughout the 3 day collection period. Feces were collected separately from urine by means of a fine screen placed above the collection tray.

Total fecal collections were dried in a low temperature oven, weighed, finely ground and stored in air tight plastic containers. Total urine collections were acidified with 6 N HCl to a pH of between 1 and 2,

the volume accurately measured and 100 ml samples stored in polyethylene bottles.

At the conclusion of each trial pigs were killed and various bones, organs and glands were removed and weighed.

1. Experiment I. Effect of level of protein on mineral utilization by baby pigs.

Twelve baby pigs were adapted to the dry diet (see table 1) and at one week of age were allotted to diets consisting of 20%, 30% or 40% of isolated soybean protein. These diets analyzed by the Kjeldahl method 16%, 24% and 32% crude protein, respectively. All diets in the experiment were supplemented with only 6.25 μg of ergocalciferol/kg of diet in an attempt to show the rachitogenicity of high levels of soy protein, if they were, indeed, rachitogenic.

Two pigs per treatment were killed to obtain bone and organ weight data. The remaining 6 pigs were returned to the University herd.

2. Experiment II. Effect of level of protein and vitamin D on mineral utilization by baby pigs.

Eight baby pigs were adapted to the dry diet and at one week of age were allotted to treatments of 30% soy protein with 6.25 or 12.50 μg of ergocalciferol/kg of diet or 40% soy protein with 6.25 or 12.50 μg of ergocalciferol/kg of diet. Kjeldahl analyses showed these diets to contain 24% or 32% crude protein.

3. Experiment III. Effect of level of protein and vitamin D on mineral utilization by baby pigs.

Sixteen baby pigs were adapted to the dry diet and at one

week of age were allotted to dietary treatments of 20% soy protein with 6.25 or 12.50 μg/kg of ergocalciferol or 40% soy protein with 6.25 or 12.50 μg/kg of ergocalciferol. Analyzed crude protein in these diets was 16% and 32%.

In this experiment 3 pigs per treatment were killed for bone and organ weight data. The remaining pig per treatment was returned to the University herd.

4. Experiment IV. Effect of source and level of protein.

Sixteen baby pigs were adapted to the dry diet and at one week of age were allotted to dietary treatments of 20% soy or casein protein or 40% soy or casein protein. All treatments in this experiment, as in Experiment I, received 6.25 μg ergocalciferol/kg of diet. Kjeldahl N x 6.25 showed these diets to contain 16% and 32% crude protein.

In this experiment pigs were fed 0.5% chromic oxide in the diet for a period of 4 days prior to beginning the balance study and were maintained on this feeding regime until they were killed following the collection period. Pigs were then killed one and one-half hours or three hours post feeding. The alimentary tract was quickly exposed, the sections tied to prevent movement of chyme and the tract removed and separated into stomach, cranial small intestine, caudal small intestine, cecum and colon. Digesta was cleaned semi-quantitatively from each section, weighed, pH determined on a small portion and the remainder thoroughly mixed in a Waring blender. If the digesta was too thick for proper mixing, it was appropriately diluted with deionized distilled

water. A 20 g portion of this material was then placed in a plastic bag and frozen for later analysis.

The indicator method for determining digestibility was adopted to measure absorption and/or secretion of nutrients along the GI tract of pigs. The following equation, % digestibility = $100 - \left[\left(\frac{\%\text{ indicator in feed}}{\%\text{ indicator in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in food}}\right)100\right]$, as given on page 303 of the textbook by Maynard and Loosli (1962) was used. In order to measure the degree of absorption and secretion in the different sections of the GI tract as the digesta moved posteriorly, two consecutive sections of the tract were used in relation to the above equation. The digesta in a given organ was considered to be the feed for the next posterior organ. The digesta in the second organ would be equivalent to the feces for the calculation using the ratio technique equation. Using 2 consecutive organs in relation to the above equation the degree of digestion in the following alimentary sections was calculated.

a. Stomach -- using feed as fed and stomach digesta as feces;
b. Cranial small intestine (cranial s.i.) -- using stomach digesta as the feed and cranial s.i. digesta as feces;
c. Caudal small intestine (caudal s.i.) -- using cranial s.i. digesta as the feed and caudal s.i. digesta as feces;
d. Cecum -- using caudal s.i. digesta as the feed and cecal digesta as the feces;
e. Colon -- using cecal digesta as the feed and digesta in the colon as the feces;
f. Rectum -- using the digesta in the colon as the feed and the feces voided as the feces.

With this method, a positive value indicates net absorption from

the GI tract and a negative value would indicate net secretion into that section of the tract.

C. Chemical Analyses

1. Serum. After the blood was drawn from the animals, it was put into acid washed test tubes. After the blood had coagulated, the clot was removed and the serum spun in an international centrifuge at 1500 RPM for 15 minutes. The cell free serum was then poured into acid washed vials. Serum alkaline phosphatase and serum protein were determined within 24 hours of serum collection. The remaining serum was frozen at -10° C for future analysis.

a. Calcium

In Experiments I and II serum calcium was determined by the complexiметric method of Mori (1959). A Jarrell-Ash atomic absorption spectrophotometer with a hydrogen-air flame was used for Ca determinations in Experiments III and IV. The Ca absorption line at 4227 A° was used to determine concentration of Ca in standards and unknowns. 10,000 ppm Sr was added to all samples to suppress any phosphate interferences (Appendix 1).

b. Phosphorus

All serum P analyses were determined by the method of Gomori (1942) and read on a Bausch and Lomb spectronic 20.

c. Magnesium

Serum Mg in Experiments I and II was determined by the colorimetric method of Orange and Rhein (1951). In

Experiments III and IV the Jarrell-Ash atomic absorption spectrophotometer was used to make these determinations. A wave length of 2852 A^{o} was used to determine absorption, and 10,000 ppm of Sr was added to suppress phosphate interference (Appendix 1).

d. Alkaline phosphatase

All serum alkaline phosphatase values were determined by the Sigma method (Sigma, 1963).

e. Total protein

Serum total protein values were determined by the method of Waddell (1956) and read on a Beckman DU spectrophotometer.

2. Feed, feces and digesta. In Experiments I and II, one g of oven dried fecal material was weighed into a 50 g acid washed crucible and ashed in a muffle furnace by slowly raising the temperature to 550° C over a 6 hour period and allowing to remain at 550° C for an additional 6 hours. This resulted in a clean ash which was then taken up to 5 ml 6 N HCl and diluted to 100 ml with deionized distilled water in an air tight polyethylene bottle which had been acid washed.

A wet ashing procedure was used in Experiments III and IV. A 0.5 g sample was placed in a 250 ml extraction flask and 20 ml of concentrated HNO_3 were added. After allowing to stand for 15 minutes, the sample was heated to near dryness on a hot plate. The nearly dry sample was then cooled, 7 ml of perchloric acid were added and digestion continued over the hot plate. A watch

glass over the flask acted to reflux the moisture and thus minimize losses. As the sample became clear the flask was removed from the heat, cooled and diluted to 100 ml with deionized distilled water and stored in an air tight acid washed polyethylene bottle.

Standards and blanks were prepared in the same manner as the unknowns.

a. Calcium

In Experiments I and II feed and fecal Ca was determined by the method of Mori (1959). A micro spatula tip full of ascorbic acid added to the samples to be titrated resulted in a sharper end point. Calcium in the feed and feces samples prepared in Experiments III and IV was determined as described for serum Ca using atomic absorption (Appendix 1).

b. Phosphorus

All P determinations were made by the colorimetric method of Gomori (1942).

c. Magnesium

Feed and fecal Mg in Experiments I and II was determined on a Perkin Elmer 303 atomic absorption unit. A Jarrell-Ash atomic absorption spectrophotometer was used for these determinations in Experiments III and IV.

d. Sodium, Potassium and Chromium

In Experiment IV, Na, K and Cr were determined by flame emission spectrophotometry. Samples were wet ashed

as described and were diluted and read as shown in Appendix 1.

e. Iron, Zinc, Manganese, Copper and Cobalt

These minerals were determined only in Experiment IV and were analyzed from a single wet ashing of material (Appendix 1). Manganese gave a sharper response when 10,000 ppm Sr was added; therefore, this was used to suppress interferences when Mn was determined.

f. Nitrogen

All N determinations were carried out on oven dried samples using the semi-micro Kjeldahl technique.

g. Energy

Energy was determined on feed and feces in Experiment IV by the use of a Parr adiabatic oxygen bomb calorimeter.

h. Phytin Phosphorus

In Experiment IV a modification of the method of Earley (1944) was used to determine the amount of dietary P that is bound as phytin phosphorus. One g of feed or feces was extracted with 20 ml of 0.15 N HCl containing 10% Na_2SO_4, for 2 hours on the shaking machine. The acid extract was centrifuged and then filtered through a filter paper (Watman #5) using suction. Fifteen ml of ferric chloride solution, which was prepared in 0.07 N HCl and contained about 0.2% Fe, was then slowly added to the supernatant. The container was rotated gently until the ferric phytate formed. The ferric phytate was allowed to

stand over night before it was centrifuged and the supernatant poured off. The precipitated ferris phytate was washed with cold distilled water, recentrifuged and then taken up in 20 ml of 15.8 N HNO_3. The washed precipitate was then subjected to the perchloric acid digestion described above. Inorganic P was then determined as described previously.

3. Urine. With the exception of Ca, all determinations were carried out as described for the elements in feed or feces. Urinary Ca was determined on 2 ml of urine after the Ca had been precipitated at a pH of 4.0 over night with 1 ml of saturated sodium oxalate. The precipitate was spun down, the supernatant poured off, and the Ca taken up with 2 ml diluted nitric acid. The Ca determinations were then carried out as described previously.

Urinary energy was calculated using 5.41 KCal/g N (Kleiber, 1961). Urinary N was determined by the Kjehldahl method.

4. Bone analysis. Femurs and the 8th rib from the right side were taken from each pig. The bones were scraped free of all flesh and the periosteum was trimmed from the bone. The cleaned bones were placed in an air tight plastic bag and stored at 5° C until analyzed.

a. Specific gravity

Both femurs and the rib from each pig were weighed in air and water and the specific gravity of the bones calculated.

b. Strength tests

Breaking tests were made on each femur using a Tinius-Olsen universal testing device. Strength characteristics were calculated as described by Miller et al. (1962).

c. Bone ash

After the femur was broken in the strength tests, it was sawed into small pieces, the water and fat were extracted in a Soxhlet extractor and the dried bone ground through a 40 mesh screen. A 2 g sample of the finely ground sample was then ashed in a muffle furnace. Ash was calculated and Ca, P, and Mg were determined by methods described for feed and feces.

D. Statistical Analyses

Statistical analyses were carried out by analysis of variance and treatment differences were determined by application of the multiple range test of Duncan (1955). In Experiment IV, in order to analyze statistically for the effects of time after feeding, as well as the effects of litter, sex, protein level and protein source, the digestibility data were subjected to least squares analysis. Duncan's multiple range test was then applied to determine mean differences.

IV. RESULTS AND DISCUSSION

A. Experiment I. Effect of protein level on mineral utilization by the baby pig.

The growth and serum data from Experiment I are presented in table 2. Pigs receiving the higher levels of dietary protein (24% and 32%) tended to gain as well or better than pigs receiving the lower level of protein (16%). Feed intake, however, was lower at the high protein concentration which resulted in improved feed utilization at the higher levels of protein intake. Serum inorganic P values were higher in pigs receiving 16% crude protein than in pigs receiving higher levels. The higher levels of serum alkaline phosphatase values shown in pigs on high protein diets, along with the lowered serum inorganic P could be indicative of the onset of rickets.

Data from the balance trial (table 3) show that maximal mineral retention occurred in pigs receiving 24% protein diets. Feeding high levels of protein resulted in decreased retention of nitrogen by the pig. Apparent digestibility of nitrogenous matter, however, was not affected by level of dietary intake.

Relatively constant amounts of feed intake between lots and the wide differences in mineral intake were due to analyzed values being used rather than calculated values. Mineral composition of all diets was calculated to be equal, however, table 3 shows that this was not accomplished. Throughout the remainder of this report all values reported are as analyzed, unless stated otherwise.

Table 4 shows that density, mineral content and strength of bone were depressed by increasing levels of dietary protein. Lowered serum

Table 2. Growth and serum analyses of baby pigs fed different levels of protein.

Dietary protein, %	16	24	32	
No. of pigs	4	4	4	±SE[1]
Initial wt, kg	2.84	2.85	2.86	0.14
Daily gain, kg	0.19	0.25	0.19	0.02
Daily food intake, kg	0.39	0.40	0.34	
Gain/food	0.49	0.62	0.56	
Serum Ca, mg/100 ml				
Initial	10.9	11.3	11.3	0.3
3 weeks	10.2	11.5	10.4	0.3
5 weeks	10.6	10.5	11.4	0.3
Serum P, mg/100 ml				
Initial	7.3	7.8	7.1	0.4
3 weeks	8.5[aa,b]	5.5	4.5	0.6
5 weeks	6.0[aa,b]	4.8	3.9	0.3
Serum Mg, mg/100 ml				
Initial	2.6	2.2	1.8	0.1
5 weeks	2.7	2.5	2.3	0.1
Serum alkaline phosphatase, Sigma units				
Initial	13.9	12.1	15.7	1.1
3 weeks	7.9	8.9	10.3	1.1
5 weeks	8.0	7.2	14.1[aa,b]	1.1
Serum protein, g/100 ml				
Initial	5.3	5.1	5.1	0.2
5 weeks	4.6	4.9	4.7	0.1

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$).

Table 3. Daily calcium, phosphorus, magnesium and nitrogen excretion and retention as affected by level of dietary protein.

Dietary protein, %	16	24	32	
No. of collections	3	3	3	±SE[1]
Daily food intake, g	372	375	372	2
Daily water intake, ml	840	897	824	5
Daily feces, g	27.8	27.2	31.2	1.3
Daily urine, ml	384	429	438	23
Ca balance, daily				
Ca intake, g	2.91	3.01	3.40	0.06
Fecal Ca, g	1.15	1.07	1.24	0.06
Urinary Ca, mg	18	33	24	3
Ca retention, g	1.75	1.91	2.14	0.07
Ca retention, %	60	63	63	2
Ca apparent digest., %	61	65	64	2
P balance, daily				
P intake, g	2.31	2.46	2.51	0.03
Fecal P, g	0.99	0.82	0.94	0.05
Urinary P, g	0.10	0.10	0.01	0.02
P retention, g	1.22	1.54	1.56	0.07
P retention, %	53	63	62	3
P apparent digest., %	57	67	62	3
Mg balance, daily				
Mg intake, mg	189	210	232	4
Fecal Mg, mg	100	93	125	6
Urinary Mg, mg	18	11	5	3
Mg retention, mg	71	106	102	8
Mg retention, %	38	51	44	4
Mg apparent digest., %	47	56	46	4
N balance, daily				
N intake, g	9.07	14.17[aa]	18.51[bb]	1.18
Fecal N, g	0.49	0.48	0.79[bb]	0.04
Urinary N, g	2.21	5.54[aa]	8.84[bb]	0.83
N retention, g	6.37	8.15[aa]	8.88[aa]	0.34
N retention, %	70	58	48	4
N apparent digest., %	95	97	96	0

[1] Standard error of the mean.
[aa] Significantly greater than least value ($P < 0.01$).
[bb] Significantly greater than least two values ($P < 0.01$).

Table 4. Weight, density, composition and strength of bones from baby pigs fed different levels of protein.

Dietary protein, %	16	24	32	
No. of observations	2	2	2	±SE[1]
Femur wt, g	59.4	58.8	50.4	1.4
Femur density	1.18[a]	1.15	1.14	0.01
8th rib wt, g	6.0	6.3	6.9	0.2
8th rib density	1.28[aa]	1.20	1.14	0.01
Humeral analyses, dry, fat-free basis, %				
Ash	46.1	45.1	42.5	2.0
Ca	16.6	16.2	15.4	0.8
P	8.6	8.4	7.8	0.4
Mg	0.35[a]	0.34[a]	0.24	0.02
Femur strength				
Breaking load, kg	62[a]	51	41	4
Bending moment, kg-cm	118[a]	91	68	10
Moment of inertia, cm^4	0.08	0.08	0.09	0.00
Breaking stress, kg/cm^2	880[bb]	681[aa]	470	77
Young's modulus of elasticity, 1000 kg/cm^2	10.6[aa,b]	7.0[a]	3.1	1.4

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$.

P and increased serum alkaline phosphatase values indicated that this may be expected. Carlson et al. (1964a, b) had observed this same effect of high levels of isolated soy protein on bone development in turkey poults.

Figure 1 shows load-deflection curves of these bones. Femurs from pigs receiving low levels of protein had higher breaking loads and bent less before breaking than femurs from pigs fed high levels of isolated soy protein in the diet.

