RUMINAL AVAILABILITY OF PHOSPHORUS AND ITS EFFECT ON DIGESTION

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CHAPTER I

INTRODUCTION

Phosphorus in the form of some inorganic phosphorus supplement is added to most livestock rations to help meet the phosphorus requirement of animals. With nonruminants, the animal's phosphorus requirement is often assessed by feeding trials and growth measurements. Ruminants, however, require phosphorus both for growth of tissue and bone as well as for multiplication of microorganisms within the rumen. Thus, phosphorus availability becomes important at two sites for the ruminant. Rate of growth of bacteria and, thus, rate of digestion of feed components within the rumen could be limited by an inadequate ruminal phosphorus supply. Through reduced microbial activity, a deficiency of phosphorus could precipitate deficiencies of energy or protein. This concept is suggested by the observation that loss of appetite is one of the first clinical signs of a phosphorus deficiency in ruminants.

Two other factors in phosphorus metabolism are peculiar to ruminant animals. First, ruminal phytase liberates one type of bound phosphorus-phytin phosphorus. This complex appears in many plants and the bound phosphorus has very low availability for nonruminants. Secondly, pancreatic

ribonuclease, which is secreted into the small intestine of ruminant animals in large amounts, releases the phosphorus present in nucleic acids reaching the small intestine for absorption and recycling to the rumen. In this manner, phosphorus used by ruminal microbes is well recovered. Phosphorus in ruminal fluid is derived from solubilized or degraded feed ingredients as well as from recycling to the rumen of endogenous phosphorus through saliva and by diffusion through the ruminal wall.

Since it plays many critical roles in the animal's metabolism, phosphorus is supplemented to most ruminants diets. However, phosphorus is one of the more expensive minerals to supplement in a ration. Therefore, much research has concerned optimal feed concentrations of phosphorus and the relative value of various phosphorus sources for ruminants (Long et al., 1956; Long et al., 1957; Wise et al., 1961; Webb et al., 1975). Availability for animal performance of different phosphorus sources has been studied in many trials as well (Lofgreen, 1960; Ammerman et al., 1957; O'Donovan et al., 1965; Hemingway and Fishwick, 1975; Arrington et al., 1962; Tillman and Brethour, 1958a).

Phosphorus availability for the animal could theoretically differ from availability in the rumen. The term, ruminal phosphorus availability, in this thesis reflects the concentration of phosphorus in the rumen produced by 1) the solubilization of phosphorus sources and 2) phosphorus recycled from the lower gastrointestinal tract. Little

information has been published on ruminal phosphorus concentrations and ruminal availability of phosphorus sources. Therefore, a series of trials were designed 1) to determine if ruminal phosphorus concentration was linearly related to phosphorus intake so that regression could be used to evaluate ruminal availability of phosphorus from various phosphorus sources, 2) to estimate ruminal availability of various phosphorus sources for mature cattle, 3) to measure the solubility of phosphorus sources in a buffer solution and in abomasal fluid, and 4) to correlate *in vitro* solubilities to ruminal availability.

Several workers using an artificial rumen technique have found rate of cellulose digestion decreased with a low level of phosphorus in the medium (Burroughs *et al.*, 1950; Burroughs *et al.*, 1951; Anderson *et al.*, 1956; Hall *et al.*, 1961; Chicco *et al.*, 1965). This phenomenon could explain partially the anorexia exhibited by ruminants consuming inadequate phosphorus diets. Thus, a final trial was conducted to determine if low dietary phosphorus levels decrease the extent of digestion in the rumen or the total gastrointestinal tract.

CHAPTER II

REVIEW OF LITERATURE

Availability of Phosphorus Sources

Commercial Phosphorus Sources. Livestock rations are often supplemented with phosphorus. Commercial sources of phosphorus include, dicalcium phosphate, defluorinated rock phosphate, soft phosphate with colloidal clay, Curacao Island phosphate, steamed bone meal, and phosphoric acid. The phosphorus present in these different supplements differs in availability to animals and must be considered.

Long et al. (1956) fed beef heifers a basal (.09% P) ration plus supplemental phosphorus (.05% P) from dicalcium phosphate or soft phosphate with colloidal clay. Soft phosphate is a waste product from phosphorus washing plants formed during the processing to produce high grade rock phosphate. Both phosphorus supplements increased weight gains of cattle above the gain of cattle fed the basal ration. Heifers fed the dicalcium phosphate gained significantly more weight than those fed the soft phosphate. Phosphorus from soft phosphate was 17% as available as phosphorus from dicalcium phosphate using growth rate as the response criterion. Feed intake of cattle fed the basal ration decreased throughout the experiment for heifers receiving soft phosphate. The

dicalcium phosphate supplemented ration consistently was consumed at greater levels than the other two rations. Plasma phosphorus levels, an index of phosphorus availability, indicated that phosphorus from soft phosphate was only 45% as available as phosphorus from dicalcium phosphate. Dicalcium phosphate also produced a significantly greater percent ash as a percentage of dry, fat free weight of the mandibular and cannon bones, than the other two treatments. Therefore, in this particular study phosphorus from dicalcium phosphate proved to be more available than phosphorus from soft phosphate.

Further studies by Long et al. (1957) examined the availability of phosphorus from steamed bone meal, Curacao Island phosphate, and dicalcium phosphate for 200 kg beef heifers. A .07% phosphorus basal ration was supplemented with each of the phosphorus sources to increase the level of phosphorus in the ration to .15%. Weight gains were significantly greater for all three phosphorus supplemented rations, with the Curacao Island phosphate showing slight superiority. All three phosphorus sources significantly increased feed intake over the basal ration. Intake of the basal ration decreased during the trial. Plasma inorganic phosphorus levels were significantly greater for cattle fed the phosphorus supplemented rations. Over six times as much feed was required per unit of gain with the basal level of phosphorus ration. Long et al. (1957) concluded that steamed bone meal, Curacao Island phosphate and dicalcium phosphate were equally

available as sources of phosphorus in this study.

