THE AVAILABILITY OF CALCIUM AND PHOSPHORUS IN FEEDSTUFFS FOR RUMINANTS

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INTRODUCTION

The need for minerals in the diet of animals has long been established. Two of the macro or major elements required are calcium and phosphorus. These two elements are essential to the growth, maintenance, reproduction and production requirements of all animals.

The nutrient requirement tables for cattle list the requirements for protein as both total protein and digestible protein while calcium and phosphorus requirements are listed as only total grams or as a percentage of the total ration. Feed composition tables indicate that feeds vary in the amounts of total and digestible protein that they contain. The ratio of these two components is not always the same. It seems logical therefore to expect the amount of digestible calcium and phosphorus to show seasonal and other variations, however, the composition tables show only the total percentage of calcium and phosphorus with no regard to differences in availability which may occur.

This report was prepared in an attempt to determine if experiments have been conducted to examine the amounts of calcium and phosphorus that are available from various feedstuffs and to summarize some of the factors which may affect availability.

The availability of calcium and phosphorus for a particular species depends on both the source feedstuff and the animal. Feedstuffs vary greatly in total calcium and phosphorus depending on their species, maturity, origin and other factors. Calcium and phosphorus are found in many chemical forms and combinations; some more readily available to the animal than others. The levels as well as ratios of calcium to phosphorus vary and may have an effect on the amount of these minerals utilized by the animal. Both in vivo and in vitro methods have been used to study the availability of these minerals in various feedstuffs. The ability of an animal to absorb and utilize calcium and phosphorus supplied in its diet may depend on species, age, and various nutritional and physiological factors.

BODY STORES OF CALCIUM AND PHOSPHORUS

Calcium and phosphorus comprise a major portion of total body ash. Crampton and Lloyd (1959) estimated that over 70 percent of total body ash is calcium and phosphorus. These elements are constantly being incorporated into body tissues and excreted from the tissues in an endogenous form. There is a constant exchange of calcium and phosphorus between the bones and the soft tissues. Approximately one percent of these elements are exchanged daily. Ellenberger et al. (1950) reported that 98.5 percent of total body calcium and 87 percent of total body phosphorus are found in the skeleton of adult cows. In younger cattle the amount of phosphorus in the bones was less, averaging 83 percent.

Mitchell (1947) stated that one of the functions of minerals in the diet of animals was to maintain the status quo of body tissues against the "constant erosion of the life processes". He further indicated that phosphorus is considered the most important mineral to the animal, and even though 75-85 percent of total body phosphorus is located in bone, the phosphorus in the soft tissues is considered to be of primary importance. The body stores of phosphorus are not readily available in times of dietary phosphorus deficiency. This is the reason blood phosphorus levels change and deficiency symptoms occur so rapidly.

Mitchell (1962) noted that "inorganic ions liberated in the course of metabolism from functional combination in the tissues are not changed in character or rendered unavailable to the tissues in any sense of the word. They are just as available for the reconstruction of functional combinations for the various functions that they serve in the body as are the inorganic ions picked up by the blood from the intestinal tract."

Duckworth and Hill (1953) considered a Ca:P ratio in bone of 2.15:1 in cattle normal. They found the skeleton to be a highly labile storage area for minerals, and the source of calcium and phosphorus mobilized by action of the parathyroid glands. Cancellous bone such as ribs and vertebrae were more susceptable to resorption than some of the compact bones of the extremities.

Duncan (1958), in a review of literature, concluded that within wide limits the retention of calcium and phosphorus is more dependent on stage of life cycle and body reserves than on intake. The body Ca:P ratio tends to increase with age. The principle bone salt involved in resorption is $CaCO_3 \cdot Ca_3(PO_4)2$. Phosphorus and calcium were reported to be independently removed from the bone of rats. Bone mineral is more readily mobilized to maintain serum calcium than serum phosphorus therefore serum inorganic phosphorus level is one of the first symptoms of phosphorus deficiency.

Young _et_ _al_. (1966a) used a balance study and the radioisotopes Ca^{45} and P^{32} to study the affect of phosphorus depletion on calcium exchange in the whole skeleton of sheep and also to determine the size of the readily exchangeable calcium pool.

The control sheep in this experiment had an exchangeable calcium pool that averaged 6 percent of the total body calcium. Phosphorus depletion resulted in a 50 percent decrease in the size of this pool. The total skeletal ash weight was reduced by approximately one-third. A similar study of the exchangeable phosphorus pool was not deemed practical because of the large amount of extraskeletal phosphates. Sheep in a state of phosphorus depletion had slower rates of calcium deposition and removal from bone than did the control sheep.

Westerlund (1956) in reviewing United States and Swedish literature found that calcium losses were increased by a reduction of phosphorus in feed. He concluded that as cattle drew upon their skeletal reserves of phosphorus, calcium was removed from the skeleton at the same time.

The literature seems to be in agreement that calcium and phosphorus comprise the major portion of body ash. These minerals are absorbed from the gastrointestinal tract and may be incorporated into skeletal bone or may become part of the soft tissues. The calcium and phosphorus in bone apparently forms an exchangeable pool which is drawn upon as needed.

ENDOCRINE INFLUENCE

Mayer (1968) in his review of recent research found that the parathyroid glands influence calcium homeostasis of cows in much the same way as other animals. This was not indicated by several earlier experiments. However he felt that the effect of the parathyroid glands may have been masked by other factors. Because a cow's diet is composed of large amounts of calcium rich materials such as legumes, the parathyroid glands may not be relied on much for maintaining day to day calcium homeostasis but play an important role during periods of adjustment to acute calcium drains.

Rasmussen _et al_. (1963) conducted an _in vivo_ study of normal and vitamin D deficient rats to determine the rationship between vitamin D and parathyroid hormone. They reported that the parathyroid glands of the vitamin D deficient rats were uniformly enlarged. Moderate doses of parathyroid hormone had an effect on phosphate metabolism but little influence on calcium mobilization in the vitamin D deficient rats. Vitamin D appeared to increase the renal tubular reabsorption of phosphate. Results of this experiment indicated that parathyroid hormone affects the translocation of calcium only in the presence of vitamin D, but will continue to exert an effect on phosphate metabolism in the vitamin D deficient animal.

Rasmussen (1959) reported the ability of inverted sacs of rat small intestine to actively transport Ca^{45} from the mucosa to the serosa was decreased by parathyroidectomy 3 or more hours before isolation of the intestinal sac. However the addition of parathyroid hormone _in vitro_ produced inconsistent results.

The thyroid glands may also play a role in calcium homeostasis according to Care (1968). He showed that the hormone, thyrocalcitonin, is secreted in response to hypercalcemia in the young of several species including sheep. The hypocalcemic response to thyrocalcitonin appears to decrease with age possibly due to the reduced plasma thyroxine content and decreased vascularity of bone.

THE ROLE OF VITAMIN D

Vitamin D plays a significant role in the metabolism of calcium and phosphorus. Wasserman et al. (1966) in reviewing the literature stated, "current hypothesis of vitamin D action on the intestinal absorption of calcium suggest that vitamin D is either directly essential for the active efflux transport of calcium, or that vitamin D decreases the diffusional barrier of the cellular surfaces to calcium. Another theory . . . postulates the existence of calcium pumps located on both the serosal and mucosal surfaces of the intestinal mucosa cell, with each pump directing the transfer of calcium from cell interior to cell exterior."

Gaster et al. (1967) in a trial conducted with rats fed normal and calcium deficient diets reported vitamin D supplementation increased the uptake of radiocalcium from the low calcium diet. Wasserman et al. (1966) found that vitamin D significantly increased the transfer of calcium from plasma to the intestinal lumen of chick ileum and rat duodenum by factors of 2.7 and 1.9, respectively. The movement of calcium from the lumen to plasma was also favorably enhanced. Data indicated that vitamin D increased diffusional permeability by changing the membrane structure or had an effect on calcium carrier synthesis rather than influencing the calcium transport system.

Harrison and Harrison (1960) conducted an in vitro study of rat intestine. They found that vitamin D increased the rate of calcium diffusion across the intestinal wall along its entire length and was not affected by inhibition of oxidative metabolism while active transport of calcium was found to be localized in the cranial portion of the intestine and to be dependent on oxidative metabolism. Vitamin D increased the permeability of the intestinal wall to calcium possibly by incorporation of vitamin D into cell surfaces. Schachter et al. (1961) reported vitamin D affected the transfer of calcium in all segments of the intestine with maximum influence in the duodenum where active transport is greatest. They found vitamin D influenced calcium transport by affecting the active transport mechanism rather than simple diffusion as reported by Harrison and Harrison (1960).

Forbes (1967) in a review of current research on vitamin D reported evidence that the administration of vitamin D induced the intestinal mucosa to form a calcium-binding factor identified as a protein.