Organ weights shown in table 5 may indicate some of the physiological effects of a high protein diet. Although not statistically significant,

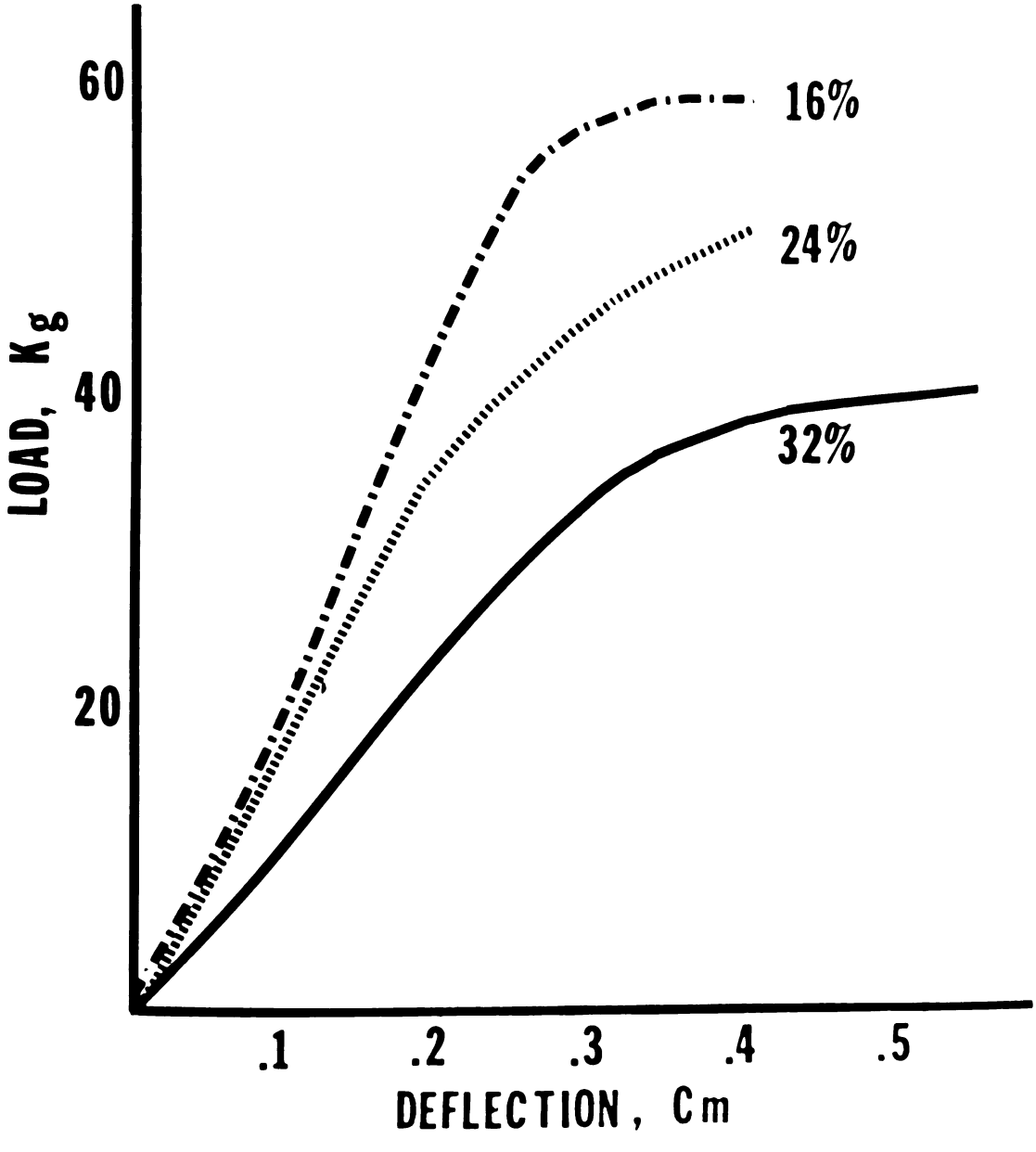

Figure 1. Exp. 1.-- Load-deflection curves for femurs from pigs fed different levels of soy protein (16%, 24%, or 32%) with 6.25 μg ergocalciferol/kg diet.

Table 5. Organ weights of baby pigs fed different levels of protein.

Dietary protein, %	16	24	32	
No. of observations	2	2	2	±SE[1]
Thyroid wt, g	1.25	0.92	0.89	0.11
Thyroid, % BW[2]	0.009	0.007	0.009	0.001
Adrenal wt, g[3]	1.37	1.15	1.25	0.05
Adrenal, % BW	0.011	0.009	0.012	0.001
Kidney wt, g[3]	62	70	62	3
Kidney, % BW	0.48	0.58	0.57	0.05
Spleen wt, g	25	16	16	3
Spleen, % BW	0.19	0.13	0.16	0.03
Heart wt, g	56	59	52	2
Heart, % BW	0.43	0.48	0.51	0.02
Liver wt, g	319	334	307	6
Liver, % BW	2.44	2.76	3.00	0.13

[1] Standard error of the mean.
[2] Expressed as a percent of live body weight.
[3] Combined weight of both organs.

due to the small number of observations made, there is a tendency toward increased kidney and liver size with increasing levels of protein. These observations are not surprising when we consider the deamination and excretory function of these organs. Note in table 3 the increase in volume of urine with increasing dietary protein level. Nitrogen loss by urinary excretion is significantly greater on high protein intake.

B. Experiment II. Effect of level of protein and vitamin D on mineral utilization by the baby pig.

Only 2 animals per treatment were used in Experiment II; therefore, it was very difficult to show statistically significant differences. Trends, however, can be noted even in small numbers of observa-

tions per treatment. From the performance and serum data shown in table 6, it would appear that there is little difference in the response of these criteria to 24% or 32% protein or to 6.25 or 12.50 μg ergocalciferol per kg of diet.

Table 7 shows balance trial data from this experiment. The data would suggest that neither dietary protein level nor level of vitamin D activity in the diet affected the absorption or retention of Ca, P or Mg.

Mineral deposition in the bone and bone development occur during the entire experimental period. For this reason data derived from bones would more truly reflect the mineral status of an animal as it is growing. Table 8 shows that, although not statistically significant, there is a trend for increased mineral content in bones from pigs fed lower levels of protein. Femur strength, however, was affected more by ergocalciferol intake than by protein intake. Figure 2 shows that bones from pigs fed high levels of ergocalciferol break easier and bend less than bones from pigs fed lower levels of vitamin D.

This data is in apparent contradiction to the effect of dietary vitamin D intake on bone deflection curves reported by Miller et al. (1964b). A close look at table 8, however, shows that femurs from pigs receiving 12.50 μg ergocalciferol/kg diet weighed less than femurs from pigs receiving 6.25 μg ergocalciferol/kg diet. Under these conditions, breaking stress is perhaps a better measure of compact layer development because it is a measure of femur strength per unit of cross-sectional area. Miller et al. (1964a) had observed similar results when studying the vitamin D_2 requirement of the baby pig.

Table 6. Growth and serum analyses of baby pigs fed two different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	24	32	24	32	
No. of pigs	2	2	2	2	±SE[1]
Initial wt, kg	3.11	3.49	2.92	3.07	0.14
Daily gain, kg	0.30	0.30	0.26	0.29	0.01
Daily food intake, kg	0.56	0.52	0.49	0.53	
Gain/food	0.53	0.58	0.53	0.55	
Serum Ca, mg/100 ml					
Initial	10.0	9.8	10.2	10.8	0.2
3 weeks	11.4	9.0	9.8	9.4	0.5
5 weeks	11.6	12.8[a]	11.2	13.2[b]	0.3
Serum P, mg/100 ml					
Initial	5.7	6.5	6.0	4.6	0.4
3 weeks	10.1	9.2	9.5	9.2	0.2
5 weeks	9.4	7.8	8.3	9.9	0.7
Serum Mg, mg/100 ml					
Initial	2.0	2.5	2.5	2.5	0.1
3 weeks	2.9	3.3	2.9	3.1	0.1
5 weeks	2.0	2.3	2.0	2.4	0.2
Serum alkaline phosphatase, Sigma units					
Initial	10.6	11.9	7.5	7.9	0.8
3 weeks	9.6	7.8	5.6	5.9	0.8
5 weeks	5.0	5.9	4.6	5.2	0.3
Serum protein, g/100 ml					
Initial	5.0	4.8	4.8	4.6	0.1
5 weeks	5.1	5.3	5.2	5.5	0.1

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$).
[b] Significantly greater than least two values ($P < 0.05$).

Table 7. Daily calcium, phosphorus, magnesium and nitrogen excretion and retention as affected by level of dietary protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	24	32	24	32	
No. of collections	2	2	2	2	±SE[1]
Daily food intake, g	450	450	450	450	0
Daily water intake, ml	900	900	900	900	0
Daily feces, g	41.7	46.3	36.7	42.7	2.2
Daily urine, ml	390	422	387	442	19
Ca balance, daily					
Ca intake, g	3.78	4.68	4.09	4.13	0.12
Fecal Ca, g	1.61	1.84	1.59	1.79	0.14
Urinary Ca, mg	45	79	22	41	9
Ca retention, g	2.13	2.77	2.48	2.30	0.16
Ca retention, %	56	59	61	56	3
Ca apparent digest., %	58	61	61	57	3
P balance, daily					
P intake, g	3.01	3.01	3.11	3.10	0.02
Fecal P, g	0.58	0.69	0.56	0.60	0.06
Urinary P, g	0.12	0.10	0.11	0.04	0.02
P retention, g	2.31	2.22	2.44	2.46	0.06
P retention, %	77	74	78	79	2
P apparent digest., %	81	77	82	81	2
Mg balance, daily					
Mg intake, mg	259	294	263	278	4
Fecal Mg, mg	157	179	139	165	14
Urinary Mg, mg	15	22	16	16	2
Mg retention, mg	87	93	108	97	13
Mg retention, %	34	32	41	35	5
Mg apparent digest., %	39	39	47	41	5
N balance, daily					
N intake, g	17.36	22.59	17.90	23.95	0.97
Fecal N, g	1.11	1.24	0.99	1.20	0.09
Urinary N, g	5.41	7.92	6.00	9.26	0.76
N retention, g	10.84	13.43	10.91	12.49	0.59
N retention, %	62	60	61	52	2
N apparent digest., %	94	95	95	95	0

[1] Standard error of the mean.

Table 8. Weight, density, composition and strength of bones from baby pigs fed different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	24	32	24	32	
No. of observations	2	2	2	2	±SE[1]
Femur wt, g	64.7	67.4	60.2	60.1	3.8
Femur density	1.17	1.17	1.17	1.15	0.01
8th rib wt, g	6.2	5.9	5.2	5.6	0.3
8th rib density	1.23	1.24	1.36[c]	1.22	0.02
Humeral analyses, dry, fat-free basis, %					
Ash	46.9	45.8	45.9	44.4	0.5
Ca	16.4	15.9	16.1	15.5	0.2
P	8.7	8.5	8.5	8.2	0.1
Mg	0.35	0.34	0.35	0.33	0.10
Femur strength					
Breaking load, kg	74	64	49	43	6
Bending moment, kg-cm	147	126	94	84	13
Moment of inertia, cm^4	0.16	0.15	0.09	0.11	0.00
Breaking stress, kg/cm^2	671	598	628	532	47
Young's modulus of elasticity, 1000 kg/cm^2	7.1	7.5	6.0	6.0	0.6

[1] Standard error of the mean.
[c] Significantly greater than all other values ($P < 0.05$).

Table 9 shows organ weight data from Experiment II. A trend toward increased weight and relative size of kidneys and liver in response to high levels of dietary protein can be observed.

C. Experiment III. Effect of level of protein and vitamin D on mineral utilization by the baby pig.

Growth and serum data from Experiment III are presented in table 10. Although serum inorganic P and alkaline phosphatase levels were higher in pigs receiving lower levels of vitamin D and protein, results were varied and no specific conclusions can be drawn. It is interesting

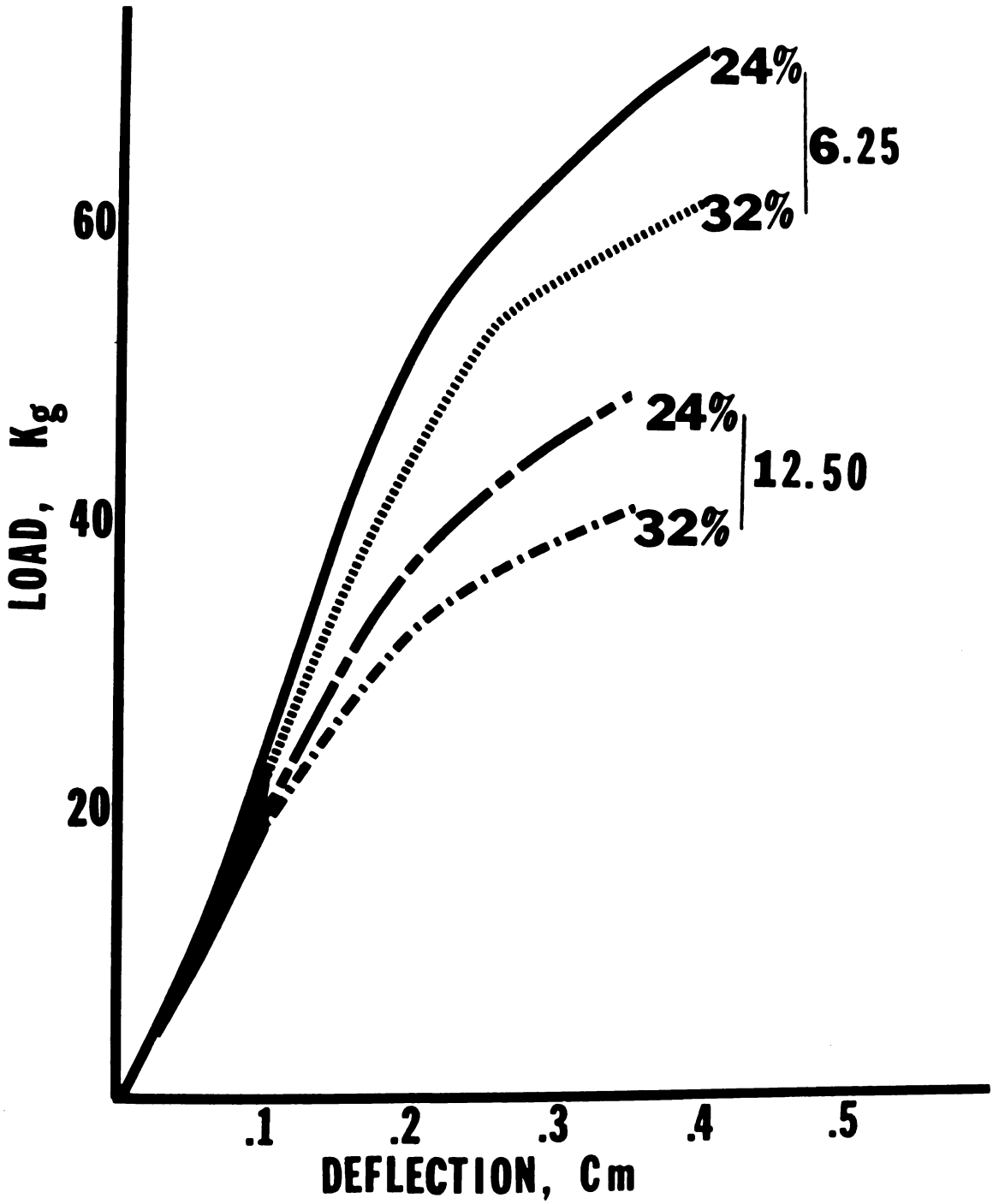

Figure 2. Exp. II. -- Load-deflection curves for femurs from pigs fed two different levels of soy protein (24% or 32%) and ergocalciferol (6.25 or 12.50 μg/kg diet).

Table 9. Organ weights of baby pigs fed different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	24	32	24	32	
No. of observations	2	2	2	2	±SE[1]
Thyroid wt, g	1.39	1.40	1.49	1.37	0.13
Thyroid, % BW[2]	0.010	0.010	0.011	0.009	0.001
Adrenal wt, g[3]	1.28	1.19	1.03	1.19	0.05
Adrenal, % BW	0.009	0.008	0.007	0.008	0.001
Kidney wt, g[3]	78	91	79	93	5
Kidney, % BW	0.55	0.62	0.57	0.64	0.02
Spleen wt, g	22	24	20	21	1
Spleen, % BW	0.15	0.17	0.14	0.14	0.02
Heart wt, g	58	58	51	55	2
Heart, % BW	0.41	0.40	0.37	0.38	0.03
Liver wt, g	402	453	389	450	22
Liver, % BW	2.85	3.06	2.80	3.08	0.18

[1] Standard error of the mean.
[2] Expressed as a percent of live body weight.
[3] Combined weight of both organs.

to observe that in this experiment dietary protein levels were reflected in the serum protein analysis.

Data in table 11 shows that mineral balance was not affected by dietary treatment in this experiment. Magnesium retention and digestibility values appear to be affected by dietary protein level but one can observe that this was a reflection of dietary intake rather than a treatment effect. Daily urine excretion from pigs receiving 32% protein diets was about double that excreted by pigs receiving 16% protein diets. Water intake, however, was the same for both treatments. Pigs receiving high levels of protein excreted about 4 times as much N in the urine as

Table 10. Growth and serum analyses of baby pigs fed different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	16	32	16	32	
No. of pigs	4	4	4	4	±SE[1]
Initial wt, kg	2.64	2.64	2.66	2.64	0.04
Daily gain, kg	0.30	0.30	0.29	0.30	0.01
Daily food intake, kg	0.58	0.53	0.62	0.54	
Gain/food	0.51	0.56	0.48	0.55	
Serum Ca, mg/100 ml					
Initial	9.1	9.0	9.2	8.9	0.1
2 weeks	9.6	9.8	9.2	10.1	0.2
4 weeks	10.7	10.2	10.1	10.0	0.1
Serum P, mg/100 ml					
Initial	7.4	7.3	7.9	7.6	0.2
2 weeks	8.1	6.6	8.8	6.9	0.4
4 weeks	10.2[c]	8.6	8.8	8.3	0.3
Serum Mg, mg/100 ml					
Initial	2.4	2.1	2.5	2.3	0.1
2 weeks	1.9	1.7	1.8	1.7	0.1
4 weeks	2.1	1.9	1.5	1.8	0.1
Serum alkaline phosphatase, Sigma units					
Initial	14.1	14.2	13.3	15.2	0.8
2 weeks	6.9	8.7	7.0	9.8[b]	0.5
4 weeks	5.5	5.1	4.7	4.0	0.3
Serum protein, g/100 ml					
Initial	3.8	3.8	4.1	4.0	0.1
2 weeks	3.6	4.0	3.7	4.2	0.1
4 weeks	4.7	4.9	4.7	5.1[b]	0.1

[1] Standard error of the mean.