Lofgreen (1960) measured the availability of phosphorus in dicalcium phosphate, bone meal, soft phosphate and calcium phytate by calculating absorption or true digestibility using an isotope dilution technique. Compared with true digestibility, apparent digestibility of phosphorus is not valid as an indicator of availability since absorbed phosphorus is largely reexcreted into the intestinal tract. In this trial, mature wethers were limit fed a basal ration supplemented with each of the four phosphorus sources to provide phosphorus intakes above the sheep's requirement. The true digestibility of phosphorus was 50% for dicalcium phosphate, 46% for bone meal, 14% for soft phosphate and 33% for calcium phytate. These results indicate that soft phosphate was not as available as dicalcium phosphate and agrees with the findings of Long et al. (1956). Long et al. (1957) found that bone meal and dicalcium phosphate were equally available which also is supported by this study. Lofgreen (1960) demonstrated that less phosphorus was absorbed from the soft phosphate, but nevertheless, phosphorus retained from the basal ration and soft phosphate ration was equal to the retention of phosphorus in the other supplements. This indicates that the surplus phosphorus absorbed from the dicalcium phosphate, bone meal and calcium phytate supplemented rations was excreted in the feces. Significantly less phosphorus was absorbed from calcium phytate than from dicalcium phosphate

and bone meal. Phytate is the form of phosphorus found in many feeds. Because the majority of phosphorus excretion is in feces, apparent absorption can be used as an indicator of retention of phosphorus. Since the wethers apparently retained twice as much phosphorus with the supplemented rations as on the basal ration, Lofgreen (1960) concluded that the basal ration did not furnish sufficient available phosphorus to meet the animal's requirements.

An artificial rumen technique was employed by Anderson et al. (1956) to examine the availability of phosphorus from supplements fed to ruminants. Dicalcium phosphate, acid phosphate, steamed bone meal, Curacao Island phosphate and soft phosphate with colloidal clay were incubated in a phosphorus deficient fermentation medium. Cellulose digestion was measured during a twenty-four hour fermentation period. Phosphorus from dicalcium phosphate appeared as available as the standard, sodium-potassium phosphate mixture. Acid phosphate and steamed bone meal were intermediate in availability, whereas Curacao Island phosphate and soft phosphate had a low availability. Using the sodium-potassium phosphate as a standard reference of 100% availability, the phosphorus supplements were ranked on a relative scale. Dicalcium phosphate was 93% available, acid phosphate 64%, steamed bone meal 28%, Curacao Island phosphate 12%, and soft phosphate 2%.

Holstein calves (seven weeks old) were used to study

the availability of phosphorus from dicalcium phosphate, defluorinated rock phosphate, Curacao Island phosphate and colloidal clay phosphate in a trial by Wise et al. (1961). Calves had available ad libitum either a basal (.085-.11% P) ration or rations which were supplemented to .19% phosphorus with each of the four phosphorus sources. Autoradiographs were used to determine bone growth, and were found to be a sensitive indicator of the amount of phosphorus supplied. The length of rib growth, from greatest to least was for calves fed the defluorinated rock phosphate, dicalcium phosphate, Curacao Island phosphate, basal and soft phosphate rations. Body growth was determined to be significantly ($P<.05$) greater for calves supplemented with dicalcium phosphate, defluorinated rock phosphate and Curacao Island phosphate than other calves. The basal and soft phosphate rations produced significantly lower ($P<.01$) plasma inorganic phosphorus levels. Calves on the basal ration excreted 17.7% more phosphorus than they consumed. The dicalcium phosphate supplemented calves retained 56.6% of their dietary phosphorus. The general phosphorus deficiency symptoms of a rough hair coat and a stiff gait were exhibited by calves consuming the basal and soft phosphate supplemented rations. From this study, Wise et al. (1961) concluded that phosphorus from defluorinated rock phosphate, Curacao Island phosphate and dicalcium phosphate were similar in availability. Soft phosphate with colloidal clay was the least satisfactory phosphorus supplement, with less than 50% of the phosphorus

being available to the calf.

Webb et al. (1975) studied the utilization by growing-finishing beef steers of phosphorus from an 87% mono-13% dicalcium phosphate mixture, two sources of defluorinated rock phosphate, Mexican rock phosphate, sodium tripolyphosphate, mono-sodium phosphate, and a 1:1 mixture of a defluorinated rock phosphate and sodium tripolyphosphate. The basal (.12-.15% P) ration was supplemented with each phosphorus source to a level of .22% phosphorus, and rations were available ad libitum to the steers housed in individual stalls. Weight gains were lower and feed efficiency poorer for unsupplemented steers. Steers receiving Mexican rock phosphate exhibited the highest daily gains, feed intake and efficiency of feed use, while steers fed mono-dicalcium phosphate had the lowest daily gains, feed intakes and efficiency of feed use. These differences were not significant at the 5% probability level. The basal ration produced the lowest blood phosphorus and the highest blood calcium concentrations. Feed intake of steers fed the basal ration tended to decrease with time. In a second trial, mono-sodium phosphate and one of the defluorinated rock phosphates produced the highest feed intakes. The other defluorinated rock phosphate and Mexican rock phosphate gave intermediate feed intakes, while mono-dicalcium phosphate produced the lowest feed intake. Supplemented steers graded low to average choice and the defluorinated rock phosphate supplemented steers exhibited the most marbling. Webb et al. (1975) concluded that

phosphorus from these phosphorus supplements was available for growing-finishing cattle.

Yearling beef steers were used by Ammerman et al. (1957) to study seven different sources of phosphorus. These were two dicalcium phosphates, two defluorinated rock phosphates, bone meal, Curacao Island phosphate, and soft phosphate. No statistically significant differences were observed for phosphorus retention, though retention ranged from 28% for soft phosphate to 33% for one of the dicalcium phosphates. Blood calcium and phosphorus levels were not altered by treatment. The seven commercial phosphorus sources appeared to be equally effective for yearling steers. Ammerman et al. (1957) conducted a similar trial using weanling wethers to compare defluorinated rock phosphate and soft phosphate. Phosphorus retention was 46% for dicalcium phosphate, 38% for Curacao Island phosphate, 13% for soft phosphate and 11% for defluorinated rock phosphate. Calculated "true" absorption values were 100±6%, 90±18%, 50±10%, and 54±12% respectively for dicalcium phosphate, Curacao Island phosphate, soft phosphate, and defluorinated rock phosphate. This indicated that soft phosphate and defluorinated rock phosphate were only about half as useful as Curacao Island phosphate or dicalcium phosphate as sources of phosphorus for lambs.

A comparison of the availability of phosphorus in dicalcium phosphate and defluorinated phosphate for steers was conducted by O'Donovan et al. (1965). They found that the apparent digestibility of phosphorus from dicalcium phosphate

was significantly ($P<.05$) greater than phosphorus from defluorinated phosphate. However, calculated "true" digestibilities were not significantly different for the two phosphorus sources.