Capen (1968) conducted an experiment where cows were fed 30 million units of vitamin D for 30 days. Serum calcium increased from a base level of approximately 11.3 mg. calcium per 100 ml. to 14.8 mg. calcium per 100 ml. on the 20th day of feeding vitamin D. Serum phosphorus was raised from the 2nd through the 20th days of the experiment but had returned to normal by the 30th day. The calves from the cows fed the high level of vitamin D did not have serum phosphorus, ionized calcium or total calcium levels different from those of the calves from the control cows. In a trial with four groups of weanling rats, Harrand et al. (1966) fed diets containing either 0.12 or 0.24 percent of both calcium and phosphorus in the presence or absence of added ergocalciferol to determine if there was an interaction between the effects of dietary levels of calcium and phosphorus and vitamin D. Dietary levels of both calcium and phosphorus were below optimal levels making it possible to study independently the effects of adding vitamin D and increasing mineral intake. Both the increased dietary mineral level and vitamin D increased serum calcium and phosphorus concentrations without significant interaction. Dunham (1969) reported that vitamin D usually increased both calcium and phosphorus retention in dairy cows.

THE EFFECT OF pH ON CALCIUM AND PHOSPHORUS METABOLISM

Ali and Evans (1967) in a study of growing rats found that an increase in H+ ion concentration in the intestinal secretions caused by an increase in lactose resulted in an increased percentage of calcium absorption. Dietary increases of calcium buffering capacity or EDTA resulted in a decrease in calcium absorption. Oke and Adekoje (1967) reported that lactic acid may increase availability of calcium in ruminants. Smith and McAllen (1966) conducted a trial with ruminating calves. They studied the digesta from the ileum of calves fed three types of diets; milk fed, stall fed and grazing. The amount of ultrafilterable calcium and inorganic phosphorus was used as an estimate of the amount of these elements available for absorption compared to the total concentration. Almost all the calcium and inorganic phosphate was ultrafilterable at a pH of 2 to 3. As the pH increased to 5.5 calcium became progressively less ultrafilterable while the amount of inorganic phosphate did not change. Davis (1959a) indicated that feeding a high level of calcium carbonate may increase the pH of the intestinal tract, favoring the formation of insoluble tricalcium phosphates. Therefore feeding a high level of calcium with only marginal phosphorus levels may result in a phosphorus deficiency condition.

ANATOMICAL SITES OF ABSORPTION AND ENDOGENOUS SECRETION OF CALCIUM AND PHOSPHORUS

Although most absorption investigations have been conducted with small laboratory animals, similar mechanisms are thought to be involved with ruminants since most of the calcium and phosphorus is absorbed from the small intestine. Schachter and Rosen (1959) found calcium to be actively transported from the small intestine of the rat. Schachter et al. (1966) demonstrated that the transfer of calcium across everted sacs of rat duodenum in vitro occurred in two steps: uptake at the mucosal surface and transfer toward the serosal surface. The first was hypothesized to be a facilitated diffusion but the second was dependent on oxidative metabolism and the generation of phosphate-bond energy. Both steps were markedly influenced by vitamin D. Rats deficient in vitamin D increased both steps involved in calcium transfer when supplemented with vitamin D. Wasserman et al. (1961) conducted an experiment with living rats in which ionic calcium was transferred against a concentration gradient and an electropotential gradient by duodenal membrane providing evidence that active transport of calcium occurs.

Kimberg et al. (1961) conducted an experiment with rats in which a diet low in calcium was shown to facilitate the transfer of calcium against concentration gradients throughout almost the entire intestine when normally this action takes place primarily in the duoenum.

Sheep rumen epithelium was found to be relatively impermeable to calcium by Phillipson and Storry (1965), even when concentration was increased to six times the normal level. Calcium was absorbed in the cranial jejunum and the middle of the small intestine relative to the calcium concentration. No absorption was observed from the duodenum between the pylorus and the opening of the common biliary and pancreatic duct or from the caudal ileum.

Hansard et al. (1952) administered radiocalcium orally or intravenously to 21 head of Hereford cattle ranging in age from seven to thirty-six months. The animals were all in slight positive phosphorus balance. After balance studies had been completed the animals were slaughtered and the various tissues and organs were counted for ca^{45}. Calcium ions in the soft tissues were generally interchangeable with those in the plasma. This circulating calcium was secreted into all parts of the gastrointestinal tract but primarily into the small intestine. Shirley et al. (1951) conducted a similar experiment with phosphorus in which a 420 pound steer was fed a grass mixture of fescue and oats harvested off a plot of ground which had been fertilized with superphosphate 39 hours after a feeding of the grass mixture. The isotope was found widely distributed in most of the organs and tissues, although 45 percent of the isotope was found in the alimentary tract, with 36.3 percent in the rumino reticular contents.

The literature concerning the mode of phosphate transfer from the lumen of the gastrointestinal tract is not as complete as that of calcium transport. It appears that inorganic phosphate is at least partially absorbed by passive diffusion.

Harrison and Harrison (1961) used everted loops of small intestine from rats to study the transfer of inorganic phosphate from the lumen of the gut to the plasma. Transport of phosphorus from the solution bathing the serosal surface was against a concentration gradient. The presence of calcium, potassium, and vitamin D enhanced the transfer of phosphate. Data suggested that diffusion did not entirely account for phosphate transfer. Young et al. (1966b) conducted an experiment with sheep in which they presented data which indicated phosphorus absorption may have occurred via passive diffusion.

Berndt and Gosselin (1962) used isolated rabbit mesentery to study transfer of phosphate ions. P^{32}-orthophosphate was shown to move across the membrane apparently by passive diffusion with no evidence of an operative carrier mechanism. Data indicated that transfer of phosphate occurred by facilitated diffusion or facilitated penetration.

Mature dairy cows and two-month old calves were injected with radiophosphate and slaughtered in intervals from one-half to seventy-two hours after injection by Smith et al. (1956). Tissues and contents of the gastrointestinal tract were analyzed. Radiophosphate was found in the gastrointestinal tract, indicating a large secretion of endogenous phosphorus. The rumen was the main G. I. tract location of P^{32} secretion in the mature cows. Most of this phosphorus was probably accumulated via saliva. More phosphorus was secreted into the small intestine and less into the rumen of calves than mature cows. Based on other studies they concluded that intestinal phosphorus exchange is generally less in the cow than in the sheep.

The work of Lofgreen et al. (1952) indicated that in calves injected with P^{32} the phosphorus was excreted throughout the gastrointestinal tract but primarily in the jejunum.

In summary, the literature indicates that calcium is actively transported from the gastrointestinal tract while phosphorus enters by means of passive diffusion. Although endogenous calcium and phosphorus are secreted along the whole tract most of the secretion as well as absorption occurs in the small intestine. Large amounts of phosphorus are secreted into the rumen, however most of this enters via the saliva.

RELATIONSHIP OF DIETARY INTAKE LEVELS TO AVAILABILITY AND UTILIZATION OF CALCIUM AND PHOSPHORUS

Hansard and Plumlee (1954) in an experiment with rats found an inverse relationship between calcium intake and efficiency of utilization. The calcium maintenance requirements of the rats varied with the nutritional status of the animal. These requirements were closely correlated with the animals ability to adapt its metabolic losses to its calcium intake level. Davis (1959b) in a review of literature concluded that most species can adapt to various dietary calcium levels. Percent absorption generally decreased as calcium intake increased but total absorption and retention increased. Calcium requirement figures may be more a reflection of dietary pattern than an indication of absolute requirements. Swenson <u>et</u> <u>al</u>. (1956) fed an excess amount of calcium in the form of ground limestone added to the ration of growing Hereford heifers. The heifers without the excess calcium in their ration gained 38.9 pounds more during the 12 month experiment. During the last one-third of the experiment however, the calves appeared to adjust to the higher levels of calcium carbonate with as much as 1.1 pounds of ground limestone consumed per head daily.

Visek <u>et</u> <u>al</u>. (1953) conducted an isotope dilution trial to determine if variation in calcium intake and calcium to phosphorus ratio would affect endogenous fecal calcium. Within the same animal, endogenous fecal calcium values were not significantly changed when dietary calcium was varied from normal to high levels. The daily endogenous fecal calcium losses for three 8-year old cows averaged 7 grams compared with 4 grams for two yearling Hereford steers. Comar <u>et</u> <u>al</u>. (1939) found that most of the calcium secreted into the gastrointestinal tract had been previously involved in bone and soft tissue exchange.

Lindsey <u>et</u> <u>al</u>. (1931) conducted a trial with two groups containing four dairy heifers each. One group was fed a diet high in calcium and the other a diet low in calcium for the first three years of life. Short balance trials were run periodically. The group given the higher calcium diet retained a larger amount of calcium but the lower calcium group retained a higher percentage of dietary calcium, indicating that at a lower intake level the calcium was more efficiently utilized after the first year of life. A smaller percent of dietary phosphorus was retained by the group receiving the low calcium diet.

Hansard _et al_. (1954) investigated the effects of age on calcium absorption and excretion. Thirty-four Hereford cattle ranging in age from 10 days to 190 months were used. Animals over 36 months of age were in negative calcium balance. There was little difference between apparent and true digestibility of calcium with the younger animals. As the age of the animal increased, apparent digestibility decreased much more than true digestibility. The percent of calcium intake absorbed decreased with age from 99 ± 0.5 at ten days of age to 22 ± 4.0 percent in the aged animals. The fecal calcium from body stores increased from 3 ± 2.0 percent at ten days of age to 45 ± 9.0 percent in aged animals. The daily endogenous fecal calcium expressed as milligrams per kilogram of body weight was not significantly different between 30 days of age and maturity.