[b] Significantly greater than least two values ($P < 0.05$).

[c] Significantly greater than all other values ($P < 0.05$).

Table 11. Daily calcium, phosphorus, magnesium, and nitrogen excretion and retention as affected by level of dietary protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	16	32	16	32	
No. of collections	3	3	3	3	±SE[1]
Daily food intake, g	520	522	499	519	6
Daily water intake, ml	743	746	712	742	8
Daily feces, g	29.7	38.4	30.4	37.3	1.8
Daily urine, ml	294	542[bb]	310	563[bb]	43
Ca balance, daily					
Ca intake, g	4.08	4.12	3.92	4.10	0.46
Fecal Ca, g	1.40	1.57	1.41	1.58	0.59
Urinary Ca, mg	3	6[a]	2	5	1
Ca retention, g	2.68	2.55	2.51	2.52	0.63
Ca retention, %	66	62	64	62	1
Ca apparent digest., %	66	62	64	62	1
P balance, daily					
P intake, g	2.93	2.81	2.82	2.80	0.32
Fecal P, g	0.94	0.93	0.95	0.89	0.32
Urinary P, g	0.18	0.22	0.16	0.27	0.23
P retention, g	1.81	1.66	1.71	1.64	0.43
P retention, %	62	59	61	59	1
P apparent digest., %	68	66	67	69	1
Mg balance, daily					
Mg intake, mg	223	282[bb]	215	281[bb]	10
Fecal Mg, mg	82	131[b]	86	125[b]	8
Urinary Mg, mg	10	9	6	6	2
Mg retention, mg	131	142	123	150	6
Mg retention, %	59	50	58	53	2
Mg apparent digest., %	63	55	60	56	2
N balance, daily					
N intake, g	14.06	28.46[bb]	13.51	28.34[bb]	2.21
Fecal N, g	0.53	1.21[b]	0.55	1.24[b]	0.12
Urinary N, g	3.69	13.55[bb]	3.78	14.02[bb]	1.57
N retention, g	9.84	13.70[b]	9.18	13.08[b]	0.70
N retention, %	70[bb]	48	68[bb]	46	4
N apparent digest., %	96	96	96	96	0

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$).
[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$.

pigs fed the lower level of protein.

Skeletal development as shown in table 12 was depressed by high protein intake. Specific gravity, which reflects bone mineralization,

Table 12. Weight, density, composition and strength of bones from baby pigs fed different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	16	32	16	32	
No. of observations	3	3	3	3	±SE[1]
Femur wt, g	58.3	57.6	59.0	58.3	1.3
Femur density	1.17	1.16	1.17	1.16	0.00
8th rib wt, g	5.0	6.1	5.4	5.4	0.3
8th rib density	1.25[a]	1.22	1.25[a]	1.18	0.01
Humeral analyses, dry, fat-free basis, %					
Ash	46.1[b]	42.1	45.6[b]	41.8	0.7
Ca	16.7	15.8	16.5	15.6	0.2
P	8.6[b]	7.9	8.6[b]	7.9	0.1
Mg	0.37	0.37	0.35	0.38	0.01
Femur strength					
Breaking load, kg	67	57	67	57	3
Bending moment, kg-cm	122	104	123	104	7
Moment of inertia, cm^4	0.14	0.14	0.14	0.14	0.01
Breaking stress, kg/cm^2	594	514	605	526	27
Young's modulus of elasticity, 1000 kg/cm^2	5.4	4.4	5.6	4.6	0.3

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$).
[b] Significantly greater than least two values ($P < 0.05$).

and the humeral mineral analyses show that mineral deposition was significantly greater in pigs receiving 16% crude protein than in pigs receiving 32% crude protein in the diet. Figure 3 shows load-deflection curves for femurs of pigs in this experiment. High levels of protein intake depressed the breaking strength at either level of vitamin D

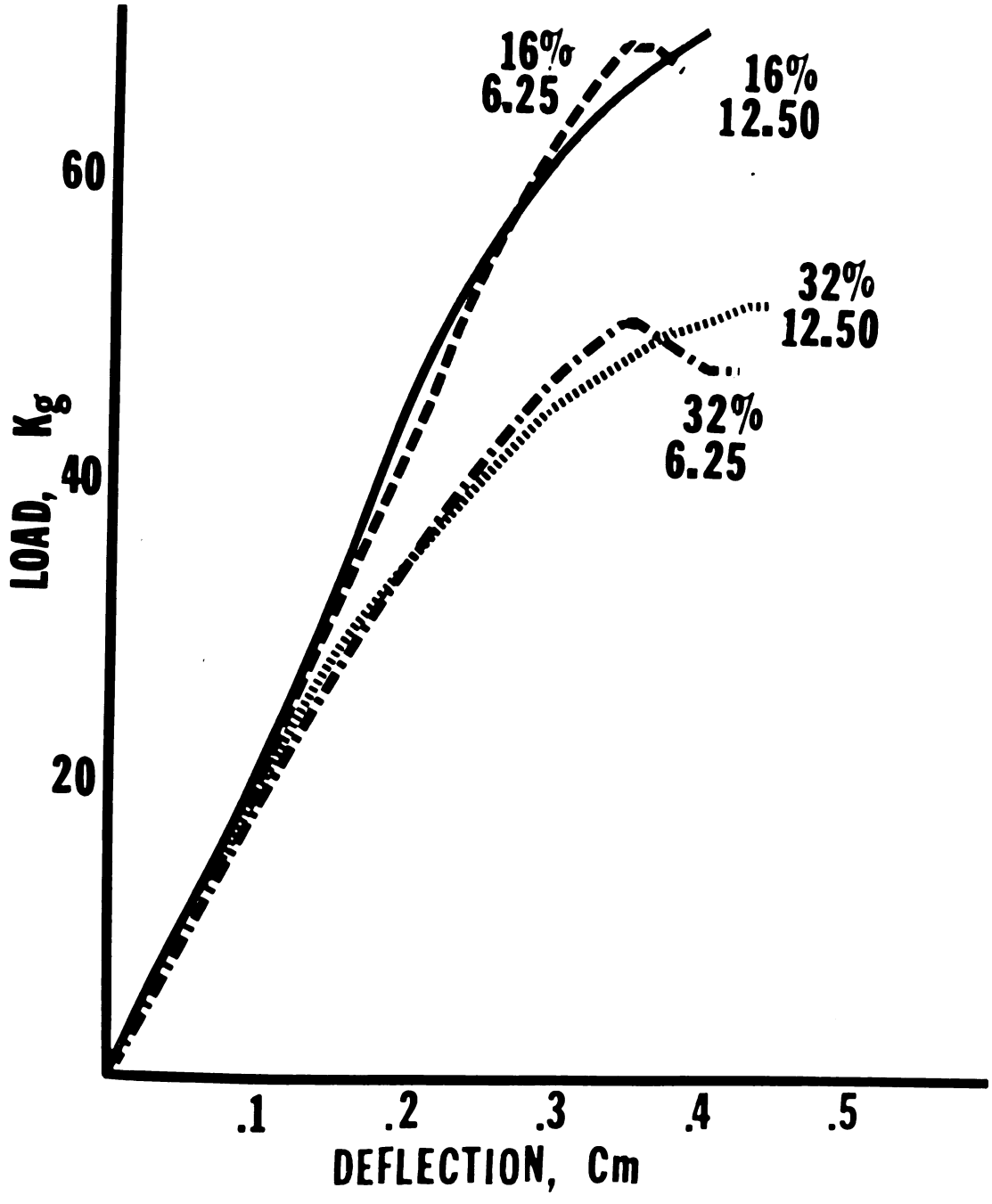

Figure 3. Exp. III. -- Load-deflection curves for femurs from pigs fed two different levels of soy protein (16% or 32%) and ergocalciferol (6.25 or 12.50 μg/kg diet).

intake. Carlson et al. (1964a, b) reported that increasing the vitamin D intake of turkey poults would overcome the rachitogenic effect of high levels of isolated soy protein. They used 800 to 3520 IU vitamin D_3/kg diet. Photographs of the broken femurs (Figure 4) show that high levels of protein intake interfered with normal compact bone formation. Note the narrow band of compact bone and the inner ring of spongy matrix seen in the femurs from pigs receiving a 32% protein diet (C & D). Bones from pigs fed 16% soy protein protein diets do not show this area of poor mineralization.

Table 13 shows that kidneys were somewhat hypertrophied in response to the high levels of protein intake. Liver size in this experiment did not reflect any treatment differences. Thymus and pancreatic tissue weights increased linearly with protein intake. Spleen weight, however, was inversely related to dietary protein level.

D. Experiment IV. Effect of source and level of protein on mineral utilization by the baby pig.

Data presented in table 14 show that pigs receiving 16% crude protein in the diet ate 20% more feed than pigs receiving 32%. When analyzed by the Chi Square method, mean daily intake by treatments differed significantly ($P < 0.01$) from the overall mean intake. Although not statistically significant, there is a trend toward increased feed efficiency at higher levels of protein intake. Krauss and Mayer (1965) and Anderson et al. (1967) reported that food intake of rats decreased with increasing dietary protein content. Krauss and Mayer suggested that the limitation imposed on the rats by their ability to metabolize protein acts to reduce the total food intake in such a manner

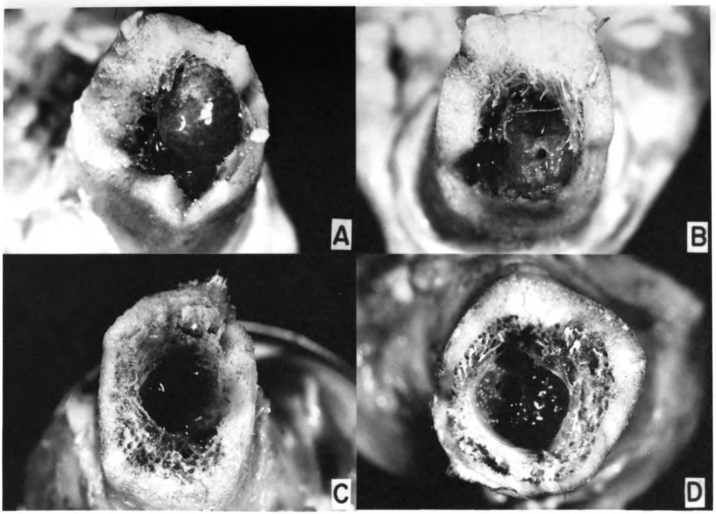

Figure 4. Exp. III. -- Photographs (4x) of cross-sections of femurs from pigs fed (A) 16% soy protein diet with 6.25 μg ergocalciferol/kg, (B) 16% soy protein diet with 12.50 μg ergocalciferol/kg, (C) 32% soy protein diet with 6.25 μg ergocalciferol/kg, (D) 32% soy protein diet with 12.50 μg ergocalciferol/kg.

Table 13. Organ weights of baby pigs fed different levels of protein and ergocalciferol.

Dietary ergocalciferol, μg/kg	6.25		12.50		
Dietary protein, %	16	32	16	32	
No. of observations	3	3	3	3	±SE[1]
Thyroid wt, g	0.90	0.69	0.82	0.74	0.05
Thyroid, % BW[2]	0.007	0.006	0.006	0.006	0.000
Adrenal wt, g[3]	1.15	1.16	1.10	1.01	0.03
Adrenal, % BW	0.009	0.010	0.009	0.009	0.000
Kidney wt, g[3]	66	74	67	72	3
Kidney, % BW	0.52	0.65[bb]	0.52	0.63[b]	0.02
Spleen wt, g	26[b]	18	32[bb]	15	2
Spleen, % BW	0.21	0.16	0.25[a]	0.14	0.02
Heart wt, g	54	48	54	48	2
Heart, % BW	0.42	0.43	0.42	0.42	0.01
Liver wt, g	373	351	361	330	11
Liver, % BW	2.93	3.11	2.80	2.88	0.07
Pancreas wt, g	21	22	20	22	1
Pancreas, % BW	0.17	0.19[b]	0.15	0.19[b]	0.01
Thymus wt, g	29	39	39	43	3
Thymus, % BW	0.22	0.35[a]	0.30	0.36[a]	0.02
Lungs wt, g	145[b]	126	138	128	3
Lungs, % BW	1.14	1.12	1.07	1.13	0.04

[1] Standard error of the mean.
[2] Expressed as a percent of live body weight.
[3] Combined weight of both organs.
[a] Significantly greater than least value ($P < 0.05$).
[b] Significantly greater than two least values ($P < 0.05$); [bb] $P < 0.01$.

Table 14. Growth and serum analyses of baby pigs fed casein or soy protein at two different levels.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of pigs	4	4	4	4	±SE[1]
Initial wt, kg	2.57	2.54	2.56	2.56	0.09
Daily gain, kg	0.26	0.24	0.26	0.25	0.01
Daily food intake, kg	0.51	0.42	0.51	0.42	
Gain/food	0.50	0.56	0.51	0.60	
Serum Ca, mg/100 ml					
Initial	11.7	12.4	11.8	11.5	0.1
3 weeks	10.3	10.9	10.5	11.2[a]	0.2
5 weeks	10.9	11.2	11.3	10.5	0.2
Serum P, mg/100 ml					
Initial	5.5	5.3	5.9	5.2	0.2
3 weeks	9.8[cc]	8.6[aa]	9.7[bb]	5.0	0.5
5 weeks	10.2[aa]	9.7[aa]	10.1[aa]	6.2	0.5
Serum Mg, mg/100 ml					
Initial	2.6	2.9	2.6	2.5	0.1
3 weeks	1.8	2.0	2.1	2.0	0.1
5 weeks	1.8	2.1	2.0	1.5	0.1
Serum alkaline phosphatase, Sigma units					
3 weeks	7.5	8.1	10.7	14.6[bb,c]	0.8
5 weeks	7.2	6.6	8.3	10.6[b]	0.6
Serum protein, g/100 ml					
Initial	3.6	4.2	3.5	4.0	0.1
3 weeks	3.7	3.4	3.7	3.7	0.1
5 weeks	4.8[aa,b]	4.4[a]	4.1	4.5[a]	0.1

[1] Standard error of the mean.

[a] Significantly greater than least value ($P < 0.05$; [aa] $P < 0.01$.

[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$.

[c] Significantly greater than all other values ($P < 0.05$); [cc] $P < 0.01$.

that the amount of protein ingested does not exceed a certain threshold.

Serum inorganic P values (table 14) were reduced significantly ($P < 0.01$) when a high level of soy protein was fed. Serum alkaline phosphatase values were also high in pigs receiving 32% soy protein diets. A comparison of these results with those obtained by Miller et al. (1964a, b) would suggest the possibility of a P deficiency rickets in those pigs receiving high levels of isolated soy protein in the diet.

Data from the balance trial (table 15) show that Ca and P were absorbed more readily from the casein diets than from the soy diets. Increasing dietary protein level increased Ca and P retention when casein was the protein source, but when isolated soy was the source of protein, Ca and P retention decreased with increasing levels of dietary protein. The results observed in this trial are probably a consequence of the enhancing effect of the lactose which is associated with the casein on Ca and P absorption and the suppressing effect of the phytic acid in the isolated soy protein on these same minerals. An analysis of the isolated soy preparation used in these studies showed that about 40% of the P in the protein was in the form of phytin phosphate. Table 15(B) shows that Mg tended to follow the absorption and retention pattern of Ca and phosphorus. Sodium and K were readily absorbed from all diets. Sodium, however, was very poorly retained. Iron and Zn balance studies were somewhat variable and due to the wide differences within treatments statistical differences could be attributed only to differences in dietary intake. Manganese, Cu and Co were also highly variable and treatment effects upon the balance study, if any, were obliterated. Nitrogen balance was not affected by protein source, but as protein

Table 15 (A). Daily calcium, phosphorus, sodium, and potassium excretion and retention as affected by source and level of dietary protein.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of collections	4	4	4	4	±SE[1]
Daily food intake, g	297	341	302	327	10
Daily water intake, ml	794	896	789	856	26
Daily feces, g	19.6	24.6	17.1	25.9	2.2
Daily urine, ml	443	532	474	564	24
Ca balance, daily					
Ca intake, g	2.50	2.80	2.45	2.73	0.09
Fecal Ca, g	0.65	0.51	0.94	1.46[aa,b]	0.13
Urinary Ca, mg	8	7	8	10	1
Ca retention, g	1.84[a]	2.28[bb,c]	1.50	1.27	0.12
Ca retention, %	75[aa]	82[aa,b]	62	47	4
Ca apparent digest.,%	75[a]	82[bb]	62	47	4
P balance, daily					
P intake, g	1.93	2.14	1.80	1.81	0.07
Fecal P, g	0.39	0.24	0.54	0.75[aa,b]	0.06
Urinary P, g	0.42[aa,b]	0.31[aa]	0.17	0.04	0.05
P retention, g	1.12	1.59[aa,c]	1.09	1.02	0.08
P retention, %	57	74[bb,c]	60	57	2
P apparent digest., %	81[aa]	89[bb]	70	59	3
Na balance, daily					
Na intake, g	1.33	1.60	1.47	1.60	0.05
Fecal Na, g	0.07	0.09	0.06	0.09	0.01
Urinary Na, g	1.05	1.05	1.37	1.16	0.07
Na retention, g	0.21	0.46[a]	0.04	0.34	0.07
Na retention, %	14	29	3	21	4
Na apparent digest.,%	95	95	96	94	1
K balance, daily					
K intake, g	1.09	1.22	1.19	1.14	0.04
Fecal K, g	0.05	0.07	0.04	0.10	0.01
Urinary K, g	0.37	0.19	0.45[aa]	0.24	0.04
K retention, g	0.67	0.96	0.70	0.80	0.06
K retention, %	60	78	58	70	4
K apparent digest., %	95	94	97	91	1

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$.
[c] Significantly greater than all other values ($P < 0.05$).