Hemingway and Fishwick (1975) studied the apparent retention and blood phosphorus concentrations of growing sheep supplemented with dicalcium phosphate or defluorinated rock phosphate. Compared to sheep fed a low phosphorus basal diet, the two supplements were equally superior. From this work, it was concluded that defluorinated rock phosphate was as efficient a source of phosphorus as dicalcium phosphate for growing sheep.

The availability of dicalcium phosphate, Curacao Island phosphate and defluorinated rock phosphate for dairy calves was examined by Arrington et al. (1962). All phosphorus sources were determined to not differ statistically. Dicalcium phosphate had a true absorption of 98.2%, Curacao Island phosphate 85.7%, and defluorinated rock phosphate 82.7%.

Labelled inorganic phosphates were employed by Arrington et al. (1963) to examine the absorption, retention and tissue deposition of phosphorus from dicalcium phosphate, defluorinated rock phosphate and soft phosphate with colloidal clay. A single oral dose of the irradiated phosphorus source was administered to Jersey calves (144 kg) and steers (357 kg) which were subsequently slaughtered 144 hours after dosing. At slaughter approximately 5% of the dosed ^{32}P was found in the gastrointestinal tract. Skeletal deposition of ^{32}P was

greatest in the femur epiphysis and rib, with the femur shaft, liver, kidney and muscle having decreasing amounts of ^{32}P. Phosphorus from the dicalcium phosphate was determined to be absorbed and retained in larger amounts than phosphorus from defluorinated rock phosphate or soft phosphate.

Tillman and Brethour (1958a) conducted a trial using radioisotopes to determine the true digestibility of phosphoric acid and dicalcium phosphate. Yearling Hereford steers were individually fed a ration containing two grams of phosphorus per one hundred pounds of body weight with 60.2% of the phosphorus coming from the phosphorus sources. There was no statistical difference between the true digestibility (75.8%) of the two phosphorus sources. Thus, the availability of phosphorus from phosphoric acid was determined to be similar to that of dicalcium phosphate. Menzies et al. (1955) added phosphoric acid mixed in molasses and soybean meal to a low phosphorus control ration and increased the retention of phosphorus by lambs.

Superphosphate, an acid phosphate commonly used as a fertilizer was studied by Agarwala et al. (1971) to discover its usefulness as a phosphorus supplement for lambs. Superphosphate, which has a high fluorine content, significantly reduced growth rate and the retention of calcium and phosphorus, while significantly increasing blood inorganic phosphorus levels. Calcination of superphosphate to reduce the fluorine levels produced responses similar to the untreated superphosphate. Agarwala et al. (1971) also supplemented

the diet with oral cobalt and parenteral vitamin B_{12} to examine if they would correct the adverse effects of the high fluorine content in superphosphate. Vitamin B_{12} was believed to be involved in mitigating fluorine toxicosis and cobalt is involved in the ruminal synthesis of vitamin B_{12}. Neither of these factors improved animal response to superphosphate supplementation.

In summary of these biological availability experiments, dicalcium phosphate consistently has proven to have readily available phosphorus as assessed by all response criteria. Curacao Island phosphate and defluorinated rock phosphate usually exhibited slightly lower phosphorus availability than dicalcium phosphate, although defluorinated rock phosphate appeared more variable. Defluorinated rock phosphate has been found to have very low phosphorus availability in some trials. When compared to the other phosphorus sources, soft phosphate with colloidal clay has shown to have consistently lower phosphorus availability. Superphosphate, a phosphorus fertilizer, was detrimental for ruminants, possibly due to its high fluoride content. Bone meal, an animal source of phosphorus, has been demonstrated to be a very available source of phosphorus, but generally has been more difficult and expensive to obtain. According to Thompson (1980), about 1670 million tons of 18% phosphorus equivalent supplements were fed in 1979. Dicalcium phosphate and defluorinated rock phosphate were fed the most at 51 and 30% of the total respectively. Curacao Island phosphate

provided 4.2%, while only 1.5% came from soft phosphate. Less than 1% of the total came from bone meal.

Inorganic Phosphates. Several studies have dealt with utilization of inorganic phosphates by ruminants. Phosphorus occurs in several different chemical forms. The compound may be ortho-, meta-, or pyrophosphate, which can greatly affect the availability of the phosphorus to the animal. Orthophosphates have the general chemical structure of $H_2PO_4^-$, metaphosphates PO_3^-, and pyrophosphates $P_2O_7^{-4}$.

Ammerman et al. (1957) studied meta- and pyrophosphates, formed during the thermal defluorination of rock phosphate, and compared these two forms to orthophosphate. Phosphorus retention of lambs was 26% for monocalcium phosphate monohydrate (ortho) compared with 0% for vitreous calcium metaphosphate and -21% for gamma calcium pyrophosphate. The pyrophosphate caused an increase in metabolic fecal phosphorus excretion, resulting in a negative phosphorus retention value. The true absorption was 63% for the ortho-form, 34% for the meta- form, and 0% for the pyrophosphate form. Apparently meta- and pyrophosphates are of low availability for lambs. Reduced availability could be due to reduced phosphorus solubility and absorption, or inability of the animal to use the absorbed phosphorus. Crystalline phosphates can form long polymers which could impede intestinal absorption. Calcium metaphosphate, in contrast, may be hydrolyzed to monocalcium phosphate (ortho) in the

intestine which would have increased availability.

Tillman and Brethour (1958b) used wethers to study monosodium phosphate, vitreous sodium metaphosphate and acid sodium pyrophosphate. Retained phosphorus, apparent digestibility and true digestibility values indicated that phosphorus from monosodium phosphate and acid sodium pyrophosphate was equally available to sheep. Vitreous sodium metaphosphate caused an increase in fecal phosphorus excretion, which was explained as an increase in endogenous loss. Apparently, the metaphosphate was absorbed, but was inefficiently utilized.