Hutton _et al_. (1967) conducted 89 balance trials with 6 lactating dairy cows fed fresh cut pasture herbage for 32 weeks. Availability of calcium and phosphorus was determined by comparing the amount of each element consumed and excreted. Calcium availability varied widely between weeks but only slightly between cows. There seemed to be no relationship between percentage of calcium in the feed and the concentration in the feces, urine and milk. The amounts of calcium in milk and feces remained relatively constant. The mean apparent availability of calcium in these experiments was 22.5 percent and varied from -7 to 40 percent for individual cows. The level of phosphorus in the forage remained relatively constant as did the percent in the feces. Seventy-five percent of the available phosphorus was excreted in the milk. The mean apparent availability of phosphorus was 34 percent and ranged from 12 to 55. There was a high correlation between the amount of ingested phosphorus and the amount of phosphorus excreted in the feces.

Tillman et al. (1959) concluded that low, medium or high dietary levels of phosphorus did not significantly affect the amount of endogenous fecal phosphorus or the true digestibility of phosphorus. Urine calcium content increased at the lower phosphorus levels indicating that if sufficient phosphorus is not available for bone calcification the excess calcium is excreted in the urine.

Apfelbaum and Brigant (1964) used a balance study with rats to investigate the absorption and excretion of phosphorus as influenced by dietary phosphorus level and the calcium to phosphorus ratio. They found that when intake of calcium was high the excretion of phosphorus in urine decreased and fecal excretion of phosphorus increased over the levels excreted when dietary levels of calcium were low, indicating the possible formation of insoluble calcium phosphates. Unabsorbed phosphorus in feces increased as dietary intakes of calcium and phosphorus increased.

Archibald and Bennett (1935) conducted a trial with 2 groups of dairy heifers receiving different levels of phosphorus. The heifers on the high phosphorus diet retained more phosphorus but the low dietary phosphorus heifers retained a higher percent of their dietary phosphorus. The low dietary phosphorus intake had no significant effect on the retention of calcium.

Preston and Pfander (1964) divided 24 whether lambs into 3 groups and fed complete rations containing 0.12, 0.15, and 0.29 percent phosphorus. Both the percent and the total amount of endogenous phosphorus in the rumen fluid increased with increasing dietary phosphorus levels. It was concluded that the decrease in plasma inorganic phosphorus levels which was apparent within one week caused the endogenous phosphorus secretion into the rumen to be decreased.

Compere _et al_. (1967) studied the true digestibility of phosphorus in lambs during the period of transition to functional ruminants. They reported that the true digestibility of phosphorus decreased during weaning. Phosphorus content of the feces increased sharply as the rumen became functional while urinary phosphorus elimination decreased. Young _et al_.

(1966c) added phosphorus to the ration of phosphorus depleted sheep and found a marked increase in the excretion rate of metabolic fecal phosphorus. This indicated that the intestinally secreted phosphorus was not being reabsorbed as readily rather than a change in rate of secretion. When additional phosphorus was added to the diet of control sheep, the decrease in amount of metabolic fecal phosphorus appeared to be due to a decrease in the secretion of phosphorus into the intestine.

One of the first experiments using radioisotopes to determine "true" digestibility of phosphorus in cattle was conducted by Klieber et al. (1951). Two Jersey cows were fed a diet of alfalfa hay and concentrates. They found that the maximum specific activity of the feces occurred 2 to 3 days after injecting P^{32} into the bloodstream. It was assumed that the P^{32} secreted into the intestinal contents would appear in the feces 2 days later. Endogenous phosphorus secretion was positively correlated with the level of feed intake or rate of fecal excretion. The cow on the high level of feed intake showed an apparent digestibility of phosphorus of 12 percent while the true digestibility was 50 percent. The cow on the lower feed level excreted more phosphorus in her feces than she consumed but the "true" digestibility of phosphorus was 64 percent.

Suttle and Field (1966) studied the effect of water intake on mineral metabolism in sheep. Infusion of 10 liters of deionized water per day into the rumen of fistulated sheep resulted in an increased urinary phosphorus excretion of 45.1 percent but only slightly increased urinary calcium excretion. Fecal calcium and phosphorus excretions were not significantly affected. Serum concentrations of calcium and phosphorus were not affected by the increased water intake. The apparent availability of dietary phosphorus from a ration composed of concentrate and chopped hay increased from 66.2 percent to 77.3 after infusion of water and on a diet of dried grass from lush spring pasture, increased from 34.5 to 56.7 percent. These figures indicate that since normal apparent availability of dietary phosphorus is quite low, the infusion of water caused a considerable increase in absorption from the diet. Calcium metabolism was not significantly affected by the increased water intake, apparently due to re-absorptive mechanisms in the kidney which allow only a small amount of calcium in urine.

Lewis <u>et</u> <u>al</u>. (1951) fed 38 steer calves individually for 120 days on rations containing what they considered adequate, borderline, or deficient phosphorus with normal or excess calcium. Borderline and deficient phosphorus rations decreased feed consumed per 100 pounds body weight and rate of gain. An excess of calcium added to the borderline ration further increased these effects. Excess calcium added to the deficient phosphorus ration decreased feed consumption but did not affect rate of gain.

In summary, a large number of experiments have been conducted to investigate the relationship of intake to utilization and excretion of calcium and phosphorus. Most of these studies have shown that as dietary levels are increased the total amount of mineral absorbed by the animal increases but the percentage of ingested mineral actually absorbed may decrease. A low dietary level of one mineral will usually have an adverse effect on the absorption of the other. Animals have varying capacities to adapt to different dietary mineral levels. There is disagreement in the literature as to whether or not dietary level of calcium and phosphorus is correlated with the amount of endogenous mineral excreted.

RECOMMENDED DIETARY LEVELS OF CALCIUM AND PHOSPHORUS

The National Research Council (1963) regard their recommendations for beef cattle of 0.20 and 0.15 percent phosphorus in finishing and other rations respectively as minimum and with no margin of safety. Calcium requirements range from 0.15 to 0.37 percent of the ration. Calcium deficiency in beef cattle is seldom seen except in cattle on rations with only limited amounts of non-legume forage. Calcium is a critical element in dairy cattle nutrition. Mineral requirements in addition to maintenance include milk production and pregnancy and are listed in National Research Council (1966) recommendations.

Tillman et al. (1959) fed Herford steers weighing 350 pounds three levels of phosphorus after an adjustment period of 47 days on a basic diet containing 0.12 percent phosphorus.

The steers were fed individually for 70 days phosphorus levels of 1.5, 2.0 and 2.5 grams per 100 pounds of body weight which were equivalent to 0.14, 0.17 and 0.20 percent phosphorus on a dry ration basis. Dicalcium phosphate and calcium carbonate were used to keep the calcium to phosphorus ratio at 3.75:1. When bone growth and plasma inorganic phosphorus were used as criteria, the 2.0 gram level appeared to meet the phosphorus requirement. However, if growth rate, feed consumption and efficiency were considered, the increasing phosphorus levels caused a linear response indicating that 2.0 gram per 100 pounds of body weight did not meet the phosphorus requirement.

A 150 day feeding trial with long-yearling Hereford steers indicated a ration containing 0.2 percent phosphorus was adequate when daily gain, feed consumption and feed required per 100 pounds gain were used as criteria, according to Pope _et al_. (1958).

Sixty-three pregnant yearling beef heifers were divided into five lots in a study conducted by Thomas _et al_. (1965). One lot was given no winter supplement, the other four lots were fed a 20 percent protein supplement containing 0.5, 1.0, 1.5 or 2.0 percent phosphorus during the wintering period for three years. The cows and their calves were turned together during the summer grazing period and were given no supplement except salt. A summary of the three years' data show the adjusted average weaning weights of the calves to be 183, 200, 208, 200 and 205 kg. and percent calf crops of 90, 81, 88, 98 and 95 respectively for the 4 phosphorus levels. The average weaning weights appear to favor the rations containing some supplemental phosphorus.

Wise _et_ _al_. (1958) reported that 0.22 percent phosphorus was the minimal level for 200 to 275 pound calves with an additional 0.08 percent supplying an adequate safety factor. Burroughs _et_ _al_. (1956) fed twenty-four 600 pound steers in a 167 day trial to determine if additional amounts of phosphorus over the 0.18 percent recommended level would increase weight gain and efficiency on a fattening ration. The basal ration composed of ground shelled corn, ground corncobs, molasses and a protein supplement, contained 0.18 percent phosphorus. The steers were divided into four lots as follows: basal ration, basal ration supplemented with dicalcium phosphate to 0.25 and 0.33 percent phosphorus, and basal ration supplemented with colloidal clay to 0.33 percent phosphorus. The results of this experiment indicate that for top feedlot performance, the ration should contain more than 0.18 percent phosphorus. The blood phosphate levels of all the cattle were statistically similar, suggesting that this may not be a sensitive criteria to use in establishing phosphorus requirements. The ration containing 0.25 percent phosphorus appeared adequate to support maximum feedlot performance. The ration supplemented with colloidal clay to a phosphorus level of 0.33 percent did not give any better results than the basal ration containing 0.18 percent. These researchers concluded that the availability of phosphorus may be as important as the actual percentage of phosphorus in the ration.