Table 15 (B). Daily magnesium, iron, zinc, manganese, and copper excretion and retention as affected by source and level of dietary protein.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of collections	4	4	4	4	±SE[1]
Mg balance, daily					
Mg intake, mg	101	111	103	113	4
Fecal Mg, mg	36	30	39	58	4
Urinary Mg, mg	8	4	9[b]	3	1
Mg retention, mg	57	77	55	52	4
Mg retention, %	57	70[b]	52	46	3
Mg apparent digest.,%	65	73[a]	62	49	3
Fe balance, daily					
Fe intake, mg	33.5	35.9	43.4	64.3[cc]	3.4
Fecal Fe, mg	15.6	23.1	17.8	31.7	2.6
Urinary Fe, mg	0.3	0.3	0.3	0.6[bb,c]	0.4
Fe retention, mg	17.6	12.5	25.3	32.0[b]	2.7
Fe retention, %	55	35	58	50	5
Fe apparent digest.,%	56	36	59	51	5
Zn balance, daily					
Zn intake, mg	26.6	42.0[hh]	27.1	37.2	2.6
Fecal Zn, mg	15.1	26.8	14.2	18.0	2.6
Urinary Zn, mg	0.3	0.3	0.2	0.6	0.6
Zn retention, mg	11.2	14.9	12.7	18.6	3.4
Zn retention, %	44	28	46	47	8
Zn apparent digest.,%	45	29	47	49	7
Mn balance, daily					
Mn intake, mg	7.43	8.53	7.55	8.18	0.26
Fecal Mn, mg	5.82	11.54	5.57	8.68	1.18
Urinary Mn, mg	0.06	0.08	0.07	0.04	0.01
Mn retention, mg	1.55	-3.09	1.91	-0.54	1.11
Mn retention, %	22	-36	24	-6	13
Mn apparent digest.,%	23	-35	25	-6	13
Cu balance, daily					
Cu intake, mg	6.37	6.99	7.25	8.51[b]	0.31
Fecal Cu, mg	5.07	6.49	4.69	6.70	0.59
Urinary Cu, mg	0.14	0.19	0.20	0.23	0.01
Cu retention, mg	1.16	0.31	2.36	1.58	0.56
Cu retention, %	21	2	33	18	8
Cu apparent digest.,%	24	5	36	21	8

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$).
[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$.
[c] Significantly greater than all other values ($P < 0.05$); [cc] $P < 0.01$.

Table 15 (C). Daily cobalt, nitrogen, energy, and chromium excretion and retention as affected by source and level of dietary protein.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of collections	4	4	4	4	±SE[1]
Co balance, daily					
Co intake, mg	5.23	5.80	5.14	5.98	0.20
Fecal Co, mg	3.83	5.17	2.97	3.52	0.49
Urinary Co, mg	0.60	0.60	0.69	1.18[cc]	0.08
Co retention, mg	0.80	0.03	1.48	1.18	0.43
Co retention, %	17	1	28	20	8
Co apparent digest., %	29	11	42	42	8
N balance, daily					
N intake, g	8.4	18.9[bb]	8.6	18.7[bb]	1.4
Fecal N, g	0.2	0.4[a]	0.2	0.5[b]	0.0
Urinary N, g	2.4	8.1[bb]	2.3	8.6[bb]	0.8
N retention, g	5.8	10.4[bb]	6.1	9.6[bb]	0.6
N retention, %	67[a]	55	71[b]	51	3
N apparent digest., %	98	98	98	97	0.0
Energy balance, daily					
Energy intake, KCal	1200	1468[a]	1184	1461	51
Fecal energy, KCal	73	101	62	94	9
Urinary energy, KCal	12	45[bb]	12	46[bb]	4
Metabolic energy, KCal	1115	1317	1110	1321	45
Metabolic energy, %	92	90	94	90	1
Digestible energy, %	94	93	95	94	1
Cr balance, daily					
Cr intake, g	0.97	1.23	1.04	1.04	0.04
Fecal Cr, g	0.84	1.17	0.67	0.90	0.11
Urinary Cr, g	0.00	0.00	0.00	0.00	0.00
Cr retention, g	0.13	0.06	0.37	0.14	0.10
Cr retention, %	15	3	34	14	9
Cr apparent digest., %	15	3	34	14	9

[1] Standard error of the mean.
[a] Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$); [bb] $P < 0.01$
[cc] Significantly greater than all other values ($P < 0.01$).

level increased, N retention also increased. When expressed as percent retention, however, a larger proportion of N from the low protein diets was retained. Energy intake was directly related to protein intake. Urinary energy, calculated from urinary N, was also in direct proportion to protein intake. In order to determine the accuracy of the chromic oxide indicator method of determining nutrient digestibility, Cr balance was determined. The wide variation in Cr retention may be due in part to variation in fecal excretion (Appendix 2). A look at the fecal Cr concentration (Appendix 16) would lead one to believe that variation in amount of excreta rather than concentration of Cr within the excreta was the cause of the observed variation. Rate of passage may have played a part also. Maner et al. (1962) showed that casein diets moved through the tract more slowly than soy protein diets. This effect, however, should have been minimal due to the four day adjustment period prior to collection and the age of the pigs (6 weeks). Maner et al. reported that mean passage time for casein diets was 42 hours and that for soy diets was 19 hours when pigs were 4 weeks of age. At 10 weeks of age, however, there was no difference in rate of passage due to protein source. Ali (1967) has shown in rats that Cr concentration increases from the ileocecal valve to the apex of the cecum. He suggested that due to the sluggish action of the cecum, Cr, a heavier metal which is not absorbed, slowly builds up in concentration toward the apex of the organ. (Appendix 17 shows a comparison of the results of the total collection vs. indicator method for determining apparent digestibility of nutrients.)

Table 16 shows that skeletal development of pigs receiving 32% soy protein was depressed in comparison to that of pigs receiving other

Table 16. Weight, density, composition and strength of bones from baby pigs fed casein or soy protein at two different levels.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of observations	4	4	4	4	±SE[1]
Femur wt, g	64.1	70.8	62.4	61.8	2.7
Femur density	1.20[aa]	1.20[aa]	1.19[aa]	1.15	0.01
8th rib wt, g	8.1	8.4	7.0	7.5	0.7
8th rib density	1.30[a]	1.29[a]	1.31[a]	1.22	0.01
Humeral analyses, dry, fat-free basis, %					
Ash	49.4[aa]	48.5[aa]	47.8[aa]	39.4	1.1
Ca	20.9[a]	20.1[a]	20.2[a]	16.9	0.6
P	7.4[aa]	7.3[aa]	7.3[aa]	6.0	0.2
Mg	0.36[aa]	0.34[aa]	0.34[aa]	0.27	0.01
Femur strength					
Breaking load, kg	84[aa]	89[aa]	81[aa]	48	5
Bending moment, kg-cm	162[aa]	175[aa]	159[aa]	89	10
Moment of inertia, cm^4	0.20	0.22	0.17	0.17	0.01
Breaking stress, kg/cm^2	613[aa]	619[aa]	694[aa]	375	34
Young's modulus of elasticity, 1000 kg/cm^2	15.7[aa,b]	12.1[aa]	14.2[aa]	4.6	1.2

1 Standard error of the mean.
2 Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
3 Significantly greater than least two values ($P < 0.05$).

dietary treatments. This is in contrast to the observed retention of Ca, P and Mg which was generally lower in both lots receiving soy protein. It does, however, reflect very accurately the serum P and alkaline phosphatase values seen in table 14. Figure 5 shows the load-deflection curves of femurs from pigs in Experiment IV. Femurs from pigs fed diets containing 32% crude protein from isolated soy protein bent more and took less pressure to break than those from pigs on any other dietary treatment. Figure 6 shows that this was due to the lack of mineral deposition within the matrix of the bone. Note the spongy appearance of the cross section of the femur from a pig receiving the 32% soy diet (D) as compared to the more compact mineral deposition observed in the femurs of pigs fed 20% or 40% casein or 20% soy diets (A, B, C).

From table 17 one can see that, as has been previously observed, kidney, liver and pancreas size was in direct proportion to dietary protein level. Shilling and Rostell (1964) have shown that high levels of protein fed over an extended period of time resulted in increased liver weights in cattle. No pathological changes were observed in the enlarged livers. Imondi and Bird (1964) showed that as dietary protein increased there was a corresponding increase in the size of the pancreas of chicks. Stoewsand and Scott (1964b) observed an enlargement of the adrenal glands in chicks consuming high levels of isolated soy protein in their diets. Protein source did not show any specific effects on organ weights in this study.

Figures 7 - 17 show the absorption or secretion of various nutrients along the GI tract of baby pigs receiving casein or soy diets at two different levels of protein intake. Absorption and secretion values were calculated as described using data from Appendix 5 - 16. The

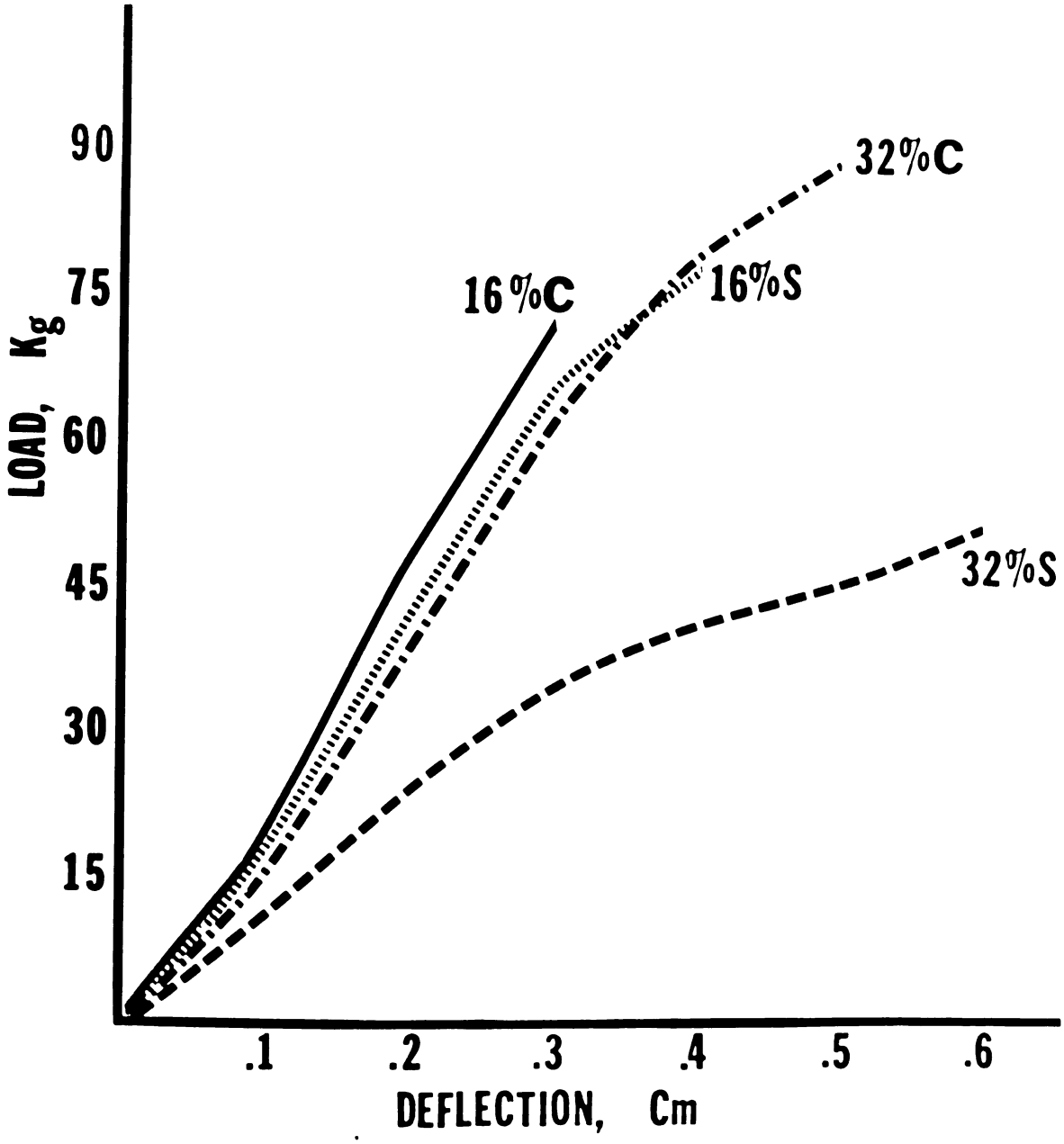

Figure 5. Exp. IV. -- Load-deflection curves for femurs from pigs fed casein (C) or soy (S) at two different levels (16% or 32%) with 6.25 μg ergocalciferol/kg diet.

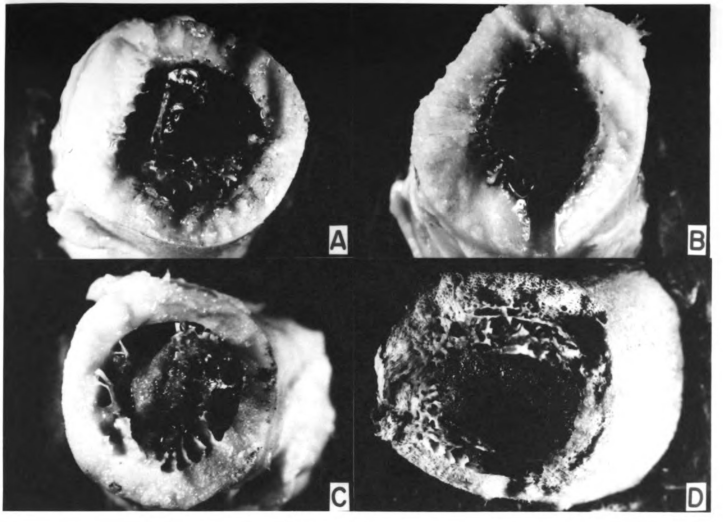

Figure 6. Exp. IV: -- Photographs (5x) of cross-sections of femurs from pigs fed (A) 16% casein, (B) 32% casein, (C) 16% soy, (D) 32% soy, with 6.25 μg ergocalciferol/kg diet.

Table 17. Organ weights of baby pigs fed casein or soy protein at two different levels.

Protein source	Casein		Soy		
Dietary protein, %	16	32	16	32	
No. of observations	4	4	4	4	±SE[1]
Thyroid wt, g	0.86	1.01	0.93	0.96	0.04
Thyroid, % BW[2]	0.007	0.007	0.007	0.007	0.000
Adrenal wt, g[3]	1.09	1.15	0.94	1.06	0.04
Adrenal, % BW	0.008	0.009	0.007	0.008	0.000
Kidney wt, g[3]	53	65[aa,b]	47	56[a]	2
Kidney, % BW	0.42	0.47[aa]	0.37	0.43[a]	0.01
Spleen wt, g	23	20	18	19	1
Spleen, % BW	0.18	0.14	0.14	0.15	0.01
Heart wt, g	73	61	64	63	3
Heart, % BW	0.57	0.44	0.50	0.49	0.02
Liver wt, g	298	346[aa]	269	352[aa,b]	12
Liver, % BW	2.32[aa]	2.51[aa]	2.10	2.70[bb]	0.06
Pancreas wt, g	24[a]	28[aa,b]	21	27[aa,b]	1
Pancreas, % BW	0.18	0.20	0.17	0.21[a]	0.01

[1] Standard error of the mean.
[2] Expressed as a percent of live body weight.
[3] Combined weight of both organs.
[a] Significantly greater than least value ($P < 0.05$); [aa] $P < 0.01$.
[b] Significantly greater than least two values ($P < 0.05$).