Chicco et al. (1965) compared in vivo and in vitro techniques for measuring the utilization of ortho-, meta-, and pyrophosphates. Phosphorus availability was determined in vivo by dosing lambs with ^{32}P isotope and measuring phosphorus absorption and retention. Vitreous sodium metaphosphate, sodium pyrophosphate, and monocalcium orthophosphate were found to have the highest availabilities. Vitreous calcium metaphosphate was intermediate in availability, and gamma calcium pyrophosphate was the least available phosphorus source. In vitro cellulose digestion was used to quantitate phosphorus availability which was measured by incubating the phosphorus sources with phosphorus depleted ruminal microorganisms. The greatest cellulose digestion occurred with sodium orthophosphate, vitreous sodium metaphosphate, sodium pyrophosphate and monocalcium orthophosphate. Vitreous calcium metaphosphate produced a lower cellulolytic response and

was concluded to be less available. Gamma calcium pyrophosphate restricted the microbial breakdown of cellulose and was considered to be totally unavailable to ruminal microorganisms. Overall, the *in vitro* results gave similar rankings to the *in vivo* results. These results also reflect the conclusions drawn from studies by Ammerman *et al.* (1957) concerning the low availability of gamma calcium pyrophosphate and of Tillman and Brethour (1958b) concerning the relatively high availability of sodium pyrophosphate.

In vitro and *in vivo* studies have established that calcium and sodium orthophosphates, sodium metaphosphate and sodium pyrophosphate are all highly available phosphorus sources. Calcium metaphosphate was found to be somewhat less available biologically than the others. The least available inorganic phosphorus source was calcium pyrophosphate. Apparently the form of the phosphate salt and its chemical composition dictates its solubility and dissociation, and thereby influences absorption and utilization by the animal or by microorganisms within the rumen.

Phytate Phosphorus. Phosphorus can occur naturally in plants as phytate phosphorus. Grains and seeds are known to be high in phytate phosphorus while hays and forages have very low levels of phytate. Phosphorus in the phytate form is largely unavailable to monogastric animals except through coprophagy. Bacterial phytase enzymes hydrolyze phytate to inositol and phosphoric acids or their salts. Ruminal

microorganisms can produce phytases and, thereby, liberate the phosphorus from phytate. This is very important since 50 to 95% of the total phosphorus in ruminant feeds occurs as phytin phosphorus. Several studies have examined the availability of phytate phosphorus for ruminal microorganisms and for the ruminant. Reid and Franklin (1947) found that sheep fed different feeds under various calcium and phosphorus intakes completely hydrolyzed all phytates. Sheep were slaughtered and the contents of the rumen, abomasum, small intestine, large intestine and rectum were examined to locate the site of phytate hydrolysis. Results indicated that complete hydrolysis occurred in the rumen. The phytate of bran incubated in vitro with ruminal contents was completely hydrolyzed in eight hours.

Calcium phytate was found by Raun et al. (1956) to be as available to ruminal microorganisms as a highly available inorganic phosphorus source. A substantial amount of phytase activity by microorganisms was detected. Optimal phytase activity occured at a pH of 5.5. Both an increased incubation time and increased concentration of ruminal microorganisms increased the amount of phytate hydrolyzed. However, as the level of substrate increased, the amount of phytate hydrolyzed tended to decrease. This suggests that phytase activity may be inhibited by an excess of substrate.

A low phosphorus basal diet supplemented with calcium phytate or monocalcium phosphate was fed to sheep in a trial

conducted by Tillman and Brethour (1958c) to determine the utilization of phytin phosphorus by sheep. Results showed that the availabilities of calcium and phosphorus in calcium phytate were similar to that of monocalcium phosphate. Approximately 92% of the phytin phosphorus was hydrolyzed and would be available for use by both ruminal microorganisms and the host animal.

Two month old calves and nine month old steers were used by Nelson et al. (1976) to examine the hydrolysis of natural phytate phosphorus in the digestive tract. Traces of phytate phosphorus were recovered in the feces of the young calves, but not in feces from older steers. No phytate was detected in the rumen, abomasum or small and large intestines of the two month old calves. Thus, calves can hydrolyze phytate at an early age and phytase production appears to occur with the onset of ruminal function. Therefore, phytate phosphorus is readily available to the animal if hydrolysis occurs anterior to the site of phosphorus absorption.

Panj et al. (1969) studied the nutrient availability of phytin phosphorus in various cattle feeds. Phytin phosphorus was readily available to ruminal microorganisms. Although hydrolysis was low during the first six hours, it was totally hydrolyzed after 36 hours in an artificial rumen. Thus it can be assumed that complete hydrolysis of phytin phosphorus occurs in the rumen. The initially slow rate of hydrolysis may indicate that the microorganisms require time to attach to the feed and may need another supply of inorganic

phosphorus in the medium. Slower hydrolysis near the end of incubation suggests either a feedback inhibition, possibly through the accumulation of phosphorus, or the low amount of remaining phytin. Phytase activity of the microorganisms was found to be independent of the chemical composition of the feeds.

Phosphorus in the phytate form can be completely hydrolyzed in the rumen. Phytate phosphorus thereby is biologically available as a source of phosphorus for ruminal microorganisms and subsequently for the animal. The enzyme, phytase appears with the onset of ruminal function. The ability to effectively utilize phytate phosphorus gives ruminants a distinct advantage over nonruminants in meeting their phosphorus requirement. Nonruminants have very limited ability to hydrolyze phytate. Ruminants thereby can obtain more phosphorus from the natural feedstuffs containing phytate phosphorus than can nonruminants.

Effect of Phosphorus on Cellulose Digestion

Several workers have indicated that a low level of phosphorus can decrease the rate or extent of cellulose digestion. This could have important implications for ruminants grazing poor quality pastures and rangeland. Most of the research supporting this phenomenon has used an *in vitro* artificial rumen technique. Burroughs *et al.* (1950) examined five good quality hays (19% crude protein, 20% cellulose) and five poor

quality hays (3% crude protein, 25% cellulose), and found that the addition of a mineral mixture to the medium increased the extent of cellulose digestion of the poor quality hays. Cellulose digestion of the good quality hays was unaffected by the mineral supplementation. Further work by Burroughs et al. (1951) discovered that the addition of disodium phosphate and iron to the medium stimulated both urea utilization and cellulose digestion by ruminal microorganisms.

Graded levels of various phosphorus supplements were studied by Anderson et al. (1956) to determine their phosphorus availability via the extent of cellulose digestion occurring in a phosphorus deficient medium. An approximately linear relationship was found to exist between the amount of phosphorus added (10-40 µg phosphorus per ml medium) to the phosphorus deficient medium and the amount of cellulose digested by ruminal microorganisms. The medium devoid of phosphorus had 34% cellulose digestion. Cellulose digestion increased to 80% when 80 µg of the standard phosphorus source per ml medium was added.