The literature shows conflicting recommendations for mineral requirements. Calcium requirements for cattle have been studied less than phosphorus requirements. Much of the controversy involving recommended levels of mineral supplementation can be partially explained by a quotation from the National Research Council (1963), "Data on the availability of calcium and phosphorus in various feeds are meager and requirements are expressed in terms of gross amounts needed with little consideration being given to availability".

THE INFLUENCE OF CALCIUM TO PHOSPHORUS RATIO

The ratio of calcium to phosphorus varies widely between rations depending on the type and quantity of feedstuffs used. Several workers have approached the problem of availability by investigating the effects of Ca:P ratio.

Davis (1959b) reported that Ca:P ratios of 7:1 or even 10:1 may not cause abnormal performance if the phosphorus level in the diet is at least 0.16 percent. When phosphorus is below 0.13 percent a widening Ca:P ratio resulted in a depressed availability of phosphorus and may result in phosphorus deficiency symptoms. Similar conclusions were reached by Young *et al.* (1966b) in a feeding trial with sheep. Four to six month old whethers were fed diets low in phosphorus and adequate in calcium or adequate in both calcium and phosphorus. Phosphorus absorption was not affected by Ca:P ratio when phosphorus was adquate. However, a wide Ca:P ratio lowered phosphorus absorption when the diet was deficient in phosphorus. A diet low in phosphorus reduced calcium absorption, and when dietary phosphorus level was raised, calcium absorption increased.

Lueker and Lofgreen (1961) fed lambs rations with Ca:P rations of 0.8:1, 2.8:1 and 6.0:1 with the element present in the lowest amount sufficient to meet the National Research Council recommendations. The Ca:P ratio had no effect on the amount of calcium or phosphorus absorbed. The amount of either calcium or phosphorus absorbed was however directly related to the amount fed. A ratio of 6.0:1 caused the smallest excretion of metabolic fecal phosphorus. The ratio of calcium and phosphorus absorbed in this case was 1:1. Working with dairy cattle, Dunham (1969), compared ratios of 1.4:1 to a ratio of 2.9:1 and 1.3:1 to 2.3:1 with and without vitamin D supplementation. He reported that the Ca:P ratios used did not appear to influence phosphorus utilization. The wider Ca:P ratios favored the retention of calcium and also resulted in less phosphorus in blood cells.

Colovos et al. (1958) concluded that the digestibilities of protein and energy are reduced by the addition of calcium above the requirements when the ration had a wide Ca:P ratio. Wales

(1936) studied the effect of adding calcium carbonate to a ration supplemented primarily with prairie hay. The heifers not receiving the calcium supplementation had a higher percent calcium retention. Wise _et_ _al_. (1963) summarized the Ca:P ratio problem in an experiment in which Hereford calves were fed nine Ca:P ratios ranging from 0.4:1 to 14.3:1. Similar performance and feed conversion resulted from ratios of 1:1 to 7:1. Ratios above and below these values were detrimental to performance and feed conversion. Growth was depressed more severely at the lower ratios than at the higher ones. Optimum growth occurred at a ratio of 4.8:1. This experiment indicated that ruminants will tolerate a much wider range of Ca:P ratios than nonruminants but are less tolerant of a ratio less than 1:1.

Dowe _et_ _al_. (1957) conducted a 140 day feeding trial with Hereford steer calves fed 4 pounds of ground shelled corn and 1 pound of soybean oil meal with prairie hay free choice. Ground limestone was added in varying amounts to produce Ca:P ratios of 1.3:1, 4.3:1, and 9.1:1. Serum calcium levels were not elevated by the increased Ca:P ratio; serum inorganic phosphorus levels were depressed but not below a level considered adequate for good nutrition.

Most of the literature shows that ruminants are more tolerant of wide variations in Ca:P ratios than are nonruminants with the possible exception of high producing dairy cows. Ratios above 1:1 are more favorable than those below this value. The ratio does not seem to be as critical when the lowest element present meets the animal's requirements. Research workers have reported different ratios as being optimum. Values somewhere between 1:1 and possibly as high as 6:1 appear to be acceptable.

THE EXPERIMENTAL USE OF BLOOD CALCIUM AND PHOSPHORUS LEVELS

Long *et al.* (1957) concluded that experimental rations for beef cattle should not exceed 0.14 percent total phosphorus when trying to determine the availability of phosphorus in mineral supplements. These workers considered plasma phosphorus levels a good indicator of phosphorus availability because the plasma phosphorus levels were sensitive to change in phosphorus intake. In fifteen days the addition of 0.04 percent phosphorus to a 0.07 percent basal ration increased plasma values from 4.3 to 5.5 mg. per 100 ml. It took 4 to 5 weeks for detectable changes to appear in feed consumption and weight change. Feed intake appeared to be affected less by low phosphorus intake than did feed efficiency. Steers on the basal ration containing 0.07 percent phosphorus ate 39 percent less feed and required 88 percent more feed per unit of gain than did steers receiving 0.11 percent phosphorus. Ewing *et al.* (1957) agreed that plasma inorganic phosphorus is apparently a satisfactory indicator of the state of phosphorus nutrition of beef cattle. Wise *et al.* (1961) in an experiment conducted with fifty calves to determine the relative availability of phosphorus in various mineral supplements considered serum inorganic phosphorus level as one of the most sensitive and reliable criteria which they used. This same criterion was used by O'Donovan et al. (1965). These workers reported that serum phosphorus levels gave as good an indication of phosphorus availability as did apparent and true digestibility values.

This theory was refuted by Moir (1966) who concluded that blood inorganic phosphorus levels were more closely related to phosphorus intake than to the actual phosphorus status of the animal and therefore not satisfactory for distinguishing a deficiency condition. Arrington et al. (1963) found cattle blood phosphorus levels of little value in measuring absorption. Blood levels of P^{32} varied greatly among animals at the various time intervals after oral and intravenous administration of the radiophosphorus. Tissue samples indicated that P^{32} deposition was greatest in the femur epiphysis and ribs. These were followed by the femur shaft, liver, kidney and muscle.

Knox et al. (1941) found the inorganic phosphorus level of blood was a fairly accurate method of diagnosing phosphorus deficiency in range cattle. However blood calcium analyses were an unreliable index of calcium content of the ration. In a four year study of weanling calves on semi-desert range Bohman et al. (1961) reported plasma phosphorus was consistently increased by dietary phosphorus supplements, but plasma calcium levels were not affected by calcium supplementation. There was less variation in plasma calcium than plasma phosphorus.

Moxon et al. (1947) made calcium and phosphorus determinations on blood samples from 88 head of range cattle collected over a five year period. The inorganic phosphorus ranged from 1.19 to 9.57 mg. per 100 ml. of plasma with a mean of 4.77. Calcium content ranged from 4.8 to 19.8 mg. per 100 ml. with a mean value of 9.9 mg. Kendall et al. (1966) in an experiment with aged dairy cows reported average values of 8.96 mg. calcium and 4.17 mg. phosphorus per 100 ml. of serum in the group not receiving additional mineral supplement on a ration containing a calcium to phosphorus ratio of 2.3:1.

Umsheim and Flock (1967) studied the plasma values of inorganic phosphorus and alkaline phosphatase of 1049 head of cattle ranging in age from 1 to 12 years. In younger animals males had lower values than females. The older animals, "showed an almost linear decrease with increasing age".

Burroughs et al. (1956) felt that blood phosphorus level was not a good indicator of an animal's true phosphorus needs because the requirements for optimum bone growth are not as high as those for optimum feedlot performance. These researchers suggested that feedlot performance be used as the basis of determining phosphorus needs rather than blood studies.

A review of the literature reveals conflicting opinions among researchers on the value of blood calcium and phosphorus levels in mineral nutrition research. Blood calcium and phosphorus levels appear to be quite sensitive to changes in intake. Blood samples are relatively easy to obtain but may reflect intake levels or other factors rather than a true reflection of an animal's true calcium or phosphorus status. Values of approximately 8.5 to 14.0 mg. of calcium and 3.5 to 7.5 mg. of phosphorus would apparently be considered "normal" according to Dittmer (1961).

MICROBIAL IN VIVO AND IN VITRO RESEARCH

Rumen microorganisms have a unique set of mineral requirements separate from the requirements of the host. *In vivo* and *in vitro* techniques involving rumen organisms have been used to investigate the availability of calcium and phosphorus, and to try to establish recommendations for dietary mineral levels. In an experiment with sheep given four dietary levels of phosphorus ranging from 0.42 to 4.02 gm. per day, Tomas *et al.* (1967) reported dietary phosphorus intake was directly related to phosphorus content of rumen fluid, serum, and parotid saliva. Salivary phosphorus appeared to be the main source of rumen phosphorus. The ratio of dietary to salivary phosphorus increased with decreasing dietary phosphorus intakes. It appeared that the effect of dietary phosphorus decrease is multiplied in the rumen by a correspondingly steeper reduction in parotid salivary phosphorus concentrations and total salivary phosphorus secretion.