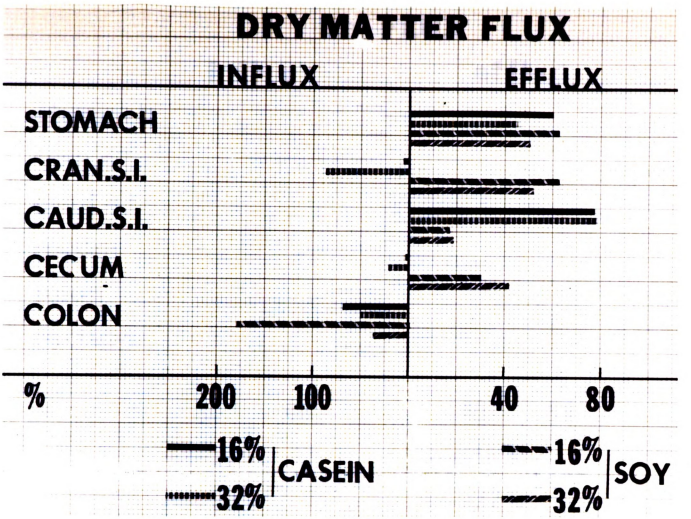

Figure 7. Exp. IV. -- Influx and efflux of dry matter along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

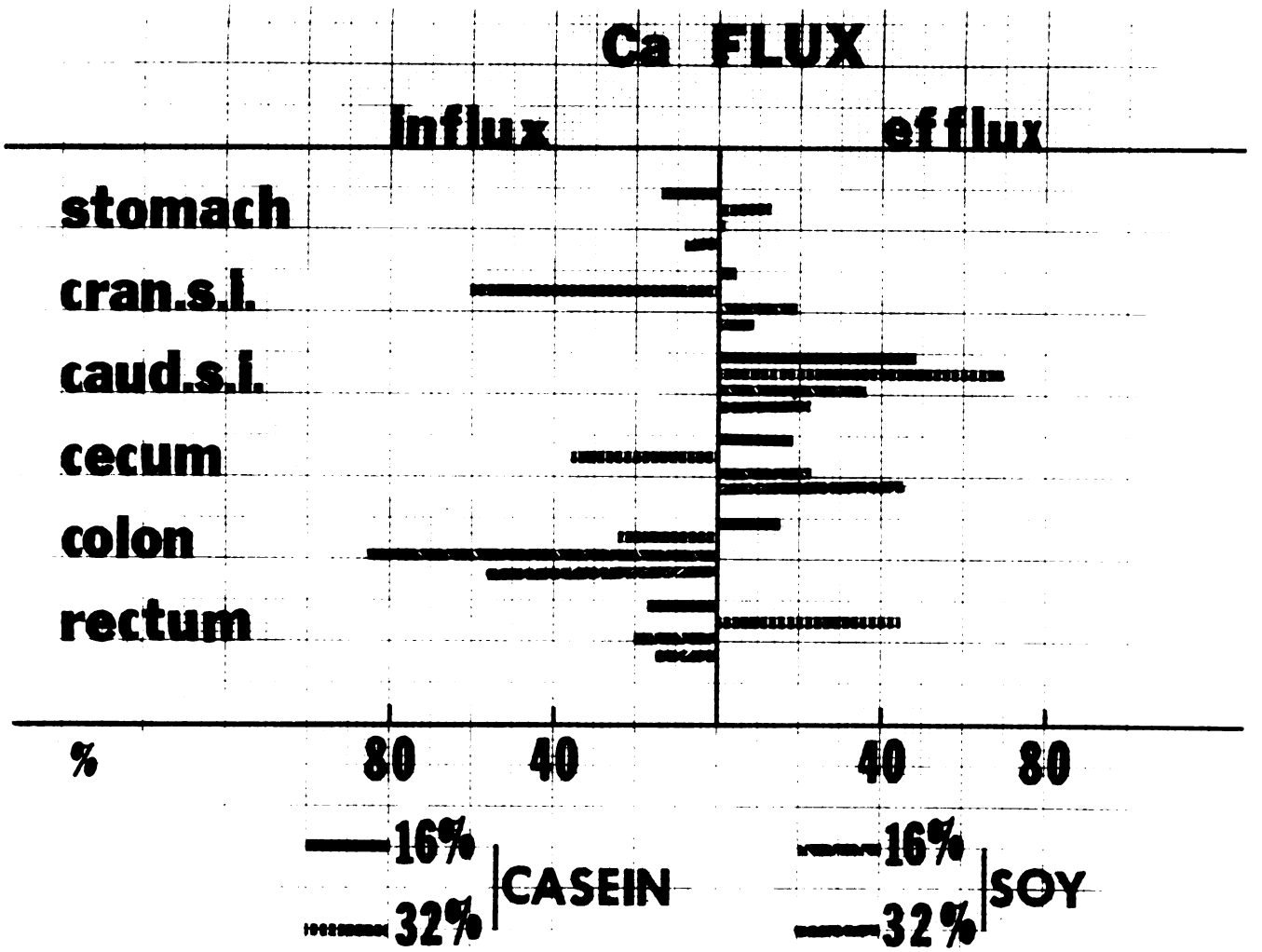

Figure 8. Exp. IV. -- Influx and efflux of calcium along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

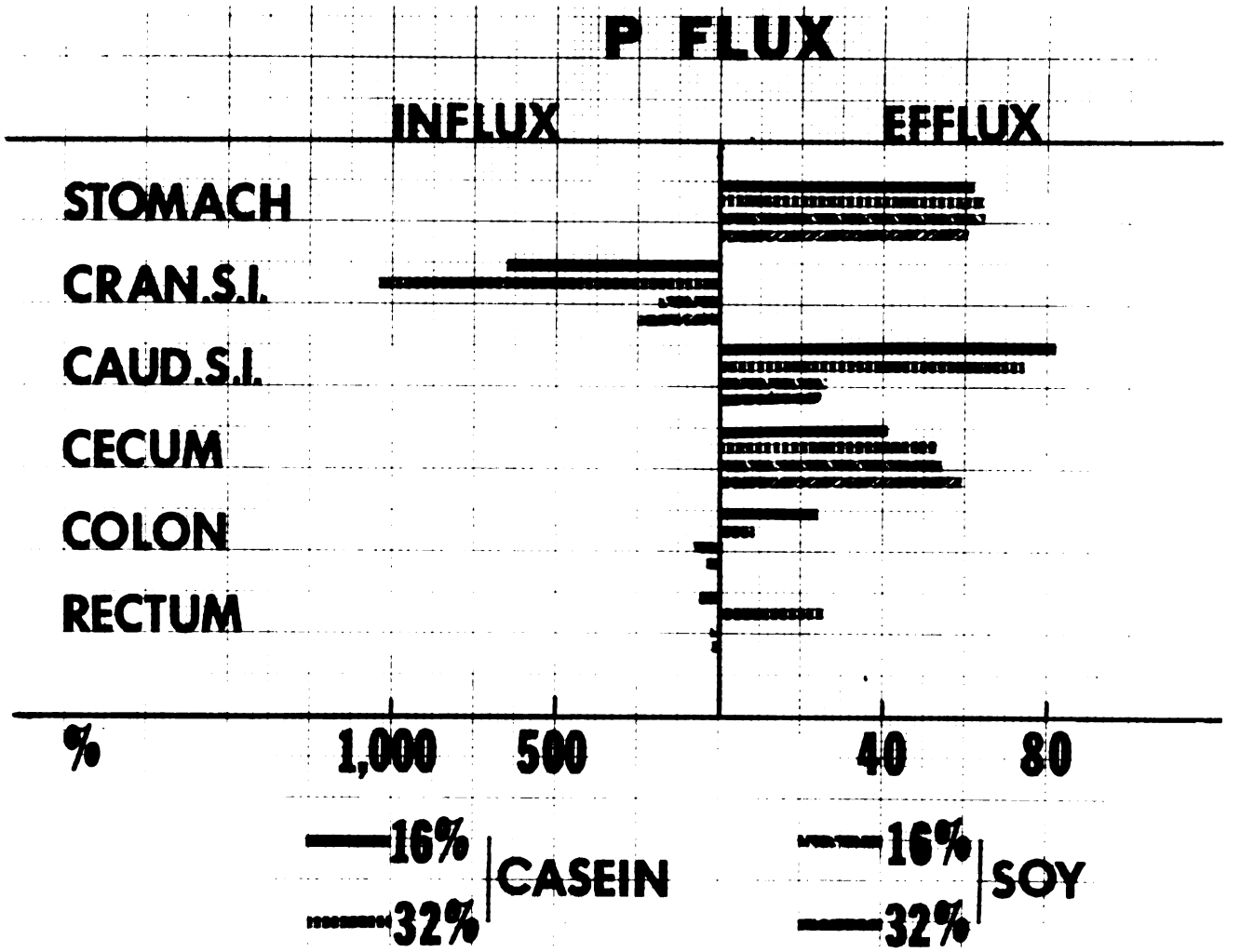

Figure 9. Exp. IV. -- Influx and efflux of phosphorus along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

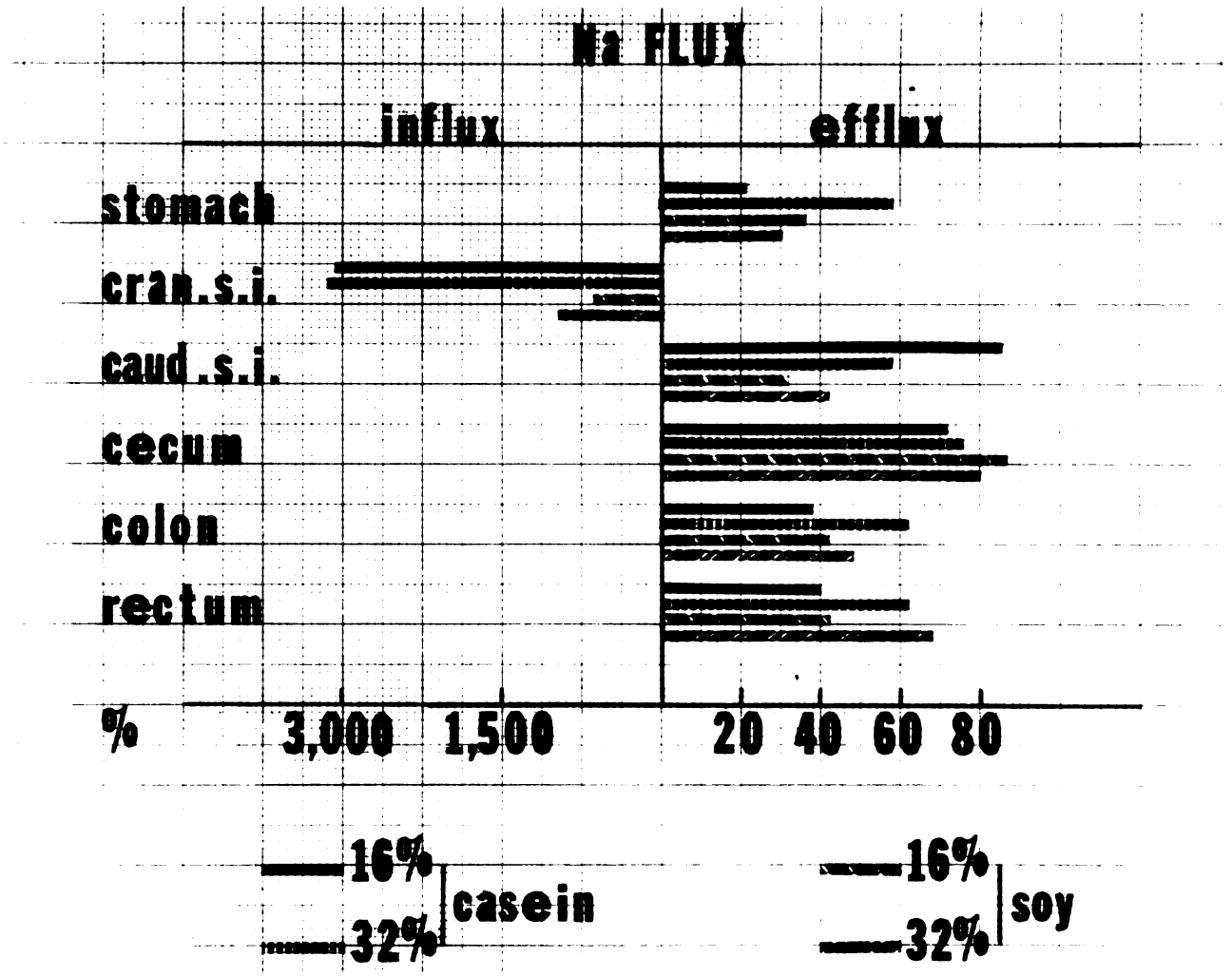

Figure 10. Exp. IV. -- Influx and efflux of sodium along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

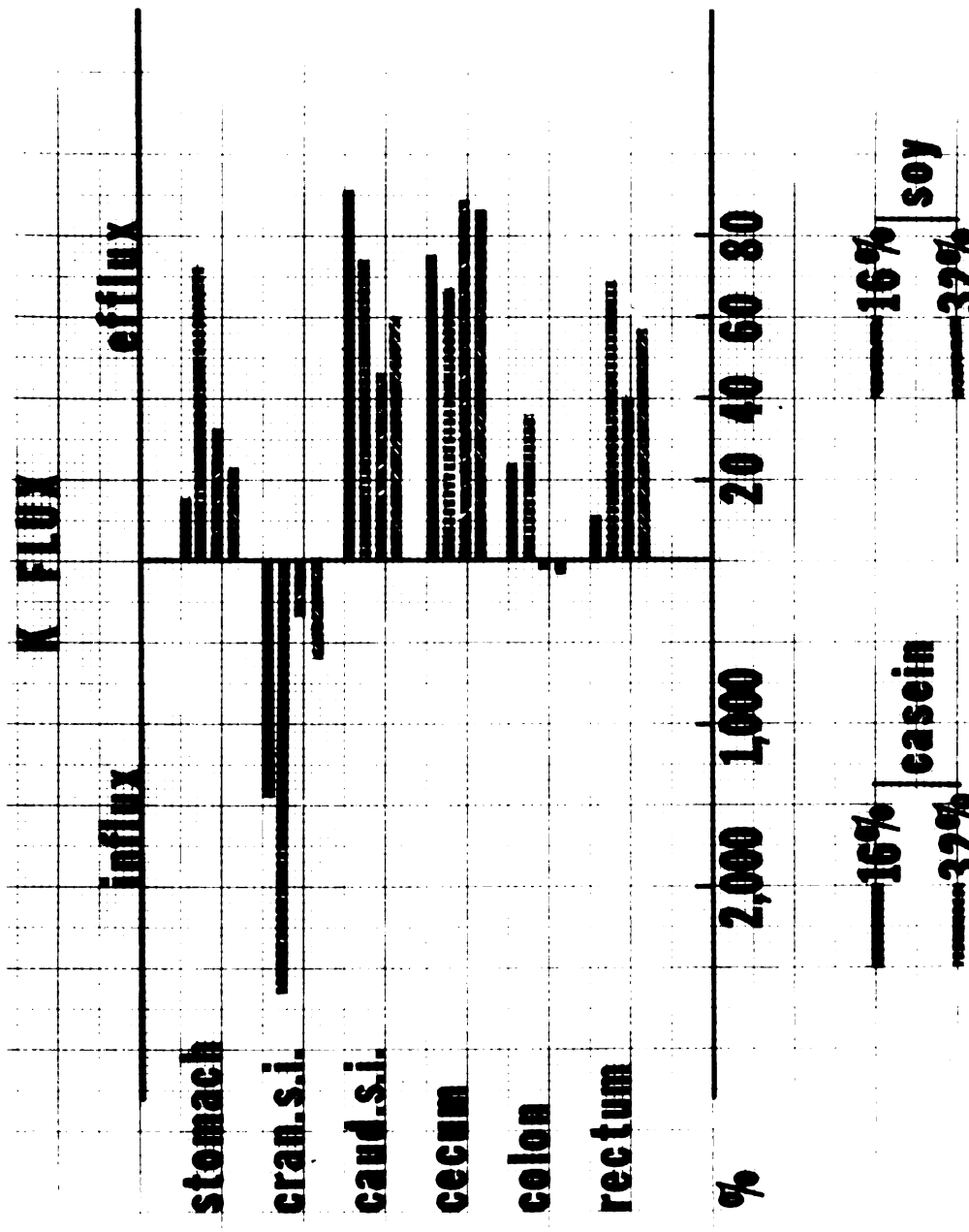

Figure 11. Exp. IV. -- Influx and efflux of potassium along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