Hubbert et al. (1958) found that an excess of phosphorus, 1,000 µg of phosphorus per ml of medium, did not decrease cellulose digestion in vitro. Anderson et al. (1956) reported that 40 to 80 µg of phosphorus per ml of medium was sufficient to meet the requirements for in vitro cellulose digestion. Maximum in vitro microbial cellulose digestion occurred between 20 and 60 µg of phosphorus per ml of medium

according to Hall et al. (1961). Sodium ortho-, meta- and pyrophosphates, and calcium phytate were all equally available sources of phosphorus. In vitro work by Chicco et al. (1965) showed that 60 µg or more of available phosphorus per ml of medium produced maximum cellulose breakdown by rumen microorganisms. This level of phosphorus supplementation agrees with the results of Anderson et al. (1956) and Hall et al. (1961). A quadratic effect was observed by Chicco et al. (1965) between the level of phosphorus in the medium and the amount of cellulose digestion. However, it should be noted that the low levels of phosphorus which caused decreased cellulose digestion in vitro could not be practically reproduced in the rumen where ruminal phosphorus levels normally do not fall below 200 µg per ml due to dietary and recycled phosphorus.

Phosphorus Metabolism in Ruminants

Knowledge concerning the metabolism of phosphorus in ruminant animals is essential for understanding the mechanisms of availability of phosphorus to the animal for maximum productive efficiency. Phosphorus is required both for the growth of tissue and bone, as well as for the multiplication of microorganisms within the rumen. Ruminants are unique because of their ability to recycle phosphorus to the early part of the gastrointestinal tract through both saliva and blood. Since the pH in the rumen is within the buffering range of phosphates, they serve as buffering agents for

volatile fatty acids produced in the rumen, although the phosphate contribution to ruminal buffering relative to carbonates appears very low (Counotte et al., 1979). The site of phosphorus absorption from the gastrointestinal tract appears to be influenced by species and, possibly, age of an animal. Studies have examined the various routes of phosphorus absorption and excretion. Their interrelationship serves to maintain phosphorus homeostasis. Resorption of phosphorus from bone also may play a role in an animal's efforts to maintain adequate phosphorus levels. Whether phosphorus can be mobilized to maintain homeostasis, or whether such mobilization simply parallels calcium mobilization and homeostasis is unknown. Excess bone resorption can lead to rickets and osteoporosis. These are symptoms of a dietary phosphorus deficiency of the nonruminant. In contrast, the first clinical sign of a phosphorus deficiency in ruminants is anorexia. This may reflect reduced microbial activity in the rumen, and a slower rate of fiber digestion and removal from the rumen.

Smith et al. (1952) injected ^{32}P intravenously into lambs which were 1, 4, and 10 months old to examine the distribution of phosphorus among the body tissues. In decreasing order of ^{32}P uptake, tissues ranked as follows: bile, thymus, liver, tongue, kidney, heart, lymph nodes, spleen, lung, rumen muscle, testes, biceps muscle, gastrocemius muscle and brain. There was no pronounced age effect on ^{32}P uptake, but phosphorus exchange was greater for the younger

sheep.

Injection of ^{32}P into 1, 4, and 10 month old lambs (with the one month old lambs still nursing) by Smith et al. (1955a) was used to study the transfer of phosphorus to the digestive tract of sheep. The highest phosphorus level was found in the omasal contents, except for the one month old lambs, and tended to decrease posterior to that point. The highest phosphorus concentration for the young lambs was present in the ruminal contents. The very young lambs had only small amounts of ^{32}P entering the digestive tract compared to the older lambs. This indicates that a change in the digestive process occurs after weaning. Data suggesting omasal phosphorus secretion agrees with the suggestion of Garton (1951). Per 100 ml, omasal fluid contained 120 mg phosphorus compared with ruminal fluid at 79 and abomasal liquor at 63 mg. The high phosphorus level in the omasal contents could be due to the direct passage of saliva to the omasum by way of the esophageal groove, by passage prior to equilibration with rumen contents, or by selective water resorption. The secretion of phosphorus into the omasum and abomasum appears to be small compared to the total phosphorus in the digesta.

Smith et al. (1955a) found the four stomach compartments of sheep to be the major sites for endogenous phosphorus secretions. This is suggested from the delay in the transfer of ^{32}P from blood to the intestinal tract. Entry of phosphorus into the rumen of a ten month old lamb was

approximately 4.8 grams per day. According to McDougall (1948) sheep saliva contains about .81 grams of phosphorus per liter. If 6 to 16 l of saliva are produced daily (Kay, 1960) phosphorus from salivary flow would cycle 4.9 to 13 g phosphorus to the rumen. Thus, the majority of the endogenous phosphorus in the rumen may be derived from saliva, with the ruminal wall resorption of endogenous phosphorus being about equal to the ruminant secretion of exogenous phosphorus.

No parallelism between the amount of ^{32}P in tissues and that in the gut contents was detected by Smith et al. (1955a). Phosphorus metabolism in tissues operated independently from phosphorus exchange between gastrointestinal tract contents and blood. However, tissue may serve as an intermediate in this exchange. The rate of uptake of ^{32}P by tissue was determined to be proportional between tissue and plasma phosphates, exhibiting a first order rate reaction. With increasing age, most tissues exhibited a decreasing rate of exchange of plasma phosphate. However, aging did not change the mineral composition of the body.

The transfer of phosphates across the digestive tract of two month old calves and three year old lactating dairy cows was examined subsequently by Smith et al. (1956). Calves exhibited a higher concentration of phosphorus in all tissues (except ruminal epithelium) and a lower concentration of phosphorus in the digesta (except ruminal contents) than the cows. This phenomenon may have been due to a higher phosphorus

content in the liquid diet consumed by the calves than the diet consumed by the cows. Ruminal phosphorus secretion was determined to be 66 grams per day. Using an estimated salivary flow of 50 to 150 liters per day (Church, 1969), and a phosphorus concentration of .81, phosphorus input from saliva would equal 40 to 121 g daily. Therefore, the majority of the endogenous phosphorus in the rumen of cattle could be derived from saliva. The level of ^{32}P in the small intestinal digesta of both calves and cows was greater than the ^{32}P in the abomasal contents. This indicates that additional phosphorus is being secreted into the intestines, or that exogenous phosphorus is more rapidly absorbed than endogenous phosphorus. Results from Smith et al. (1955b) found similar endogenous phosphorus secretion in the swine small intestine. Young calves preferentially absorbed endogenous phosphorus compared to feed phosphorus from the contents of the lower intestinal tract. Among sites along the digestive tract, the net uptake of P^{32} into plasma was greatest from the intestinal tissues of calves and from omasal tissue of cows. The rate of phosphorus exchange was greatest for the rumen epithelium and small intestinal tissue of the calf and decreased further down the tract. In contrast, for cows the small and large intestines had similar exchange rates. Thus, the phosphorus exchange by bovine intestinal tissues seems to increase with increasing age as has been observed with sheep tissues. The overall rate of phosphorus exchange between gastrointestinal tissues and

plasma appears to be much less in cattle than in sheep.