Barth and Hansard (1961) used an *in vitro* experiment to confirm the need of rumen microorganisms for calcium. Their experiment indicated that calcium to phosphorus ratios may be more critical when utilizing phytin phosphate than when inorganic phosphorus is used to supply dietary mineral supplementation.

Fonnesbeck and Harris (1961) added monosodium phosphate via rumen fistulas in sheep fed a continuous diet of coarsely chopped meadow hay, to bring the level of dietary phosphorus to 0.11, 0.22 and 0.33 percent. In this study volatile fatty acid production was not significantly affected by phosphorus level.

Evans and Davis (1966a) used four rumen fistulated Jersey steers to determine mineral composition of rumen fluid. A basal diet containing 0.04 percent phosphorus was fed and the phosphorus content was raised to 0.16 and 0.54 percent by the addition of $NaH_2PO_4 \cdot H_2O$. They found that rumen fluid phosphorus seemed to plateau just above the 0.16 percent dietary level which was equivalent to 31 mg. per kilogram of body weight. Dietary phosphorus levels of 0.04, 0.16 and 0.54 percent resulted in rumen fluid phosphorus levels of 0.87, 2.30 and 2.87 percent in rumen fluid dry matter or 198, 417 and 543 mcg. per ml. of rumen fluid. Evans and Davis (1966b) recommend that phosphorus content of rumen fluid should be 430 mcg. per ml. for optimum <u>in vitro</u> cellulose digestion.

<u>In vitro</u> studies were conducted by Hall <u>et al</u>. (1961a) to compare dicalcium phosphate, Curacao phosphate and deflourinated phosphate as sources of phosphorus for rumen microorganisms.

The dicalcium phosphate increased microbial cellulose digestion whereas the other two phosphorus sources did not. Using cellulose digestion *in vitro* as the criterion or availability, the basal ration of cottonseed hulls containing only 0.05 percent phosphorus was considered to contain ample quantities of phosphorus for the cellulose digesting rumen microbes. Hall *et al.* (1961b) compared monosodium orthophosphate, vitreous sodium metaphosphate, acid sodium pyrophosphate and calcium phytate to determine their utilization by washed suspensions of rumen microorganisms. Cellulose digestion was increased when levels of 20 to 100 mcg. of phosphorus per ml. of medium were added, regardless of the source. These results indicate that *in vitro* cellulose breakdown may not necessarily be indicative of availability of various phosphorus sources for the host animal, because Ammerman *et al.* (1957) found vitreous calcium metaphosphate a poor source of phosphorus for young lambs. It is possible that these lambs may not have had functional rumens at that age.

The literature demonstrates the need of calcium and phosphorus for rumen microorganisms. Much of the phosphorus in the rumen is obtained via saliva. Not all researchers are in agreement that *in vitro* results are valid tests of availability of calcium and phosphorus for the host animal.

AVAILABILITY OF CALCIUM AND PHOSPHORUS FROM INORGANIC SUPPLEMENTS

More research has been conducted on the availability of calcium and phosphorus in inorganic supplements than in any of the other categories of feedstuffs. In most cases mineral supplements are fed to add either one or both of these elements to the diet of ruminants. Supplements which have been most frequently used in availability studies are dicalcium phosphate, defluorinated rock phosphate, Curacao Island phosphate, colloidal clay phosphate (soft phosphate with colloidal clay), bone meal and ground limestone (calcium carbonate).

Ammerman _et al_. (1957) fed yearling steers on a low phosphorus diet of long prairie hay and one pound corn gluten meal for a 126 day period of phosphorus depletion. Two commercial dicalcium phosphates, two calcined defluorinated phosphates, bone meal, soft phosphate with colloidal clay and Curacao Island phosphate were fed at levels of approximately 1.7 or 1.0 grams of phosphorus per 100 pounds body weight. The supplements were of equal value in maintaining blood phosphorus concentration and promoting phosphorus retention in yearling steers. In young lambs the phosphorus in dicalcium phosphate and Curacao Island phosphate were well utilized but the phosphorus in the soft phosphate and the calcined defluorinated phosphate were poorly utilized.

Long _et al_. (1956a) conducted a feeding trial with beef heifers to compare dicalcium phosphate and soft phosphate with colloidal clay. A control group was fed a basal ration containing 0.09 percent phosphorus. Growth rate and feed consumption data strongly favored the group receiving dicalcium phosphate. The group fed soft phosphate exhibited pica, coprophagy, walked with difficulty, and failed to recover when placed on pasture at the end of the experiment. Differences in plasma inorganic phosphorus concentrations of the control group and the soft phosphate group were not statistically significant at the 5 percent level. The dicalcium phosphate group increased in plasma inorganic phosphorus content while the other two groups did not change during the feeding trial following the depletion period.

Burroughs et al. (1956) divided 24 yearling Hereford steers into four lots for a 167 day feeding trial to investigate the effects of supplementing an 0.18 percent phosphorus basal ration to 0.25 and 0.33 percent with dicalcium phosphate and to 0.33 percent with soft phosphate containing colloidal clay. The soft phosphate supplemented group did not produce increased gains or efficiency over the group receiving only the basal ration. The dicalcium phosphate supplement increased gain and efficiency indicating it was a more usefully available form of phosphorus. Similar results were found in an in vitro study with rumen micro-organisms. Anderson et al. (1956) used an in vitro technique to obtain the following values for phosphorus availability from various supplements using sodium and potassium phosphate as the standard phosphate solution shown in Table 1.

Table 1 - Comparative Rating of Phosphorus Availability From Supplements Tested by Artificial Rumen Technique

Supplement	Mcg. of phosphorus added per ml. of medium			
	10	20	40	Ave.
		Ratings		
Sodium and potassium phosphate, std.	100	100	100	100
Composite dicalcium phosphate	98	82	100	93
An acidulated product	61	56	74	64
Steamed bone meal	28	29	29	28
Curacao Island phosphate	17	5	13	12
Soft phosphate with colloidal clay	0	2	3	2

Dicalcium phosphate, bone meal, defluorinated rock phosphate, imported rock phosphate and colloidal phosphate were compared as phosphorus sources for yearling steers by Ammerman et al. (1954). Calcium carbonate was added to keep the calcium to phosphorus ratio of all rations at 2.5:1. Approximately one half of the total phosphorus in the ration was supplied by the basal ingredients. No significant differences were found in percent phosphorus retained.

O'Donovan et al. (1965) used a balance trial to compare the availability of phosphorus in defluorinated phosphate, dicalcium phosphate (U.S.P.) and feed grade dicalcium phosphate. The mineral supplement was added to bring the total phosphorus in the diet to 1.5 gr. of elemental phosphorus per 45 kg. of body weight daily. The formula used was:

$$\text{True Digestibility} = \frac{\left(\text{Total P Intake} - \text{P in basal ration}\right) - \left(\text{Total P Excreted} - \text{Basal P Excreted}\right)}{\left(\text{Total P Intake} - \text{P in Basal Ration}\right)} \times 100$$

Table 2 - Results of Balance Trial Conducted by O'Donovan *et al.* (1965)

	Apparent Digestibility		True Digestibility	
	Dicalcium Phosphate	Defluorinated Phosphate	Dicalcium Phosphate	Defluorinated Phosphate
Means of trials I - IV	47.8	41.1	90.6	84.6
	Dicalcium Phosphate (U.S.P.)	Dicalcium Phosphate (Feed grade)	Dicalcium Phosphate (U.S.P.)	Dicalcium Phosphate (Feed grade)
Means of trials V - VI	46.8	47.6	82.0	84.7

The availability of phosphorus in U.S.P. and feed grade dicalcium phosphate and defluorinated phosphate was not significantly different when compared using true digestibility and serum phosphorus changes as criteria. Much lower true digestibility values were reported by Lofgreen (1960) in an isotope experiment involving mature whethers. The true digestibility of phosphorus in dicalcium phosphate was 50 percent; bone meal, 46 percent; soft phosphate, 14 percent; and calcium phytate, 33 percent. He further stated, "It is interesting to note than even though only 14 percent of the phosphorus of the soft phosphate was absorbed, this amount was apparently sufficient to meet the needs of the animals because the larger amounts absorbed from the other supplements were merely excreted back into the gut as part of the metabolic fecal phosphorus".

Ammerman et al. (1955) used weanling lambs to evaluate dicalcium phosphate, soft phosphate with colloidal clay and defluorinated rock phosphate. Serum phosphorus levels fell from about 10 to about 6 mg. per 100 ml. during the depletion period but rose during repletion an average of 2.4, 2.6, 1.3 and 1.3 mg. per 100 ml. respectively ($P<.01$).

The apparent absorption, excretion, and tissue deposition of P^{32} by cattle from dicalcium phosphate, defluorinated rock phosphate and soft phosphate was investigated by Arrington et al. (1963). Net retention was calculated as follows:

$$\text{Net Retention} = \frac{(P^{32}\text{ administered}) - (\text{Fecal} + \text{Urinary } P^{32})}{(P^{32}\text{ Administered})} \times 100$$

They reported average net retention of phosphorus values of 67.6 percent for dicalcium phosphorus, 47.71 percent for defluorinated rock phosphate and 45.81 percent for soft phosphate.