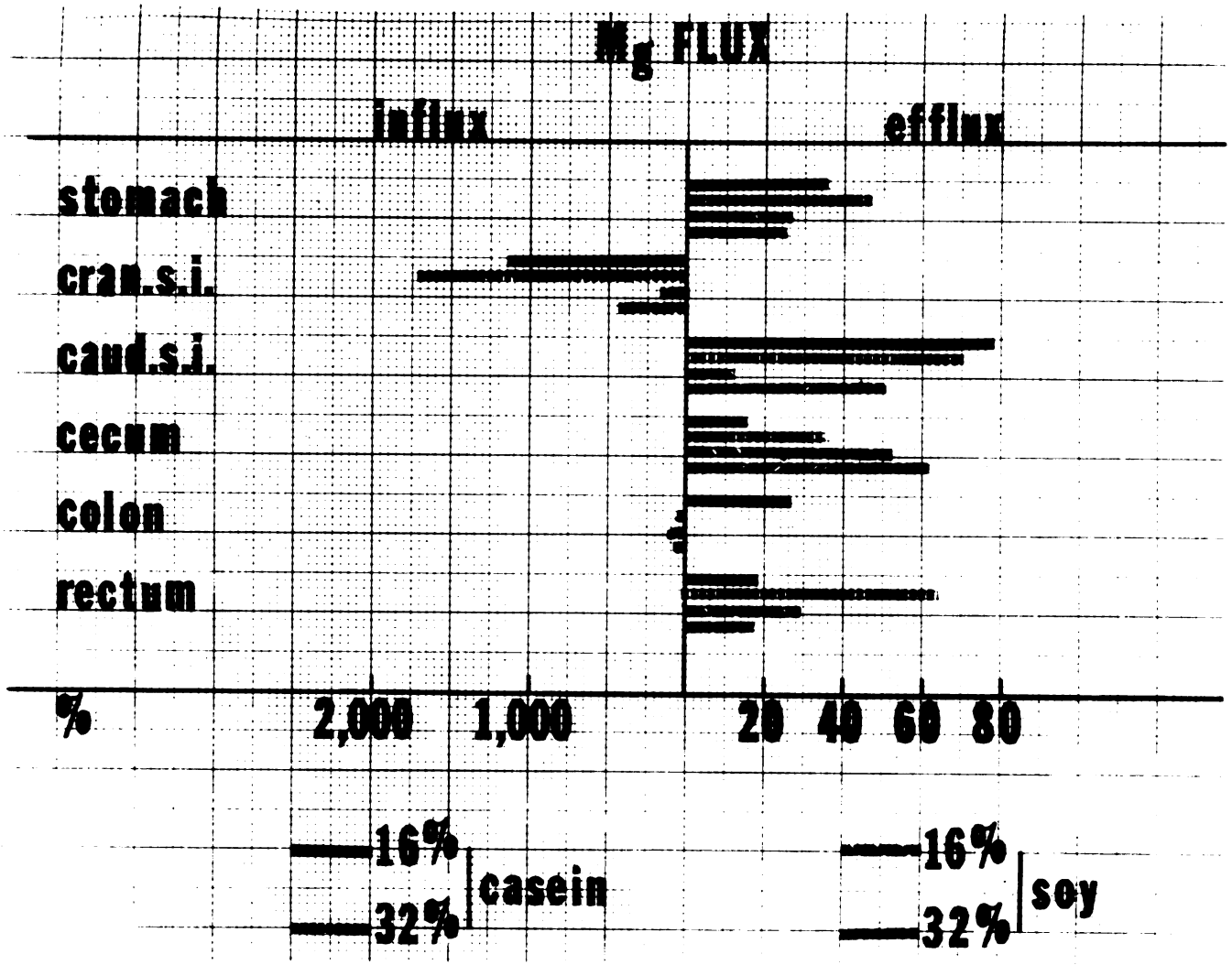

Figure 12. Exp. IV. -- Influx and efflux of magnesium along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

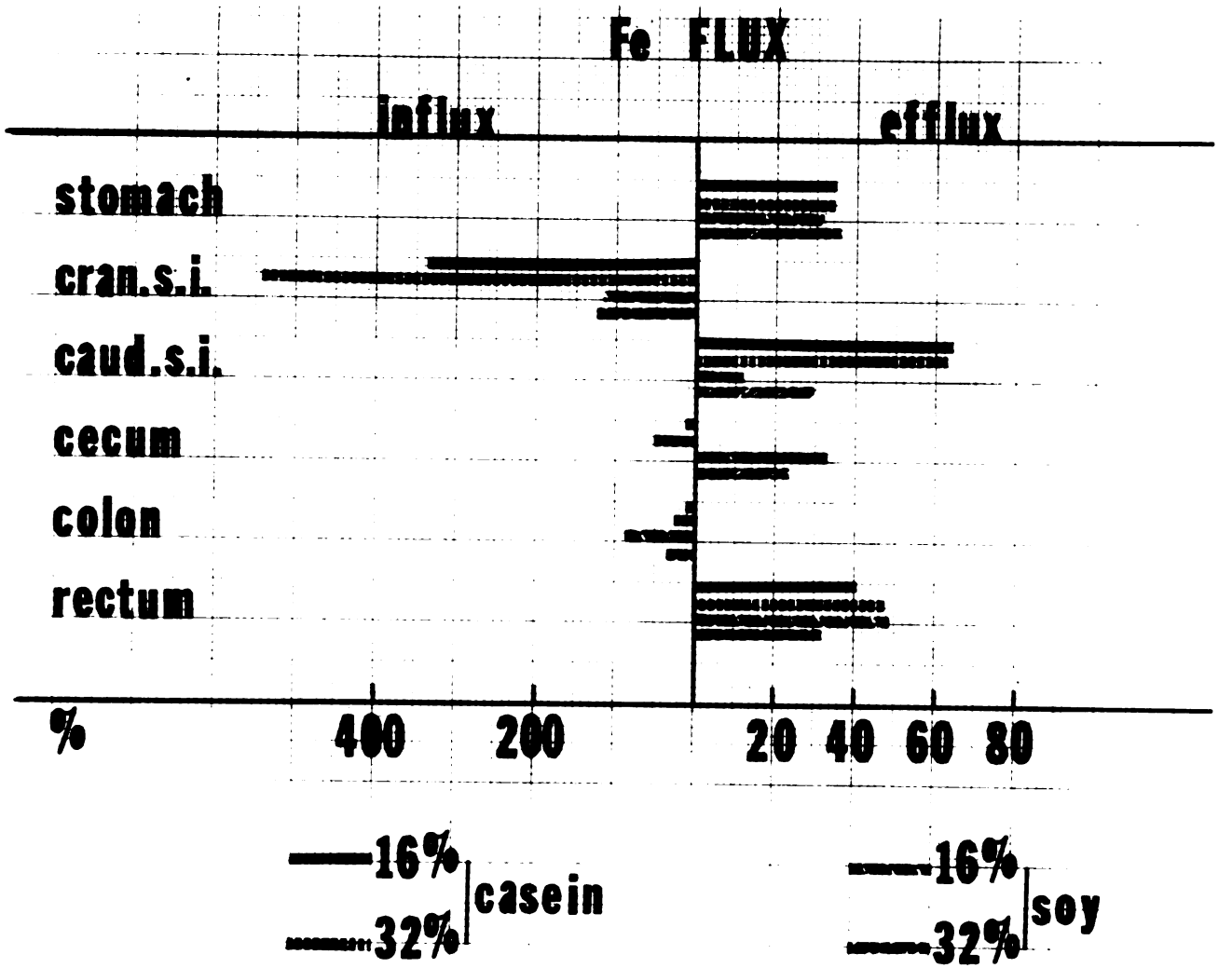

Figure 13. Exp. IV. -- Influx and efflux of iron along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

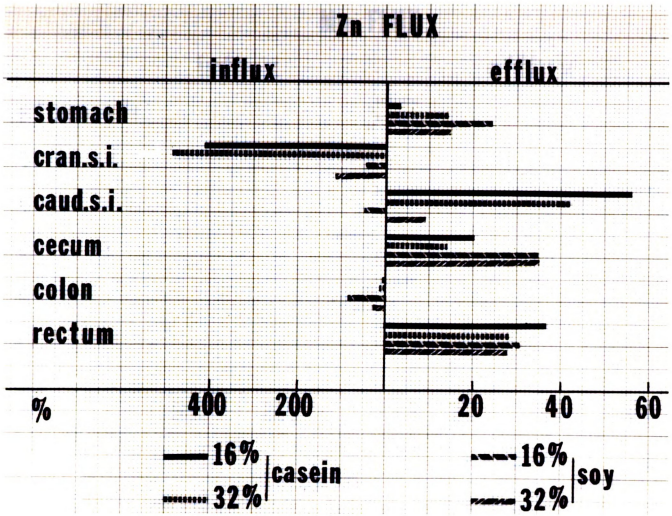

Figure 14. Exp. IV. -- Influx and efflux of zinc along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

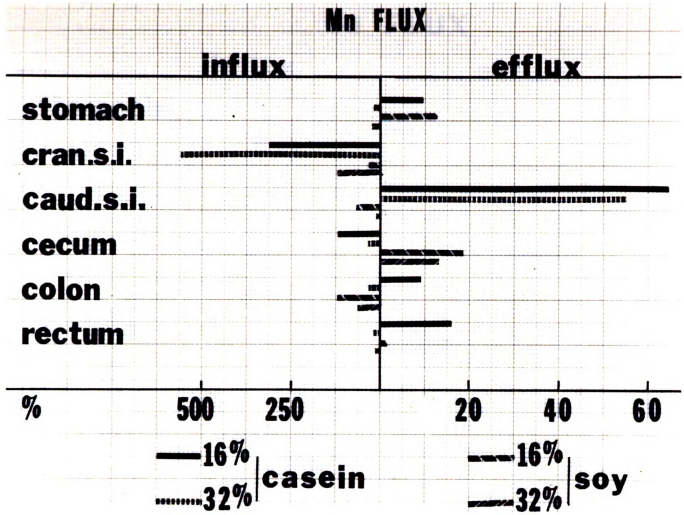

Figure 15. Exp. IV. -- Influx and efflux of manganese along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

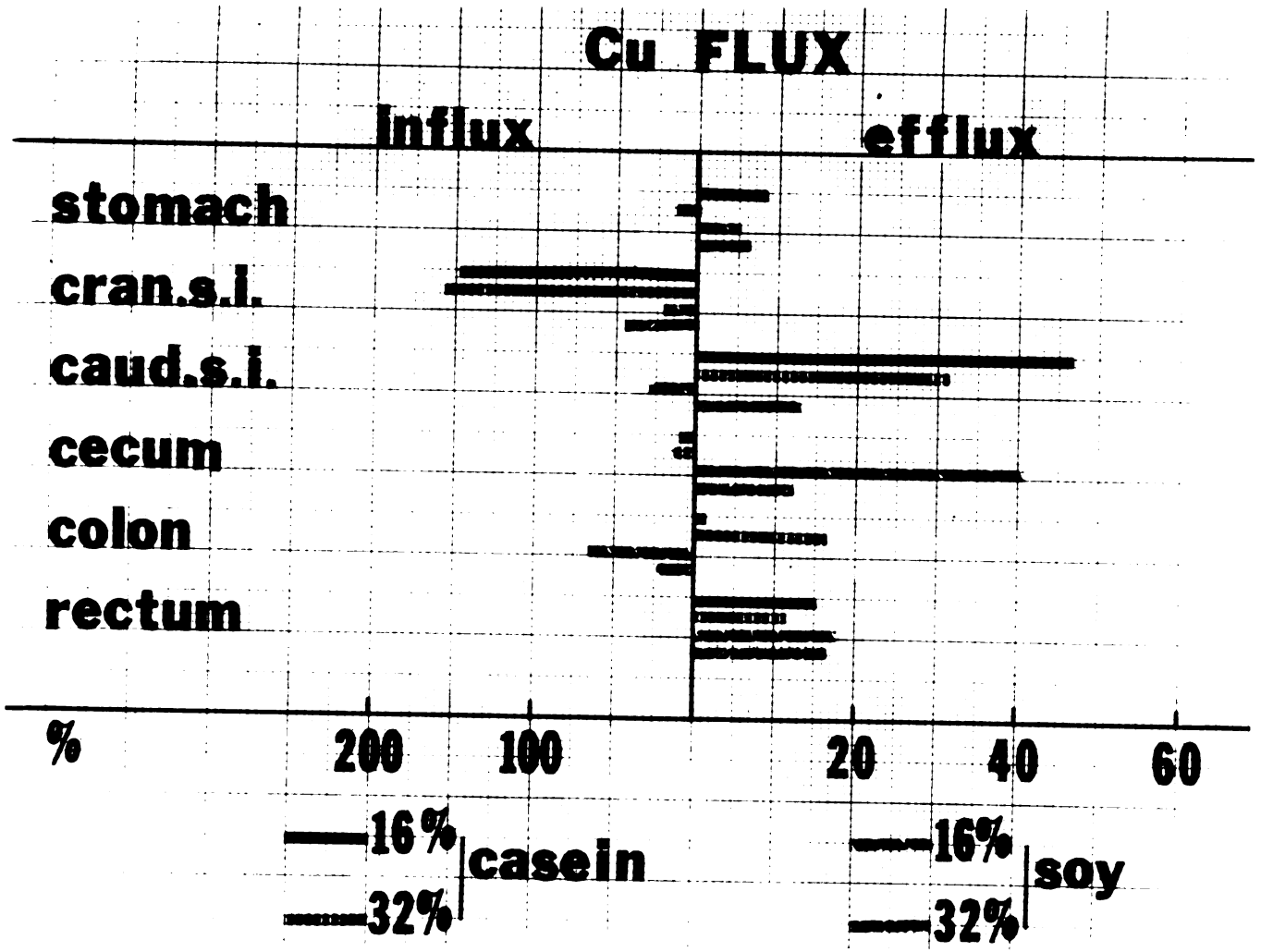

Figure 16. Exp. IV. -- Influx and efflux of copper along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

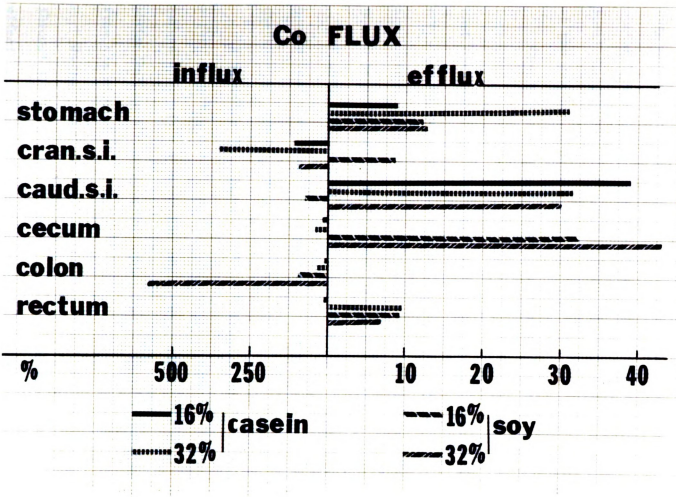

Figure 17. Exp. IV. -- Influx and efflux of cobalt along the GI tract of pigs fed casein or soy protein at two different levels of dietary protein.

length of fast prior to slaughter (Appendix 3) had a significant effect on the pH (Appendix 4) and the absorption of nutrients within the stomach and cranial small intestine. Graham et al. (1959) showed that ^{28}Mg absorption began within an hour of ingestion in man. Smith (1959) states that samples taken from the small intestine of a calf close to the cecal junction between 2 and 6 hours after feeding contained 70% to 80% of the residue from the feed.

Casein diets appear to promote secretions into the cranial small intestine, whereas the soy diets promote absorption of dry matter within that section of the GI tract. Within the caudal small intestine, however, dry matter from casein was readily absorbed. There was a highly significant difference between the rates of absorption of the dry matter due to protein source in this section of the tract.

Figure 8 shows that Ca movement within the GI tract was variable. Absorption of Ca from the casein diets was greater than Ca from the soy diets in the caudal small intestine.

Phosphorus was secreted into the cranial small intestine and absorbed from the caudal small intestine to a greater extent when the casein diet was fed than when soy protein was in the diet. Figure 9 shows that by the method used about 60% of the dietary P is absorbed from the stomach.

Sodium and K followed each other rather closely during passage through the GI tract. Figures 10 and 11 show that the only dietary effect on the assimilation of Na and K into the body was a large influx of both nutrients into the cranial small intestine when casein diets were fed.

Magnesium influx or efflux along the GI tract closely paralleled

that of P (see Figures 9 and 12). The magnitude of Mg movement, however, was generally not as great as that of P. Statistical analysis showed that Mg influx into the cranial small intestine was greater in pigs fed casein than in pigs fed soy protein diets ($P < 0.05$). Efflux of Mg from the caudal small intestine was also greater in pigs fed casein diets.

Iron absorption from the caudal small intestine was significantly greater in casein fed pigs than in those receiving soy protein diets ($P < 0.05$) (Figure 13). Zinc, Mn and Cu showed the same response to dietary treatment as observed in iron movement (Figures 14, 15, 16). In each case the influx of the respective mineral into the cranial small intestine was enhanced when casein was the source of dietary protein. As a consequence of the greater influx of minerals into the cranial small intestine, there was more mineral available to be absorbed in the caudal small intestine. Iron, Zn, Mn and Cu all show a greater magnitude of efflux from the caudal small intestine when casein was the protein source. Level of protein in the diet did not greatly affect the absorption or secretion of any of these minerals.

Results of the Co flux studies are varied (Figure 17). Cobalt appears to differ from the other minerals studied in that it was absorbed from the cecum when isolated soy was the source of protein but not when casein was fed.