Wethers fed increasing amounts of phosphorus were used by Tomas et al. (1967) to study the relationship of salivary phosphorus to dietary phosphorus and phosphorus in the rumen. Wethers had ruminal cannulas and their left parotid duct was exteriorized. A strong positive relationship was found between ruminal fluid phosphorus levels and dietary phosphorus intake. The ratio of salivary phosphorus to serum phosphorus ranged from 7:1 to 9:1 indicating that phosphorus was selectively secreted. As the rate of saliva secretion increased, its phosphorus concentration decreased. The amount of phosphorus secreted in saliva also was determined to be related directly to the phosphorus intake. Changing phosphorus intake by 10 fold caused saliva secretion to change 2 fold. This suggests that the concentration of phosphorus in the rumen is dependent primarily upon salivary phosphorus secretion rather than being directly dependent on dietary phosphorus consumed. From the earlier discussion, ruminal entry of endogenous phosphorus per day was 4.8 grams for sheep and 66 grams for cattle. This compares with daily intakes, assuming feed intakes at 2% of body weight and .20% phosphorus in the diet, of 1.6 and 16 grams per day. As dietary phosphorus intake fell below a maintenance level, both salivary phosphorus concentration and total salivary phosphorus secretion declined drastically. The effect of deficiency is multiplied in the rumen because both intake and recycling are reduced. A high correlation ($r=+.91$,

P<.001) was found between inorganic phosphorus concentrations in ruminal fluid and in parotid saliva. Inorganic phosphorus concentrations in blood serum and saliva had a correlation of r=+.64, while serum phosphorus and ruminal fluid phosphorus were also correlated (r=+.75). One parotid gland was estimated to produce 3.2 to 4.2 liters of saliva per day. With their low phosphorus intake, (.42 grams per day), 3.0 grams of phosphorus per day was present in saliva; while with their high phosphorus intake (4.02 grams per day) 5.3 grams of phosphorus per day was recycled in saliva. The ratio of salivary to dietary input of phosphorus into the rumen thereby ranged from 7.2 to 1.3.

Phosphorus homeostasis in sheep was studied by Tomas (1974a). The relationships between the amounts of phosphorus secreted in saliva and excreted via the urine and feces was examined. Saliva from the parotid gland was surgically rerouted either to the rumen or back to the blood stream. Recycling into the blood stream increased urinary phosphorus excretion by an amount equal to the amount of saliva withheld from the rumen. Phosphorus balance was unaltered. This indicates that recycling to the rumen normally substitutes for urinary excretion and recycling increases fecal loss of phosphorus. The increase in urinary phosphorus excretion, however, could be attributed partly to an increased plasma inorganic phosphorus concentration and partly to a reduced efficiency of renal tubular reabsorption of phosphorus. No effects on hormone concentrations were

detected. One important factor in phosphorus homeostasis which determines the pathway of phosphorus excretion, therefore, appears to be the rate of endogenous phosphorus secretion into the gastrointestinal tract. Data from this work supports the concept that secretion of endogenous phosphorus to the gastrointestinal tract and the quantity of phosphorus excreted in the urine would be inversely related at a given level of phosphorus intake. Renal mechanisms control phosphorus homeostasis when the flow of endogenous phosphorus to the gastrointestinal tract is reduced, but ruminants excrete phosphorus primarily via the intestines. Most of the phosphorus excreted is endogenous. Salivary phosphorus secretion, rather than absorption of phosphorus, seems to regulate phosphorus homeostasis in ruminants. The kidney plays a unique role in the ruminant as a supplement to the intestinal excretion of phosphorus. The balance between fecal and urinary excretion of phosphorus appears to be influenced by the balance between the abilities of the salivary glands and the kidneys to clear phosphorus from the blood at a particular plasma phosphorus level.

Tomas (1974b) also studied the influence of the particle size of the diet on the pathway of secretion of phosphorus in sheep. Oat hulls were fed in a coarse or a finely ground form to alter the saliva flow rate. Daily rumination time was used as an index of relative saliva flow. Rumination time was much less with fine ground than coarse roughage. Grinding reduced ruminal pH as well as the

concentration and the total amount of inorganic phosphorus in the rumen. Urinary phosphorus loss was significantly greater and fecal phosphorus loss was less for sheep fed the finely ground oat hulls. This supposedly resulted from reduced recycling of phosphorus via saliva to the gastrointestinal tract. Urine volume was unchanged by grinding of the forage. Fecal and urinary excretion of phosphorus were inversely correlated ($r=-.86$, $P<.01$), as were urinary phosphorus excretion and inorganic phosphorus concentration in the rumen ($r=-.88$, $P<.01$). Since plasma phosphorus was unaffected by feed grinding, the increased urinary phosphorus output was not a result of an increased plasma phosphorus level. The increase in urinary phosphorus could have resulted from either an increased glomerular filtration rate and/or an enhanced clearance of phosphorus filtered. However, glomerular filtration rate was not increased, and no evidence of altered parathyroid hormone action was found by Tomas (1974b). Fecal phosphorus appeared to be the primary factor determining the relationship between urinary and fecal phosphorus excretion. Therefore, the main effect of the fine particle size was to reduce the fecal phosphorus excretion and permit urinary phosphorus levels to increase. These results would suggest that recycling is quantitatively much greater with roughage rations. With high concentrate rations and lower salivary flow, ruminal phosphorus concentration should be more dependent on immediate availability of consumed phosphorus. With high roughage rations,

recycling of phosphorus will help maintain ruminal concentrations so that phosphorus availability in the total tract, not in the rumen alone, would alter ruminal phosphorus concentration.