Using feed intake, gain and plasma phosphorus levels as criteria, Long et al. (1956b) found no significant differences between phosphorus availability in steamed bone meal, Curacao Island phosphate and dicalcium phosphate when fed to beef heifers at a dietary phosphorus level of 0.15 percent. Heifers on the basal ration containing 0.07 percent phosphorus declined in feed intake, made smaller gains and exhibited decreased plasma phosphorus levels.

Wise *et al.* (1961) used fifty Holstein calves in an experiment to compare semi-purified diets supplemented with dicalcium phosphate, defluorinated rock phosphate, Curacao Island phosphate and colloidal clay phosphate. Fifty-six percent of the dietary phosphorus was retained by calves on the basal diet supplemented with dicalcium phosphate. Phosphorus from the defluorinated rock phosphate ration was only slightly less available. Curacao Island phosphate closely approached the defluorinated rock phosphate in availability. Soft phosphate with colloidal clay was the least satisfactory supplement with less than half the phosphorus in this source available to the young calf. These workers reported that serum inorganic phosphorus level was a good indicator of phosphorus availability. Wentworth *et al.* (1960) conducted a similar experiment using a basal diet containing 0.10 percent phosphorus. Calves receiving soft phosphate supplement ate less and had lower gains; their ribs grew less and had a significantly lower ash and phosphorus content than the calves receiving dicalcium phosphate, defluorinated rock phosphate and Curacao Island phosphate.

Hodgson *et al.* (1948) compared steamed bone meal and defluorinated superphosphate as phosphorus supplements during two winter feeding periods with yearling steers. Both mineral supplements were acceptable when force fed by mixing them with the feed. However, neither provided an adequate amount of phosphorus when fed free choice either alone or mixed with salt.

Both feeding trials and an isotope experiment were conducted by Matsushima _et al_. (1955) to investigate the effect of particle size on availability of calcium and phosphorus for growing beef cattle. The results of blood studies, isotope research and breaking strength of bones indicate that course and fine textured limestone and bone meal were of equal value. The apparent digestibility of calcium ranged from 10 to 36 percent and the true digestibility of calcium from 33 to 56 percent.

Sequestering phosphatic solution is an ammonium polyphosphate solution that has been used in the fertilizer industry but had not been tested as a phosphorus source for ruminants. Johnson and McClure (1967) compared a sequestering phosphatic solution with dicalcium phosphate in a steer feeding trial. They reported that the two supplements appeared to support normal performance and inorganic phosphorus serum levels. The nonorthophosphates, which were primarily pyrophosphate, are apparently available for ruminants as a phosphorus source based on their work.

Chicco _et al_. (1965) compared the utilization of inorganic ortho -, meta -, and pyrophosphates by lambs and cellulolytic rumen microorganisms. The criteria used were apparent absorption, net retention, absorbed dose retained and tissue deposition of P^{32}. Solubilities of the phosphates were determined using 0.4 percent hydrochloric acid, 2 percent citric acid and neutral ammonium citrate. Results are shown in Table 3.

Table 3 - Absorption and Retention of P^{32} From Different Phosphates

Expressed in Percent

Phosphate Source	Apparent Absorption[1]	Net Retention[2]	Absorbed Dose Retained[3]	Relative Availability: In Vivo Absorption	Relative Availability: In Vitro Cellulose Digestion
(Calcium orthophosphate was arbitrarily assigned a value of 100)					
Vitreous Sodium Metaphosphate	46.8	41.7	89.0	97	98
Sodium Pyrophosphate	43.5	38.1	87.5	82	100
Sodium Orthophosphate	-	-	-	-	107
Monocalcium Orthophosphate	48.7	48.3	99.1	100	100
Vitreous Calcium Metaphosphate	32.3	26.9	83.3	70	78
Gamma Calcium Pyrophosphate	29.9	21.3	71.2	54	0

[1]Apparent absorption was calculated as ingested phosphorus minus fecal phosphorus.

[2]Net retention was calculated as ingested phosphorus minus fecal and urinary phosphorus.

[3]Absorbed dose retained was calculated as apparent absorbed phosphorus minus urinary phosphorus.

Sodium ortho - and metaphosphate and calcium orthophosphate appeared to be equally available as phosphorus sources. Calcium pyrophosphate was least available based on absorption and tissue deposition and was apparently unavailable to rumen microorganisms.

Rats were divided into lots and given calcium in the form of carbonate, sulphate or lactate in a ten day balance study conducted by Hugot and Causeret (1957). The physiological utilization of calcium from all three sources was almost identical. Fiorentini et al. (1964) used twin lambs in an experiment to show an estimated 83 percent retention of calcium from milk.

Hansard et al. (1957) pair fed mature Hereford steers and 5- to 7- month old calves a basal concentrate mixture plus hay to determine the relative calcium availability from several organic and inorganic sources. True digestibility values were determined from 109 concurrent individual chemical and radiocalcium balance studies. Results are shown in Table 4. The mature steers had higher endogenous calcium losses which made the apparent digestibilities appear much lower than true digestibility. In the younger calves the two measures were more similar. Results of this study indicate age of the animal has more effect on the calcium absorptivity than the source of calcium.

Table 4 - Apparent and True Digestibility of Calcium From Several Sources for Mature and Young Steers

	Mature Steers		Young Steers	
Source	Apparent Digestibility	True Digestibility	Apparent Digestibility	True Digestibility
Bone meal	16	55	47	68
Calcium Carbonate	1	40	26	51
Calcium Chloride	26	53	38	60
Calcium Phosphate (di-basic C. P.)	14	50	39	64
Calcium Phosphate (monobasic C. P.)	19	56	35	61
Dicalcium Phosphate (5 sources)	-19 to 4	38 to 56	20 to 44	56 to 60
Ground Limestone	15	37	28	45

Because many of the diets normally consumed by ruminants are composed of feedstuffs high in calcium and only marginal or low in phosphorus content, research has been done to find supplements which would furnish phosphorus without addition of calcium to the ration. One of these supplements is phosphoric acid.

Richardson et al. (1961) compared phosphoric acid with steamed bone meal as a source of supplemental phosphorus in wintering and fattening rations. Seventy-four Hereford heifers initially weighing an average of 441 pounds were used. These researchers reported that phosphoric acid appeared to be an acceptable source of phosphorus. However the rations used were not considered low phosphorus type rations.

Tillman and Brethour (1958a) used twelve yearling steers divided into two lots to compare the true digestibility of phosphorus in phosphoric acid and in dicalcium phosphate with isotope techniques. The phosphorus supplements supplied 60.2 percent of the total ration phosphorus. Calcium carbonate was added to the phosphoric acid supplemented ration so that the calcium to phosphorus ratios of both would be 2.27:1. Comparison of apparent digestibility, net retention, fecal endogenous excretion and true digestibility showed no significant differences in availability of phosphorus in either supplement. The true digestibility of the phosphorus in phosphoric acid was 76.3 percent while that in dicalcium phosphate was 75.2 percent.

Forty Hereford heifer calves were wintered on bluestem grass plus 1.5 pounds of soybean oil meal and 0.2 pounds blackstrap molasses daily in a trial conducted by Menzies et al. (1955) to compare phosphoric acid to bone meal as a phosphorus source for ruminants. Lot 1 received the basal diet, lot 2 received 8 grams of phosphorus from steamed bone meal, and lot 3 and 4 received 8 and 4 grams of phosphorus respectively from phosphoric acid. During the 162 day wintering phase average daily gains of 0.41, 0.42, 0.48 and 0.53 pounds respectively were reported. Blood serum phosphorus levels were maintained at the initial level of approximately 9 mg. percent in all supplemented lots but the control group dropped to 6.12 mg. percent in February. A second phase of this experiment involved a balance trial with lambs. On the control containing 0.96 grams of phosphorus daily, 0.32 grams were retained. The addition of one gram of phosphorus from phosphoric acid increased the retention to 0.46 grams daily. The workers further reported that the phosphoric acid was highly palatable and produced no ill effects.

The literature contains a large volume of data on the availability of calcium and phosphorus in inorganic mineral supplements. Some of the research indicates that the age or physiological condition of the animal may have as great an influence on availability of these elements as does the source. This may explain why wide variations are reported in availability values. Dicalcium phosphate was the standard most frequently used in comparison experiments. The "true" digestibility of the phosphorus in the above supplements ranged from approximately 50 to 75 percent. Experiments with phosphoric acid show it to be of comparable availability. The "true" digestibility of calcium in the commonly fed supplements ranged from approximately 40 to 70 percent. Soft phosphate with colloidal clay was the least satisfactory supplement in most of the trials.

RESEARCH ON THE AVAILABILITY OF CALCIUM AND PHOSPHORUS IN ORGANIC FEEDSTUFFS

Urbányi (1959) stated, "a plant diet must contain 5 to 7 times as much calcium and 2 to 3 times as much phosphorus as the absolute requirement of the animal".