Figure 18 summarizes the absorption and secretion of nutrients along the GI tract as determined by the chromic oxide ratio technique. Due to the high degree of variation of absorption and secretion, only minerals showing net absorption or secretion of greater than 10% are shown in Figure 18. Any absorption from the cranial small intestine is

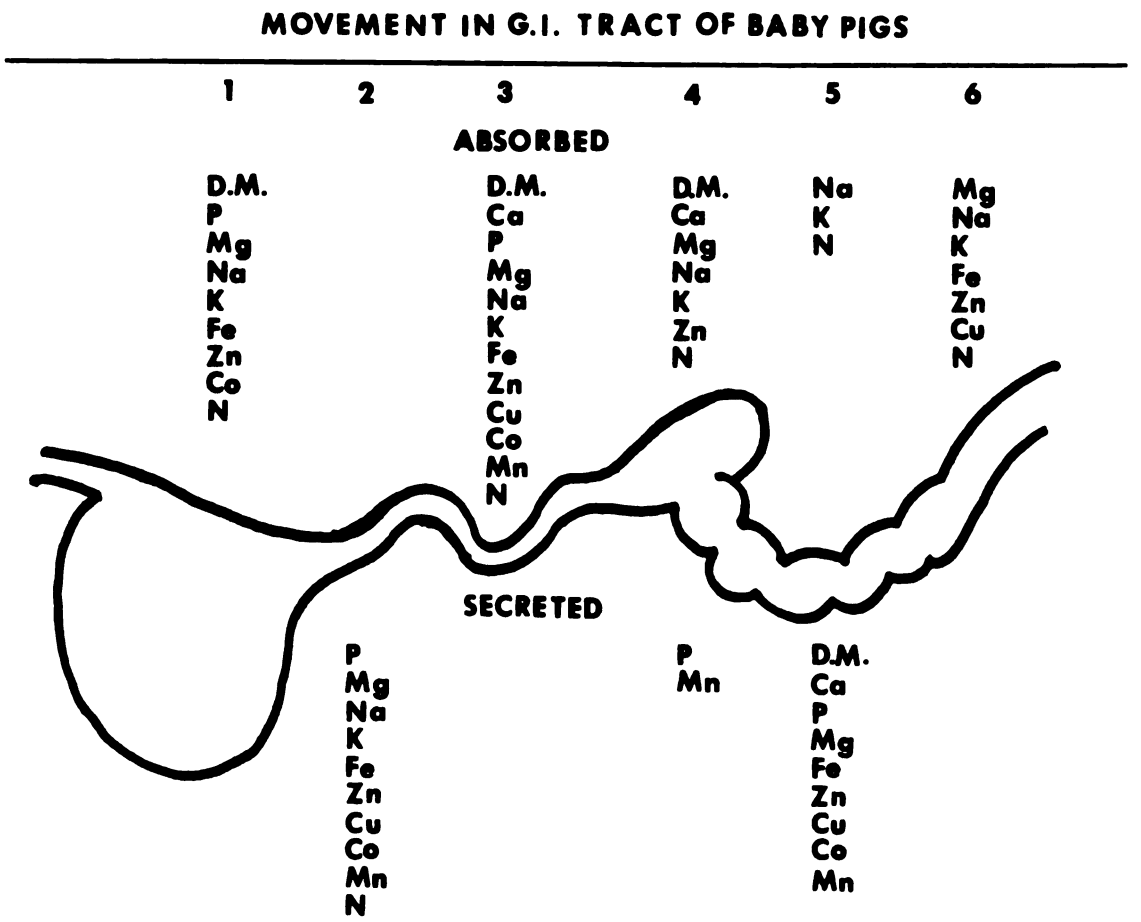

Figure 18. Exp. IV. -- Summary of net nutrient influx or efflux from sections of the gastrointestinal tract of baby pigs as determined by the Bergeim (1926) method.

masked by the large influx of nutrients via the duodenal, pancreatic and biliary secretions. What may be indicated by these data, rather than absorption within the stomach and an influx into the cranial small intestine, is a difference in the rates of nutrient passage from one section of the tract to another. Ali (1967) reported that ^{45}Ca moves out of the stomach of rats at the same rate as chromic oxide. Figure 8 shows that Ca is not absorbed in the stomach nor is there a great influx of Ca into the cranial small intestine. Phosphorus and the other minerals studied, however, appeared to be largely absorbed in the stomach and resecreted into the cranial small intestine. If these nutrients were to pass out of the stomach more rapidly than the indicator, this same pattern of absorption and secretion would be observed.

Gorrill, et al. (1967) have shown that volume of pancreatic secretion of calves fed milk diets was significantly greater than that of calves fed soy protein diets. The magnitude of the difference in the two diets appeared to increase with age. If these data can be applied to pigs, it would partly explain the greater influx of nutrients into the cranial small intestine when the casein diet was fed.

V. SUMMARY

Four experiments, involving a total of 52 baby pigs, were conducted to study the effects of level of protein (16%, 24%, 32%), level of ergocalciferol (6.25 or 12.50 μg/kg diet) and source of protein (C-1 isolated soy protein or vitamin-free casein) on mineral utilization by the baby pig.

Experiment I

Twelve baby pigs were assigned to 3 levels of dietary protein, with 4 pigs per treatment. Diets contained 20%, 30%, or 40% isolated soy protein and 6.25 μg/kg ergocalciferol. Growth response was the same for all treatments. Serum inorganic P and alkaline phosphatase concentration indicated superior P utilization occurred in pigs receiving only 16% crude protein in the diet. Femur mineral and strength data showed that bones of pigs from the low protein lot contained more mineral and were stronger than those from pigs fed either 24% or 32% crude protein in the diet. A 72-hour mineral balance study did not bear this out.

Experiment II

Eight baby pigs were assigned, 2 per lot, to the following dietary treatments: 24% crude protein with 6.25 μg ergocalciferol/kg diet, 32% crude protein with 6.25 μg ergocalciferol/kg diet, 24% crude protein with 12.50 μg ergocalciferol/kg diet or 32% crude protein with 12.50 μg ergocalciferol/kg diet. The protein source in all diets was isolated soy protein. Performance was similar in all pigs irrespective of dietary treatment. Neither serum analyses nor mineral balance studies revealed

any treatment effects. Femur breaking strength appeared to be reduced at the higher level of vitamin D intake. However, when breaking strength was placed on the basis of bone cross sectional area, or correcting for bone size, it was shown that vitamin D had little effect but the higher levels of protein intake tended to reduce the femur strength.

Organ weight data showed that kidneys and liver were hypertrophied in direct proportion to dietary protein intake. Urinary N excretion was also reflected in the relative size of these organs.

Experiment III

Sixteen 7-day old pigs were allotted in lots of 4. Dietary treatments were similar to Experiment II except that 16% crude protein was the low level of protein intake rather than 24%. Feed intake and feed efficiency was 8% to 10% higher in pigs fed 32% crude protein diets. Dietary intake of vitamin D had no effect on performance. Mineral balance studies showed a slightly reduced retention of minerals when high levels of isolated soy were fed in the diet. Vitamin D treatment had no effect on mineral retention. Bone analyses showed that mineral deposition, and consequently bone strength, were reduced when 40% of the diet was made up of isolated soy protein. High levels of ergocalciferol did not improve mineral deposition in the bones of pigs fed the high protein diets.

Weights of the kidneys, pancreas and thymus were increased in pigs fed the high protein diets. Spleen weight, in this experiment, was inversely related to level of protein intake.

Experiment IV

Four lots of 4 baby pigs each were assigned to dietary treatments

of 20% or 40% casein or 20% or 40% isolated soy. Crude protein content was 16% and 32% in the low and high protein diets, respectively. Feed intake was 20% lower when high levels of protein were fed. Growth was not affected by dietary treatment. Serum analyses showed that serum inorganic P was reduced and alkaline phosphatase correspondingly increased when high levels of soy protein were fed.

Mineral balance studies showed that Ca and Mg retention were greater when casein was the source of dietary protein. Increasing casein content of the diet enhanced the utilization of Ca, P and Mg. Increasing the amount of isolated soy protein in the diet, however, was found to suppress the utilization of these minerals. Differences in retention and excretion of Na, K, Fe, Zn, Mn, Cu, Co or Cr could not be attributed to dietary treatment.

Optimum bone mineralization did not occur when soy protein was fed at 40% of the diet. Twenty percent isolated soy protein in the diet, however, allowed proper utilization of mineral for compact bone formation as did casein diets at either level of protein intake.

Weight of kidneys, liver and pancreas increased as protein level in the diet increased. Protein source did not appear to affect the weight of any of the organs or glands that were studied.

When studying the absorption and secretion of nutrients along the alimentary tract by the indicator ratio technique, it was found that casein in the diet, regardless of level, results in an increased influx of minerals into the cranial small intestine and a compensatory efflux of minerals from the caudal small intestine. Due to a high degree of variation within treatment groups, specific conclusions pertaining to individual minerals were difficult to make.

VI. CONCLUSIONS

Within the limits of the experimental conditions employed, the results of this study have led the author to make the following conclusions:

1. When dietary P is held constant, increasing the level of isolated soy protein in the diet results in poorer utilization of Ca, P and Mg in the diet.
2. Increasing ergocalciferol in the diet from 6.25 to 12.50 g/kg diet will not overcome the suppressing effects of high levels of isolated soy protein on mineral utilization.
3. Increasing casein content of the diet results in improved retention of Ca, P and Mg.
4. Components of the protein source other than the protein fraction itself may affect mineral utilization in the diet, for example, phytate in soybean protein and lactose in milk protein.
5. Physiological responses of the body to high protein intakes include mild hypertrophy of the kidneys, liver and pancreas.
6. The rapidly growing baby pig adapts well to limited dietary alterations, therefore, single short term mineral balance trials are not always reliable as a measure of mineral utilization during an entire experimental period. A measure of the total and specific mineral deposition in bone or other tissues may be more indicative of mineral utilization.

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Appendix Table 1. Dilutions used for analyses[1].

Mineral	Serum[4]	Feed	Feces	Urine	Strontium[5]	Å[6]
Ca	5	500	4000	0	+	4227
P[2]	10	100	1000	10	-	7000
Mg	20	500	4000	10	+	2852
Na[3]	-	600	1000	200	-	5890
K[3]	-	600	1000	200	-	7665
Fe	-	100	400	0	-	2483
Zn	-	100	400	0	-	2139
Mn	-	100	100	0	+	2795
Cu	-	100	100	0	-	3248
Co	-	100	100	0	-	2407
Cr[3]	-	400	2000	0	-	4254

1 Diluted with deionized distilled water.
2 Determined spectrophotometrically.
3 Determined by flame emission.
4 Dilution after precipitation of 1 ml of serum with 4 ml 12.5% T.C.A.
5 Added at 10,000 ppm Sr + 1% NaCl to overcome interferences.
6 Wave length at which absorption or emission was read. (Robinson, 1966)

Appendix Table 2. Intake and excreta of pigs during balance trial, Exp. IV.[1]

Treatment	Pig No.	Pig wt, kg	Feed, g	Water, ml	Feces, g[2]	Urine, ml
16% Casein	8-2	12.66	1022	2700	73.3	1460
	8-8	11.94	1022	2700	73.5	1100
	9-3	11.36	702	1881	30.2	
	9-12	11.48	822	2250	58.2	1400
32% Casein	8-4	11.86	1021	2675	54.4	1410
	8-9	13.00	1021	2675	34.5	1170
	9-1	10.78	1025	2700	137.0	1885
	9-8	8.34	1025	2700	69.8	1920
16% Soy	8-5	13.26	1032	2700	38.5	1850
	8-7	12.12	1027	2675	75.1	1440
	9-2	10.80	742	1846	43.6	1180
	9-10	11.24	825	2250	48.6	1225
32% Soy	8-3	11.40	1041	2700	95.3	1560
	8-6	8.98	808	2176	62.1	1460
	9-6	13.20	1040	2700	75.6	1920
	9-9	12.28	1040	2700	77.8	1830

[1] Intake or excreta/72 hrs.

[2] Oven dry weight.

Appendix Table 3. Slaughter sequence of pigs from Exp. IV.

	Day 1	2	3	4
A M				
1½ hrs post feeding	9-12[1]	9-8	8-5	8-6
3 hrs post feeding	9-1	9-10	8-3	8-8
P M				
1½ hrs post feeding	9-2	9-9	8-2	8-9
3 hrs post feeding	9-6	9-3	8-4	8-7

[1] Pig identification number.

Appendix Table 4. pH within the GI tract of baby pigs, Exp. IV.

Treatment	Pig No.	Stomach	Cranial SI	Caudal SI	Cecum	Colon
16% Casein	8-2 F[1]	5.3	5.3	6.9	6,4	7.5
	8-8 M	4.1	5.5	6.2	6.5	6.6
	9-3 F	3.6	5.2	7.0	6.8	7.3
	9-12 M	4.6	5.1	6.8	6.9	7.1
32% Casein	8-4 F	4.4	5.8	6.6	6.6	7.2
	8-9 M	4.8	5.7	6.7	7.3	6.3
	9-1 F	4.5	5.5	5.6	6.5	6.4
	9-8 M	4.6	5.7	6.5	6.4	6.4
16% Soy	8-5 F	6.0	5.6	6.9	6.9	6.8
	8-7 M	3.0	5.4	6.5	6.2	7.2
	9-2 F	4.6	5.9	6.1	6.5	6.5
	9-10 M	4.1	5.6	6.4	6.5	6.1
32% Soy	8-3 F	4.0	5.5	6.9	6.6	6.5
	8-6 M	5.2	5.5	6.5	6.3	6.5
	9-6 F	5.1	5.5	6.3	5.0	6.7
	9-9 M	4.8	5.3	7.1	6.6	6.6

[1] F = female; M = male.

Appendix Table 5. Dry matter concentration within the GI tract of baby pigs, Exp. IV[1].

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon
16% Casein	8-2	26.9	19.1	4.8	13.2	27.6	40.0
	8-8	26.9	19.4	3.1	3.6	10.7	32.1
	9-3	26.1	17.2	10.9	16.2	22.4	41.6
	9-12	26.1	14.4	7.5	12.4	20.2	34.2
32% Casein	8-4	26.8	23.7	14.9	14.0	18.3	32.2
	8-9	26.8	22.8	6.4	12.8	39.2	25.2
	9-1	26.8	28.3	6.1	3.6	6.4	20.2
	9-8	26.8	24.5	6.3	7.1	12.4	22.2
16% Soy	8-5	27.0	16.2	6.7	12.6	19.5	42.8
	8-7	27.0	20.3	4.5	9.5	18.4	40.8
	9-2	27.0	15.6	4.5	5.3	12.9	24.8
	9-10	26.2	21.0	6.7	7.0	23.7	36.8
32% Soy	8-3	27.2	14.6	8.3	14.7	24.0	35.6
	8-6	26.4	24.6	3.6	10.4	25.8	30.4
	9-6	27.2	19.9	8.5	8.0	16.8	34.5
	9-9	27.2	23.2	5.0	6.9	10.0	14.8

[1] Expressed as a percent of material within the portion of GI tract.

Appendix Table 6. Calcium concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.83	1.52	0.33	1.58	2.74	2.38	3.87
	8-8	0.83	1.54	0.39	1.81	2.71	2.72	3.16
	9-3	0.83	1.50	0.50	1.68	2.26	2.93	2.59
	9-12	0.83	1.71	0.57	1.53	2.22	2.25	3.17
32% Casein	8-4	0.82	1.34	0.15	1.12	1.67	2.90	2.31
	8-9	0.82	1.36	0.31	0.45	1.49	1.24	1.66
	9-1	0.82	1.11	0.86	0.49	1.28	2.83	1.90
	9-8	0.82	0.89	0.62	0.79	1.66	2.53	2.36
16% Soy	8-5	0.81	1.42	1.13	1.73	2.05	3.90	5.81
	8-7	0.81	1.84	1.14	2.03	3.69	3.92	6.21
	9-2	0.81	1.19	0.89	1.11	3.29	4.02	5.37
	9-12	0.81	1.58	0.95	0.48	2.29	3.07	4.13
32% Soy	8-3	0.84	1.41	0.90	2.12	1.65	4.12	5.73
	8-6	0.84	1.44	0.78	1.80	2.82	3.55	4.91
	9-6	0.84	1.39	0.87	1.37	3.32	4.51	6.03
	9-9	0.84	1.33	0.95	0.81	2.37	3.57	5.70

[1] Expressed as a percent of dry matter within portion of GI tract.