Mayer et al. (1966) examined the effects of the parathyroid on renal phosphorus excretion in dairy cows. Parathyroid hormone causes phosphaturia, an elevated concentration of urinary phosphorus. This could result from two alterations. Parathyroid hormone could increase the amount of phosphorus filtered or alter tubular function of the kidney. Consistent results were not obtained for phosphorus filtration. However, the percentage of tubular phosphorus resorbed decreased almost to zero in the presence of parathyroid hormone. The increase in urinary phosphorus, therefore, appears to be due to direct action of parathyroid hormone on the renal tubule.

In a subsequent study of the influence of the parathyroid on phosphorus balance and homeostasis in dairy cows, Mayer et al. (1968) found that reciprocal changes in urinary and fecal phosphorus excretion occurred during altered parathyroid conditions. Parathyroid extract given to intact or parathyroidectomized cows increased urinary phosphorus and decreased fecal phosphorus. Parathyroidectomy alone decreased urinary phosphorus and increased fecal phosphorus. However, the various treatments caused minimal changes in plasma phosphorus levels and phosphorus balance. During parathyroid extract treatment, phosphorus homeostasis was

preserved primarily by a reduction in fecal phosphorus output rather than liberation of phosphorus from bones. The decrease in fecal phosphorus observed with parathyroid extract as well as the increase in fecal phosphorus after a parathyroidectomy suggest that parathyroid hormone enhances the absorption of phosphorus from the gastrointestinal tract. However, this interpretation must be considered cautiously. Since large quantities of phosphorus are secreted into the gastrointestinal tract of cows, a change in the rate of endogenous phosphorus secretion could influence the fecal phosphorus output as well as alter absorption.

Skeletal studies were conducted on sheep by Benzie et al. (1959) to study the relationship between phosphorus intake and the resorption and repair of the skeleton during pregnancy and lactation. The skeletal ash of ewes fed a low phosphorus basal diet decreased by 40% from mid-gestation to mid-lactation and was not replenished within two months after the end of lactation. Phosphorus supplementation of these ewes at mid-lactation enabled greater bone repair to occur. Ewes fed a ration supplemented with phosphorus exhibited a 19% decrease in skeletal ash by mid-lactation, but this was fully restored by two months post-lactation. Resorption occurred to a greater extent in more cancellous bone, such as the cervical vertebrae, than in compact long bones. The long bones were involved with severe resorption only. Ewes fed low phosphorus levels had very low blood phosphorus levels, especially during early lactation. Blood

phosphorus had increased by mid-lactation. Blood phosphorus concentrations increased to normal in less than four weeks post-lactation. Blood stores are replaced much more rapidly than bone stores following phosphorus depletion.

Field and Suttle (1967) examined the deposition of several minerals by developing sheep fetuses. The calcium to phosphorus ratio of the fetus was 1.3-1.8:1. On a fat free wet weight basis, calcium and phosphorus concentrations were .83% and .52%, respectively. With increasing fetal age, calcium and phosphorus levels tended to increase proportionally with skeletal growth. The ratio of calcium to phosphorus increased in developing fetuses with calcium being deposited in the skeleton and phosphorus in both the skeletal and soft tissues. The calcium to phosphorus ratio approached that of bone (2:1) as the fetus developed and more of the total phosphorus was found in the skeleton.

The effect of diets low in calcium and phosphorus on the development of growing lambs also was examined by Field et al. (1975). He fed diets low in calcium, phosphorus or both. Feed intake was reduced by 26% with a low calcium ration, decreased 41.5% for a low phosphorus diet, but was unaffected when both calcium and phosphorus were low. Dry matter digestibility was lower with the low phosphorus ration (57.7%) than for the other two rations (61.2%). All mineral deficient diets decreased calcium and phosphorus retention, but did not precipitate negative mineral balances. The deficient rations impaired bone matrix development and

mineralization of the tibia and lumbar vertebrae. These changes could lead to rickets and osteoporosis. Results indicate that besides absolute concentrations of dietary calcium and phosphorus, the calcium to phosphorus ratio may be important with either low calcium or low phosphorus diets. The interactions of these two minerals during absorption may be responsible. These interactions have been examined by several workers.

Effects of various calcium to phosphorus ratios and levels on absorption were studied by Lueker and Lofgreen (1961). Differences in absorption reflected dietary intakes. Metabolic fecal calcium excretion was constant with all calcium to phosphorus ratios and was independent of the amount of calcium and phosphorus absorbed. However, metabolic fecal phosphorus was altered by calcium and phosphorus absorption. As the amount of phosphorus absorbed was increased, metabolic fecal phosphorus excretion also increased. However, metabolic fecal phosphorus decreased as calcium absorption increased. Increased calcium absorption increased calcium retention, while an increased phosphorus absorption increased phosphorus in the feces but not the amount of phosphorus retained. The calcium to phosphorus ratio influenced the excretion of metabolic fecal phosphorus but did not affect the absorption of phosphorus and calcium. The absorption of calcium was not influenced by phosphorus intake, nor was the amount of phosphorus absorbed influenced by calcium intake.

Approximately 55% of the dietary phosphorus was absorbed while only 11% of the dietary calcium was absorbed. This low level of calcium absorption may not have been sufficient to meet the animal's calcium requirement. Lack of agreement on the importance of calcium to phosphorus ratio in diets of ruminants could be the result of a lack of absorption of specific calcium or phosphorus sources causing the ratio absorbed to differ from the ratio of the diet.

A diet with a wide calcium to phosphorus ratio did not affect phosphorus absorption when phosphorus intake was adequate in a study by Young et al. (1966b). Phosphorus absorption tended to increase with an increase in phosphorus intake. Calcium absorption was reduced by a diet low in phosphorus. Increased calcium absorption was observed when phosphorus intake increased. This disagrees with data by Lueker and Lofgreen (1961) concerning the influence of phosphorus intake on the absorption of calcium.

Ruminants can tolerate a much wider dietary calcium to phosphorus ratio than nonruminants. Since the upper small intestine is more acidic in ruminants than in nonruminants, the precipitation of phosphorus as tricalcium phosphate is unlikely. Ruminants also can utilize phytate phosphorus more efficiently than nonruminants. Thus ruminants can tolerate a wider calcium to phosphorus ratio. Recycling of phosphorus to the rumen serves as a conservation mechanism, also.