Much of the phosphorus of various plant feedstuffs is organically bound in a hexaphosphoric acid ester of inositol called phytic acid. This may occur as salts of calcium, magnesium, potassium, etc. Phytic phosphorus is not readily utilized by monogastrics. According to Taylor (1965) phytates influence calcification in monogastrics in two important ways. First insoluble calcium phytate formed in the gut interferes with calcium absorption. Secondly phytates fail to provide inorganic phosphate equivalent to what would be expected from total phosphorus analysis. Tillman and Brethour (1958b) used twelve whethers, 18 months old, to compare calcium phytate and monocalcium phosphate as phosphorus supplements. These supplements provided 70 percent of the total phosphorus in the rations. No statistically significant differences could be found between treatments when based on the criteria of apparent digestibility, net retention, fecal endogenous excretions and true digestibility of the dietary calcium and phosphorus indicating that phytin phosphate is apparently hydrolyzed by ruminants. In vitro rumen studies were conducted by Raun et al. (1956) to determine if rumen microorganisms have the ability to hydrolyze calcium phytate to free inorganic phosphorus which can be absorbed by the host animal. Results indicate that rumen microbes produce the enzyme phytase. Percent cellulose digestion was comparable for microorganisms supplied standard inorganic phosphate and phosphorus in the form of calcium phytate.

Much of the phosphorus in seeds and their by-products is in the form of phytin. Ellis and Tillman (1961) fed 5 to 6 month old lambs a ration in which 91 percent of the total phosphorus was supplied by wheat bran. The apparent digestibility of phosphorus was 14.75 percent and the true digestibility as determined by isotope procedures with P^{32} was 25.49 percent.

Forage materials fed to ruminants usually comprise much of the ration for growing or mature ruminants. Breeding herds are normally maintained on pasture and hay with mineral supplementation. Forages vary in their mineral composition between species and varieties. Even the same crop may vary widely depending on its stage of growth and maturity. Hutton *et al.* (1967) reported that the calcium to phosphorus ratio of fresh cut New Zealand pasture herbage fluctuated from 1.7:1 in October to 4.8:1 in February. Heinemann *et al.* (1957) found that an annual application of 66 pounds of available phosphorus per acre produced alfalfa hay containing 1.48 percent calcium and 0.26 percent phosphorus while the unfertilized soil produced alfalfa hay with only 1.20 percent calcium and 0.14 percent phosphorus.

Phosphorus applied to the soil as fertilizer is utilized by the animal eating the forage. Radioactive P^{32} was reported widely distributed in most of the tissues and organs of a steer fed a grass mixture of fescue and oats harvested from a plot fertilized with superphosphate fertilizer labeled with P^{32} by Shirley et al. (1951).

Black et al. (1949) found that pastures fertilized with triple superphosphate produced forage higher in phosphorus. The phosphorus and protein levels of the forage were closely related to rainfall, being lowest during winter and drought periods. They reported that supplying phosphorus to cattle via fertilizing of pastures resulted in highest returns per acre but that supplying phosphorus supplement thru drinking water produced the highest return per cow. Seasonal variations in mineral content of little bluestem grass (Andropogon Scoparius) were noted by Drake (1964). Results are shown in Table 5.

Table 5 - Seasonal Variation in Calcium and Phosphorus Content of Little Bluestem Grass

	Season			
Mineral	Spring	Summer	Fall	Winter
Calcium	0.44	0.44	0.45	0.40
Phosphorus	0.115	0.12	0.09	0.07

He reported serum phosphorus levels of cattle grazing this range showed corresponding seasonal variations.

Lofgreen and Kleiber (1954) conducted an experiment with four lambs given a single injection of radioactive phosphorus, to determine the availability of phosphorus in alfalfa hay. An average of 92 percent of the phosphorus in the feces was of metabolic origin. The average true digestibility of phosphorus in the alfalfa hay was 94 percent. In an earlier experiment Lofgreen and Kleiber (1953) fed five whether lambs a ration of chopped number 2 alfalfa hay. The lambs were put in metabolism crates and injected with 150 microcuries of P^{32} every six hours for 14 injections. Approximately 88 percent of the phosphorus in the feces was of metabolic origin leaving 12 percent that was unavailable or unabsorbed from the diet. The apparent digestibility of the phosphorus in alfalfa hay averaged only 22 percent but the true digestibility was 91 percent indicating that the phosphorus was highly available.

Weir et al. (1958) conducted feeding trials with both sheep and steers using alfalfa hay of varying phosphorus levels. Hay containing 0.10 percent phosphorus produced poor animal gain, feed utilization, and depressed serum phosphorus levels. These conditions were improved when disodium phosphate was added to the ration. Hay with 0.15 percent phosphorus appeared equal in feeding value to hay containing greater amounts of phosphorus. Digestion trials with lambs showed no difference in digestibility of the various nutrients. Variable results were obtained from calcium balance trials.

Hansard et al. (1957) reported the values shown in Table 6 for digestibilities of calcium in an experiment involving pair feeding of mature and 5- to 7- month old Herford steers.

Table 6 - Apparent and True Digestibility of Calcium From 3 Hay Sources for Mature and Young Steers

	Mature Steers		Young Steers	
Source	Apparent Digestibility	True Digestibility	Apparent Digestibility	True Digestibility
Alfalfa Hay #3	-1	31	18	41
Lespedeza Hay #2	8	36	17	50
Orchard grass #2	-7	39	26	51

These values were lower than values obtained for inorganic calcium sources in the same experiment. (See Table 4). This may have been due to the presence of calcium-binding substances in the hays that decreased availability for absorption or lowered solubility of the calcium in the hay according to the authors. Age of the animal appeared to affect availability more than the source.

Watkins (1933) conducted a feeding and digestion trial with yearling Herford steers on a ration of native New Mexico prairie hay supplemented free choice with salt and either bone meal or disodium phosphate. One group of steers received 0.75 pounds of cottonseed meal daily. He reported that the cottonseed meal was an efficient supplemental source of protein and phosphorus as indicated by daily gain. The data indicated a correlation between nitrogen balance and calcium and phosphorus balances. When the nitrogen was more nearly balanced, the phosphorus requirement appeared lower. Calcium balance was affected in the same way but to a lesser degree.

Heinemann (1965) fed thirty-six yearling beef steers in 3 groups on a ration of ground ear corn supplemented with 41 percent protein cottonseed meal, cottonseed meal enriched with a nitrogenous additive to contain the equivalent of 41 percent protein, or urea at the average rate of 21.3 grams per head daily. Each group was given 15 pounds of clean soil per week in a feeder, iodized salt and salt-bone meal (1:2) free choice. About 2 pounds of bone meal per animal were consumed monthly. Relatively low quality hay was used as the roughage. Digestibility values in Table 7 were obtained using the nylon bag *in vivo* method. Digestibility was defined as the percent of original calcium or phosphorus that disappeared from the bag. No explanation was given for the unavailability of calcium from the corn and protein supplements or for the indication that calcium from the alfalfa hay, bone meal and soil was apparently being tied up by the ear corn and supplement. The results of other research indicate that phytin may have been involved.

Table 7 - Percent Digestibility of Calcium and Phosphorus In Various Rations For Yearling Steers As Determined Using the Nylon Bag *In Vivo* Method

Feedstuff	Percent Digestibility Calcium	Percent Digestibility Phosphorus
Alfalfa Hay	74.6	61.1
Ear Corn Meal	*	78.5
Regular Cottonseed Meal	*	85.3
Nitrogen Enriched Cottonseed Meal	*	87.9
Concentrate + Supplement (CSM)	*	37.1
Concentrate + Supplement (CSM)	*	44.3
Concentrate + Supplement (N-enriched CSM)	*	71.7
Concentrate + Supplement (N-enriched CSM)	*	50.7
Concentrate + Supplement (urea)	11.1	72.8
Concentrate + Supplement (urea)	*	42.9

*Calcium content increased from pre-to-post-digestion.

The literature contains relatively little information on the availability of calcium and phosphorus in feedstuffs of plant origin. Much of the phosphorus in these feedstuffs seems to be bound in the form of phytin. The formation of calcium phytate interferes with calcium absorption. Rumen microbes produce the enzyme, phytase, which has been shown to free inorganic phosphorus from phytin phosphate. In an experiment with 5- to 6- month old lambs fed a ration in which 91 percent of the total phosphorus was supplied by wheat bran, the "true" digestibility of the phosphorus was reported to be 25.49 percent. Phosphorus content of forages vary depending on the stage of growth, fertility of the soil and other factors. True digestibility values for phosphorus in alfalfa hay have ranged from approximately 40 to 94 percent. Calcium values of approximately 31 to 74 percent have been reported. One researcher found that ear corn and cottonseed meal contained phosphorus that was approximately 78 and 85 percent digestible respectively.

SUMMARY

Minerals are essential for the normal maintenance, growth, reproduction and production of farm animals. Two of the macro elements needed are calcium and phosphorus. Nutrient requirement tables list the recommendations for cattle in terms of grams per day or as a percentage of the total ration. Feed composition tables show only the total calcium and phosphorus content. This report was prepared in an attempt to summarize research conducted by others to determine the amount of calcium and phosphorus in feedstuffs actually available to cattle and some of the factors that affect availability and utilization of these elements.