Appendix Table 7. Phosphorus concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.65	0.40	0.82	1.23	1.59	1.04	2.21
	8-8	0.65	0.45	0.51	1.24	1.61	1.62	1.97
	9-3	0.65	0.37	1.02	1.24	1.15	1.35	1.62
	9-12	0.65	0.46	1.00	1.49	1.31	1.30	1.90
32% Casein	8-4	0.63	0.38	1.06	0.94	0.61	1.05	1.03
	8-9	0.63	0.35	0.98	0.91	0.62	0.39	0.85
	9-1	0.63	0.50	0.81	0.91	1.18	1.31	0.89
	9-8	0.63	0.34	1.00	0.97	1.32	1.51	1.14
16% Soy	8-5	0.60	0.35	1.17	1.54	1.46	2.29	3.38
	8-7	0.60	0.50	0.92	1.54	2.37	2.48	3.36
	9-2	0.60	0.39	0.91	1.28	2.34	2.48	3.11
	9-10	0.60	0.38	0.88	0.81	1.70	2.01	2.74
32% Soy	8-3	0.55	0.38	1.04	1.53	1.11	2.13	2.90
	8-6	0.55	0.31	0.68	1.26	1.92	2.05	2.88
	9-6	0.55	0.32	0.85	1.15	2.06	2.35	2.86
	9-9	0.55	0.34	0.84	1.08	1.68	2.00	2.89

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 8. Sodium concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.42	0.56	2.93	1.92	0.60	0.54	0.45
	8-8	0.42	0.35	5.05	6.22	0.54	0.50	0.45
	9-3	0.42	0.58	1.98	1.58	1.04	0.41	0.17
	9-12	0.42	0.59	1.57	1.83	1.19	0.64	0.31
32% Casein	8-4	0.46	0.17	1.10	1.62	1.50	0.47	0.31
	8-9	0.46	0.36	2.46	1.74	0.33	0.64	0.41
	9-1	0.46	0.35	2.98	6.53	3.87	0.99	0.32
	9-8	0.46	0.39	3.07	3.82	2.16	0.68	0.43
16% Soy	8-5	0.48	0.70	2.59	2.13	1.33	0.39	0.44
	8-7	0.48	0.42	3.24	2.32	1.15	0.58	0.37
	9-2	0.48	0.63	4.59	5.13	2.09	0.91	0.26
	9-10	0.48	0.36	2.54	3.40	1.10	0.49	0.29
32% Soy	8-3	0.49	0.29	2.22	1.79	1.23	0.46	0.30
	8-6	0.49	0.65	4.43	2.31	0.95	0.71	0.38
	9-6	0.49	0.71	1.84	3.27	1.95	0.69	0.22
	9-9	0.49	0.38	3.78	3.94	3.01	1.90	0.51

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 9. Potassium concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.29	0.40	2.02	0.71	0.24	0.20	0.22
	8-8	0.29	0.35	1.37	1.31	0.59	0.61	0.14
	9-3	0.29	0.41	1.69	0.61	0.32	0.35	0.49
	9-12	0.29	0.44	1.63	1.13	0.43	0.32	0.33
32% Casein	8-4	0.33	0.24	1.43	1.06	0.31	0.25	0.21
	8-9	0.33	0.31	1.91	1.22	0.54	0.46	0.14
	9-1	0.33	0.18	1.25	1.62	1.14	0.83	0.30
	9-8	0.33	0.26	1.67	0.86	1.07	1.18	0.39
16% Soy	8-5	0.30	0.41	1.61	0.94	0.40	0.29	0.19
	8-7	0.30	0.34	1.40	0.72	0.31	0.22	0.21
	9-2	0.30	0.43	1.34	1.23	0.56	0.41	0.29
	9-10	0.30	0.28	0.78	0.89	0.36	0.40	0.25
32% Soy	8-3	0.29	0.40	1.50	0.74	0.40	0.65	0.28
	8-6	0.29	0.33	1.97	0.91	0.47	0.53	0.79
	9-6	0.29	0.35	1.29	1.00	0.37	0.43	0.34
	9-9	0.29	0.30	1.54	1.48	0.56	0.56	0.19

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 10. Magnesium concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.037	0.045	0.170	0.142	0.352	0.220	0.178
	8-8	0.037	0.035	0.115	0.171	0.270	0.280	0.186
	9-3	0.037	0.037	0.149	0.182	0.239	0.171	0.165
	9-12	0.037	0.038	0.107	0.207	0.153	0.194	0.202
32% Casein	8-4	0.035	0.030	0.100	0.156	0.110	0.147	0.103
	8-9	0.035	0.037	0.159	0.134	0.153	0.174	0.138
	9-1	0.035	0.020	0.113	0.159	0.155	0.241	0.108
	9-8	0.035	0.034	0.151	0.103	0.209	0.324	0.150
16% Soy	8-5	0.038	0.054	0.132	0.152	0.193	0.270	0.237
	8-7	0.038	0.059	0.142	0.236	0.463	0.328	0.223
	9-2	0.038	0.045	0.111	0.114	0.250	0.265	0.247
	9-10	0.038	0.041	0.083	0.156	0.243	0.239	0.215
32% Soy	8-3	0.040	0.041	0.166	0.185	0.142	0.222	0.236
	8-6	0.040	0.054	0.189	0.200	0.203	0.230	0.224
	9-6	0.040	0.044	0.117	0.128	0.266	0.261	0.216
	9-9	0.040	0.043	0.186	0.142	0.133	0.164	0.213

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 11. Iron concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0111	0.0117	0.0101	0.0527	0.0784	0.1295	0.0852
	8-8	0.0111	0.0126	0.0134	0.0397	0.1178	0.1202	0.0888
	9-3	0.0111	0.0096	0.0270	0.0608	0.1072	0.1295	0.0588
	9-12	0.0111	0.0139	0.0250	0.0366	0.0745	0.0952	0.0707
32% Casein	8-4	0.0106	0.0180	0.0118	0.0570	0.1288	0.1644	0.1024
	8-9	0.0106	0.0115	0.0236	0.0298	0.1197	0.1151	0.0991
	9-1	0.0106	0.0097	0.0188	0.0253	0.0366	0.0834	0.0934
	9-8	0.0106	0.0077	0.0174	0.0229	0.0494	0.0944	0.0851
16% Soy	8-5	0.0143	0.0149	0.0319	0.0376	0.1016	0.1483	0.0973
	8-7	0.0143	0.0207	0.0294	0.0725	0.1286	0.1383	0.1084
	9-2	0.0143	0.0142	0.0297	0.0360	0.1147	0.1726	0.0939
	9-10	0.0143	0.0242	0.0431	0.0613	0.1495	0.2118	0.1117
32% Soy	8-3	0.0194	0.0192	0.0390	0.0668	0.0845	0.1576	0.1298
	8-6	0.0194	0.0191	0.0241	0.0428	0.1367	0.1507	0.1078
	9-6	0.0194	0.0200	0.0271	0.0379	0.1143	0.1519	0.1311
	9-9	0.0194	0.0166	0.0260	0.0268	0.0899	0.1325	0.1163

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 12. Zinc concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0090	0.0218	0.0266	0.0856	0.0976	0.1346	0.0736
	8-8	0.0090	0.0126	0.0156	0.0513	0.1387	0.1534	0.0840
	9-3	0.0090	0.0111	0.0548	0.0819	0.0925	0.1112	0.0704
	9-12	0.0090	0.0109	0.0131	0.0575	0.0716	0.0780	0.0768
32% Casein	8-4	0.0102	0.0173	0.0242	0.0557	0.0793	0.1082	0.1021
	8-9	0.0102	0.0204	0.0192	0.0813	0.1005	0.1044	0.0990
	9-1	0.0102	0.0076	0.0130	0.0273	0.0489	0.0876	0.1202
	9-8	0.0102	0.0085	0.0104	0.0209	0.0545	0.0664	0.0978
16% Soy	8-5	0.0092	0.0104	0.0170	0.0241	0.0613	0.0920	0.0832
	8-7	0.0092	0.0173	0.0189	0.0400	0.0606	0.0793	0.0764
	9-2	0.0092	0.0111	0.0138	0.0312	0.0873	0.1144	0.0968
	9-10	0.0092	0.0142	0.0117	0.0381	0.1068	0.1352	0.0808
32% Soy	8-3	0.0094	0.0103	0.0233	0.0387	0.0423	0.0683	0.0642
	8-6	0.0094	0.0106	0.0177	0.0331	0.0631	0.0720	0.0652
	9-6	0.0094	0.0104	0.0107	0.0232	0.0638	0.1039	0.0702
	9-9	0.0094	0.0166	0.0129	0.0197	0.0661	0.0703	0.0788

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 13. Manganese concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0025	0.0026	0.0023	0.0116	0.0302	0.0299	0.0296
	8-8	0.0025	0.0026	0.0041	0.0053	0.0419	0.0439	0.0285
	9-3	0.0025	0.0034	0.0061	0.0136	0.0302	0.0316	0.0289
	9-12	0.0025	0.0028	0.0026	0.0069	0.0230	0.0324	0.0318
32% Casein	8-4	0.0025	0.0050	0.0060	0.0268	0.0283	0.0357	0.0414
	8-9	0.0025	0.0028	0.0027	0.0124	0.0359	0.0260	0.0517
	9-1	0.0025	0.0007	0.0026	0.0041	0.0123	0.0186	0.0530
	9-8	0.0025	0.0043	0.0048	0.0073	0.0084	0.0222	0.0365
16% Soy	8-5	0.0025	0.0026	0.0029	0.0051	0.0218	0.0422	0.0298
	8-7	0.0025	0.0038	0.0022	0.0103	0.0220	0.0223	0.0303
	9-2	0.0025	0.0034	0.0047	0.0063	0.0241	0.0246	0.0336
	9-10	0.0025	0.0033	0.0032	0.0106	0.0251	0.0253	0.0370
32% Soy	8-3	0.0025	0.0021	0.0049	0.0092	0.0123	0.0241	0.0273
	8-6	0.0025	0.0026	0.0011	0.0053	0.0194	0.0216	0.0335
	9-6	0.0025	0.0060	0.0030	0.0084	0.0225	0.0470	0.0394
	9-9	0.0025	0.0021	0.0046	0.0034	0.0151	0.0222	0.0355

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 14. Copper concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0022	0.0030	0.0014	0.0102	0.0209	0.0277	0.0275
	8-8	0.0022	0.0048	0.0025	0.0085	0.0258	0.0268	0.0273
	9-3	0.0022	0.0029	0.0082	0.0160	0.0261	0.0293	0.0231
	9-12	0.0022	0.0035	0.0029	0.0134	0.0224	0.0261	0.0235
32% Casein	8-4	0.0020	0.0023	0.0023	0.0098	0.0222	0.0264	0.0301
	8-9	0.0020	0.0030	0.0030	0.0120	0.0250	0.0179	0.0285
	9-1	0.0020	0.0026	0.0026	0.0053	0.0127	0.0160	0.0265
	9-8	0.0020	0.0014	0.0014	0.0057	0.0135	0.0155	0.0220
16% Soy	8-5	0.0024	0.0032	0.0032	0.0083	0.0221	0.0290	0.0302
	8-7	0.0024	0.0040	0.0040	0.0146	0.0246	0.0259	0.0286
	9-2	0.0024	0.0049	0.0049	0.0069	0.0212	0.0269	0.0225
	9-10	0.0024	0.0062	0.0062	0.0120	0.0226	0.0284	0.0275
32% Soy	8-3	0.0026	0.0040	0.0040	0.0094	0.0156	0.0250	0.0269
	8-6	0.0026	0.0041	0.0041	0.0077	0.0263	0.0239	0.0273
	9-6	0.0026	0.0027	0.0027	0.0058	0.0202	0.0279	0.0266
	9-9	0.0026	0.0039	0.0039	0.0044	0.0161	0.0231	0.0228

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 15. Cobalt concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0018	0.0028	0.0006	0.0072	0.0113	0.0172	0.0180
	8-8	0.0018	0.0022	0.0016	0.0069	0.0174	0.0175	0.0217
	9-3	0.0018	0.0027	0.0032	0.0081	0.0161	0.0173	0.0188
	9-12	0.0018	0.0029	0.0012	0.0047	0.0119	0.0145	0.0191
32% Casein	8-4	0.0017	0.0026	0.0016	0.0079	0.0154	0.0207	0.0181
	8-9	0.0017	0.0022	0.0010	0.0054	0.0177	0.0159	0.0215
	9-1	0.0017	0.0013	0.0021	0.0056	0.0055	0.0129	0.0219
	9-8	0.0017	0.0012	0.0022	0.0022	0.0061	0.0099	0.0211
16% Soy	8-5	0.0016	0.0022	0.0023	0.0042	0.0089	0.0144	0.0127
	8-7	0.0016	0.0036	0.0026	0.0080	0.0125	0.0135	0.0193
	9-2	0.0016	0.0021	0.0009	0.0038	0.0129	0.0172	0.0179
	9-10	0.0016	0.0027	0.0027	0.0042	0.0132	0.0160	0.0173
32% Soy	8-3	0.0018	0.0020	0.0033	0.0063	0.0005	0.0128	0.0137
	8-6	0.0018	0.0028	0.0027	0.0059	0.0104	0.0113	0.0120
	9-6	0.0018	0.0026	0.0017	0.0029	0.0094	0.0116	0.0142
	9-9	0.0018	0.0021	0.0031	0.0024	0.0068	0.0107	0.0141

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 14. Copper concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.0022	0.0030	0.0014	0.0102	0.0209	0.0277	0.0275
	8-8	0.0022	0.0048	0.0025	0.0085	0.0258	0.0268	0.0273
	9-3	0.0022	0.0029	0.0082	0.0160	0.0261	0.0293	0.0231
	9-12	0.0022	0.0035	0.0029	0.0134	0.0224	0.0261	0.0235
32% Casein	8-4	0.0020	0.0023	0.0023	0.0098	0.0222	0.0264	0.0301
	8-9	0.0020	0.0030	0.0030	0.0120	0.0250	0.0179	0.0285
	9-1	0.0020	0.0026	0.0026	0.0053	0.0127	0.0160	0.0265
	9-8	0.0020	0.0014	0.0014	0.0057	0.0135	0.0155	0.0220
16% Soy	8-5	0.0024	0.0032	0.0032	0.0083	0.0221	0.0290	0.0302
	8-7	0.0024	0.0040	0.0040	0.0146	0.0246	0.0259	0.0286
	9-2	0.0024	0.0049	0.0049	0.0069	0.0212	0.0269	0.0225
	9-10	0.0024	0.0062	0.0062	0.0120	0.0226	0.0284	0.0275
32% Soy	8-3	0.0026	0.0040	0.0040	0.0094	0.0156	0.0250	0.0269
	8-6	0.0026	0.0041	0.0041	0.0077	0.0263	0.0239	0.0273
	9-6	0.0026	0.0027	0.0027	0.0058	0.0202	0.0279	0.0266
	9-9	0.0026	0.0039	0.0039	0.0044	0.0161	0.0231	0.0228

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 16. Chromium concentration within the GI tract of baby pigs, Exp. IV.[1]

Treatment	Pig No.	Feed	Stomach	Cranial SI	Caudal SI	Cecum	Colon	Feces
16% Casein	8-2	0.33	0.48	0.10	2.24	4.62	4.12	4.22
	8-8	0.33	1.01	0.16	1.31	3.26	3.32	4.36
	9-3	0.33	0.41	0.58	2.56	2.56	4.40	4.46
	9-12	0.33	0.55	0.18	0.90	2.72	4.15	4.29
32% Casein	8-4	0.36	0.57	0.07	1.14	3.55	3.78	4.52
	8-9	0.36	0.46	0.15	1.45	1.44	3.38	5.12
	9-1	0.36	0.82	0.29	0.45	1.92	2.38	5.08
	9-8	0.36	0.64	0.17	0.89	1.79	2.28	4.11
16% Soy	8-5	0.33	0.41	0.44	1.03	3.98	3.29	3.42
	8-7	0.33	0.67	0.49	1.68	4.27	2.90	3.84
	9-2	0.33	0.70	0.57	0.76	6.91	3.07	3.66
	9-10	0.33	0.87	0.73	0.77	2.04	3.53	4.60
32% Soy	8-3	0.33	0.54	0.39	1.24	2.86	2.73	3.30
	8-6	0.33	0.44	0.25	0.75	3.35	2.81	3.45
	9-6	0.33	0.46	0.35	0.69	1.79	3.03	3.34
	9-9	0.33	0.60	0.41	0.44	2.34	2.56	3.78

[1] Expressed as a percent of dry matter within the portion of GI tract.

Appendix Table 17. Comparison of indicator and balance trial methods for determining apparent digestibility of nutrients, Exp. IV.

Nutrient	Method	16% Casein	32% Casein	16% Soy	32% Soy
Ca	Indicator	70	80	42	37
	Balance	75	82	62	47
P	Indicator	77	88	54	50
	Balance	81	89	70	59
Na	Indicator	94	94	94	93
	Balance	95	94	96	94
K	Indicator	92	94	93	91
	Balance	95	94	97	91
Mg	Indicator	62	72	47	47
	Balance	65	73	62	49
Fe	Indicator	47	34	37	37
	Balance	55	35	58	51
Zn	Indicator	35	20	18	27
	Balance	45	29	47	49
Mn	Indicator	-14	-74	-41	-69
	Balance	33	-35	25	- 6
Cu	Indicator	3	10	7	- 4
	Balance	24	4	36	21
Co	Indicator	18	7	10	29
	Balance	29	11	42	42
N	Indicator	97	98	96	97
	Balance	98	98	98	97

Appendix Table 18. Mineral mixtures used in purified diets.

	16% crude protein	24% crude protein	32% crude protein
	%	%	%
KCl	10	10	10
KI	0.002	0.002	0.002
$FeSO_4 \cdot 2H_2O$	0.7	0.7	0.7
$CuSO_4$	0.1	0.1	0.1
$CoCO_3$	0.1	0.1	0.1
$MnSO_4 \cdot H_2O$	0.1	0.1	0.1
$ZnSO_4 \cdot H_2O$	0.4	0.4	0.4
$MgCO_3$	2.0	2.0	2.0
$NaHCO_3$	25.0	25.0	25.0
$CaHPO_4 \cdot 2H_2O$[1]	42.4	36.0	29.6
$CaCO_3$[1]	8,8	12.5	16.2
Glucose[1]	10.4	13.1	15.8

[1] Adjusted to keep phosphorus level constant in all diets.

Appendix Table 19. Vitamin mixtures used in purified diets.

	mg/kg diet
Thiamine·mononitrate	3
Riboflavin	6
Nicotinamide	40
Calcium pantothenate	30
Pyridoxine·hydrochloride	2
p-Amino benzoic acid	13
Ascorbic acid	80
a-Tocopheryl acetate	10
Inositol	130
Choline chloride	1300
	μg/kg diet
Pteroylglutamic acid	260
Biotin	50
Cyanocobalamin	100
2-Methyl-1,4-naphthoquinone	40
Retinyl palmitate	600
Ergocalciferol	6.25 or 12.50[1]

[1] Added at 6.25 or 12.50 μg/kg for each diet.