Crampton and Lloyd (1959), Ellenberger (1950), and Mitchell (1947) have demonstrated the importance of calcium and phosphorus to normal body functions. These two elements comprise over 70 percent of total body ash. There is a continuing exchange of these elements between the skeleton and soft tissues. Crampton and Lloyd (1959) estimate that approximately 1 percent of the calcium and phosphorus in the body is exchanged daily. Mayer (1968) reported that parathyroid glands of cattle function in calcium homeostasis much the same way as in other animals but are probably not relied on as frequently because cattle usually consume large amounts of calcium rich feeds. Care (1968) indicated that the thyroid glands secrete a hormone in response to hypercalcemia in the young of several species including sheep. Capen (1968) and Dunham (1969) both reported that vitamin D increased the absorption and retention of calcium and phosphorus

in cattle. The pH of the intestinal tract plays a role in the amount of calcium and phosphorus absorbed. An increase in pH as may occur when high levels of calcium carbonate are fed may favor the formation of insoluble tricalcium phosphates according to Davis (1959a). Phosphorus deficiency could result when high levels of calcium are fed with only marginal phosphorus levels.

Calcium was absorbed by active transport while phosphorus was absorbed by passive diffusion in work reported on by Schachter and Rosen (1959) and Young et al. (1966a). Large amounts of endogenous phosphorus enter the rumen via saliva. Most of the absorption and excretion of calcium and phosphorus occurs in the small intestine primarily in the jejunum.

Dietary intake level of calcium and phosphorus has an effect on utilization of these elements. Hansard and Plumlee (1954), Davis (1959b), and Lindsey et al. (1931) found an inverse relationship between intake level and the percent absorbed. At higher dietary levels more of the elements are absorbed but the percent actually absorbed decreases. Hansard et al. (1954) and Hansard et al. (1957) have shown that as an animal becomes older apparent digestibility of calcium and phosphorus decreases more rapidly than "true" digestibility. There are conflicting opinions in the literature as to whether or not dietary levels of calcium and phosphorus are correlated to the amount of endogenous mineral excreted.

NRC (1963) list phosphorus requirements for beef cattle of 0.20 and 0.15 percent in finishing and other rations respectively and calcium requirements ranging from 0.15 to 0.37 percent. They further state, however, that these recommendations are minimum and may offer no margin of safety. Wise et al. (1958), Burroughs et al. (1956) and others have indicated that requirements for maximum performance may be above these recommendations.

The ratio of calcium to phosphorus in feedstuffs is not as critical to ruminants as it is to monogastrics. Ratios above 1:1 are more favorable than those below this value. Calcium to phosphorus ratio is more important to high producing dairy cows than to beef cattle. Beef cattle have been shown by Davis (1956b), Wise et al. (1963), and Dowe et al. (1957) to tolerate ratios of 1:1 to 6:1 and above without any decrease in performance. The ratio of calcium to phosphorus does not appear to be as critical when the lowest element present meets the animal's requirements.

Although blood calcium and phosphorus levels appear to be quite sensitive to changes in intake, researchers appear to have conflicting opinions of the usefulness of these parameters in mineral nutrition research. Values of approximately 8.5 to 14 mg. of calcium and 3.5 to 7.5 mg. of phosphorus per 100 ml. would apparently be considered "normal".

Rumen microorganisms require phosphorus to function properly. Evans and Davis (1966a) and Tomas et al. (1967) have demonstrated that rumen *in vivo* and *in vitro* experiments are valid in determining mineral requirements and availability values of various inorganic supplements for the host animal, but Hall *et al*. (1961a) and Burroughs *et al*. (1956) have shown rumen microbes to require differing quantities of minerals than the host.

Most of the research on availability of calcium and phosphorus in feedstuffs has been conducted with inorganic mineral supplements. The most commonly studied supplements are dicalcium phosphate, defluorinated rock phosphate, Curacao Island phosphate, colloidal clay phosphate, bone meal and ground limestone (calcium carbonate). Wide variations have been reported for the availability of calcium and phosphorus in these supplements. Hansard *et al*. (1957) demonstrated that age of the animal may be as important as source in determining availability. Experiments conducted by O'Donovan *et al*. (1965), Arrington *et al*. (1963), Wise *et al*. (1961), Lofgreen (1960), Ammerman *et al*. (1955), and others have found that the "true" digestibility of phosphorus in these supplements to range from approximately 50 to 75 percent. Menzies *et al*. (1955) and Tillman and Brethour (1958a) have shown that phosphoric acid is of comparable availability. The "true" digestibility of calcium in the above mineral supplements have ranged from approximately 40 to 70 percent in work done by Hansard *et al*. (1957), Matsushima *et al*. (1955) and others. Soft phosphate with colloidal clay appeared to be the least satisfactory supplement in most of the trials.

The literature contained only limited information on the availability of calcium and phosphorus in feedstuffs of plant origin for cattle. Much of the calcium and phosphorus in these feedstuffs is apparently bound in the form of phytin. The results of research by Raun *et al*. (1956) and Tillman and Brethour (1958b) indicate that ruminants have the ability to utilize the phosphorus in phytin to some extent but to a lesser degree than the phosphorus supplied by some of the inorganic mineral sources. Ellis and Tillman (1961) reported a "true" digestibility value of 25.49 percent in a ration in which wheat bran supplied 91 percent of the phosphorus. The formation of calcium phytate may interfere with the absorption of calcium. Work by Hutton *et al*. (1967), Hinemann *et al*. (1957), and Black *et al*. (1949) shows the phosphorus content of forages to vary depending on the maturity, season, fertility of the soil and other factors. Lofgreen and Kleiber (1954), Heinemann (1965) and Hansard *et al*. (1957) report digestibility values for phosphorus in alfalfa hay ranging from approximately 40 to 94 percent and calcium digestibility of 31 to 74 percent. Heinemann (1965) found that ear corn and cottonseed meal contained phosphorus that was approximately 78 and 85 percent digestible respectively. Most of the literature indicates that the feedstuffs of plant origin contain more available phosphorus and less available calcium than the inorganic mineral supplements. It appears that more research is needed on the complexity of availability of calcium and phosphorus in commonly used feedstuffs.

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THE AVAILABILITY OF CALCIUM AND PHOSPHORUS IN FEEDSTUFFS FOR RUMINANTS

by

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Calcium and phosphorus comprise over 70 percent of total body ash with approximately 1 percent of the body stores of these elements exchanged daily between the skeleton and soft tissues. Calcium and phosphorus recommendations for cattle are expressed in terms of grams per day or as a percentage of the total ration. Feed composition tables show only total calcium and phosphorus content of feedstuffs with no regard for the amount of these elements actually available to cattle. Several animal and feedstuff factors affect the availability of calcium and phosphorus.

Calcium appears to be absorbed by active transport while phosphorus is absorbed by passive diffusion. Vitamin D increases the absorption of both. Most of the absorption and endogenous excretion occurs in the small intestine, primarily in the jejunum. Research has shown that an increase in the pH of the intestinal contents which may occur when high levels of calcium carbonate are fed may result in the formation of insoluble tricalcium phosphates. As dietary mineral level is increased an animal will usually absorb more calcium and phosphorus but the percent absorbed may decrease. The apparent digestibility decreases more than "true" digestibility as an animal grows older.

Recommended phosphorus levels of 0.20 percent in cattle finishing rations have been shown to promote less than optimum feedlot performance while calcium recommendations of 0.15 to 0.37 percent have generally been reported adequate. Some researchers have found that rumen microorganisms require a level of mineral supplementation higher than the host animal.

Calcium to phosphorus ratio is not as critical for ruminants as it is for monogastrics. Ratios above 1:1 are more favorable than those below this value. Beef cattle have been shown to tolerate ratios of 1:1 to 6:1 and above without any decrease in performance. When both calcium and phosphorus in the diet meet the animal's requirements, the calcium to phosphorus ratio is not as critical as when one of these elements is below the recommended level.

Blood calcium content is quite stable while phosphorus levels appear to be quite sensitive to change in intake. Researchers have conflicting opinions as to the usefullness of these parameters in mineral studies. Blood levels of approximately 8.5 to 14.0 mg. calcium and 3.5 to 7.5 mg. phosphorus per 100 ml. would be considered "normal".

Most of the research on availability of calcium and phosphorus has been conducted using inorganic mineral supplements. Availability data varies greatly due at least partially to animal factors. "True" digestibility ranged from approximately 50 to 75 percent for phosphorus and from 40 to 70 percent for calcium in the commonly used inorganic mineral supplements. Soft phosphate with colloidal clay was the least satisfactory in most trials.

Calcium and phosphorus content of feedstuffs of plant origin vary depending on maturity, season, fertility level of the soil and other factors. Much of the calcium and phosphorus of these feedstuffs may be bound in the form of phytin. Rumen microorganisms can utilize these phytins to some extent. Phosphorus in feedstuffs of plant origin is more available than from inorganic sources while calcium is slightly less available. "True" digestibility values of approximately 40 to 94 percent for phosphorus and 31 to 74 percent for calcium have been reported. More research is needed on the availability of calcium and phosphorus in the commonly used feedstuffs.