Young and mature calcium deficient sheep were used by

Braithwaite (1975) to study the absorption and retention of calcium and phosphorus. Calcium absorption was related to calcium intake until a maximum calcium intake was reached. Young sheep absorbed dietary calcium more efficiently and at a higher rate than the mature wethers. Apparent phosphorus absorption was found to be directly related to phosphorus intake, with maximum phosphorus absorption being higher for the younger sheep. Excretion of calcium in the urine and into the intestines was not altered by the level of calcium intake, although, the calcium deficient diet did lower fecal endogenous calcium values. Excretion of phosphorus in the urine was variable, possibly due to phosphorus homeostasis. Calcium and phosphorus were determined to be retained in a constant ratio, suggesting that phosphorus retention may be controlled by the rate of calcium retention. Bone turnover was higher with young sheep than with mature sheep. Mobilization of skeletal calcium occurred on the calcium deficient diet with calcium losses being replaced by the adequate calcium diet. Relative rates of bone resorption and bone accretion were changed during depletion and repletion. The lower maximum retention of calcium for the mature wethers was due to a lower rate of bone accretion.

Manston (1967) examined the influence of dietary calcium and phosphorus on absorption of these minerals by the dairy cow. A constant percentage of dietary calcium was absorbed. Therefore, calcium absorption increased as dietary calcium intake increased. Fecal endogenous losses of

calcium increased slightly with higher calcium intakes. The amount of phosphorus absorbed was unaffected by calcium intake. A low phosphorus intake caused a negative phosphorus balance, but increasing phosphorus intakes resulted in positive phosphorus balances. Calcium and phosphorus absorption increased when dietary intakes increased, but only for a short time. Phosphorus absorption was thought to be more efficient with a higher calcium to phosphorus ratio.

Deficient, borderline and adequate phosphorus diets were fed to lambs by Preston and Pfander (1964). After two weeks, body weight gains and feed intakes had decreased with the deficient and borderline phosphorus rations. As the concentration of phosphorus in the diet increased, metabolic fecal phosphorus and phosphorus balance increased. True digestibility was related also to phosphorus intake. Total phosphorus in ruminal fluid increased markedly with increases in phosphorus intake. A deficiency of phosphorus in the diet apparently can decrease endogenous phosphorus secretion into the rumen. A phosphorus requirement for lambs was determined by regressing average daily gain on phosphorus intake. Body weight and gain were used to predict a daily phosphorus requirement.

The endogenous excretion of calcium and phosphorus by sheep was studied by Young et al. (1966a). Phosphorus depleted and control sheep were used to examine the effects of phosphorus depletion and repletion on the rates of endogenous calcium and phosphorus excretion. Metabolic fecal

phosphorus was used to indirectly estimate the total intestinal secretion of phosphorus. When phosphorus depleted sheep were fed a ration adequate in phosphorus, rate of excretion of metabolic fecal phosphorus increased markedly. This was due to decreased reabsorption of intestinally secreted phosphorus, rather than a change in the rate of secretion into the intestine. The consumption of a phosphorus deficient diet by the control sheep decreased metabolic fecal phosphorus excretion because rate of intestinal secretion of phosphorus decreased. Metabolic fecal calcium secretion into the intestinal tract was constant. However, metabolic fecal phosphorus varied with phosphorus intake. This agrees with work by Lueker and Lofgreen (1961) and Preston and Pfander (1964). Dietary phosphorus appeared to be absorbed by passive diffusion while calcium was actively absorbed.

Van't Klooster (1969) studied the physico-chemical states of minerals in the gut contents and feces of ruminants and related these factors to mineral absorption. Most of the phosphorus in the fecal non-ultrafiltrable fraction occurred as undissolved compounds which could only be dissolved at a pH below 4. Approximately one half of the undissolved calcium and magnesium in the feces was in the form of phosphates. Fecal ultrafiltrates resembled cecal ultrafiltrates indicating that little digestion occurred in the large intestine or cecum. In the first part of the small intestine, about 70% of the phosphorus was in solution, while in the last part of the small intestine, only about 31%

of the phosphorus was in solution. This may have been due to the more acidic condition of the upper small intestine compared to the alkaline state of the lower small intestine.

In summary, phosphorus homeostasis in ruminants involves several regulatory mechanisms which enable the animal to control the amount of phosphorus utilized by its system. Increasing the concentration of phosphorus in the diet causes ruminal phosphorus concentration to increase. Phosphorus concentration in saliva also is directly related with phosphorus intake and with the amount of phosphorus present in ruminal fluid. Recycling phosphorus to the rumen via saliva and blood is an important factor in the ruminant's ability to maintain phosphorus homeostasis and delay phosphorus deficiency. The endogenous phosphorus present in the rumen appears to be derived primarily from phosphorus in saliva.

Ruminants excrete excesses of phosphorus mainly via feces. However, renal mechanisms can control phosphorus homeostasis if cycling of endogenous phosphorus to the gastrointestinal tract is reduced. Apparently the extent of endogenous phosphorus secretion into the tract determines whether phosphorus excretion will occur via the urine or feces. Recycling to the rumen substitutes for urinary excretion and increases fecal phosphorus output.

Calcium and phosphorus deficient rations impair bone matrix development and mineralization of young growing animals. This can lead to the common phosphorus deficiency

symptoms of rickets and osteoporosis. However, with mature animals, long bone degeneration appears only in the case of severe bone resorption due to lack of sufficient phosphorus in the diet.

Studies concerning the interrelationships of phosphorus and calcium in absorption and excretion have shown that the calcium to phosphorus ratio of the ration does not consistently influence absorption or excretion. Calcium intake does not alter the excretion of calcium with metabolic fecal calcium levels remaining fairly constant. Metabolic fecal phosphorus was altered by both phosphorus intake and the absorption of phosphorus and calcium. With increasing phosphorus absorption, metabolic fecal phosphorus excretion also increased. However, as calcium absorption increased metabolic fecal phosphorus was found to decrease. Increased phosphorus absorption, while increasing the phosphorus present in feces, did not influence phosphorus retention by the animal. In general, low dietary phosphorus intakes will reduce feed intake. This is the first clinical sign of a phosphorus deficiency. These several factors involved in phosphorus homeostasis must be considered and applied to the interpretation of research concerning phosphorus availability and utilization by ruminants.

CHAPTER III

RUMINAL AVAILABILITY OF PHOSPHORUS SOURCES

Summary

Ruminal phosphorus availability of sodium phosphate, mono-dicalcium phosphate containing 21% phosphorus (mono-dical), mono-dicalcium phosphate containing 18.5% phosphorus (dical), and defluorinated rock phosphate was estimated by comparison to a sodium phosphate standard curve. Ruminal phosphorus concentration was directly and linearly related to sodium phosphate intake with dietary phosphorus levels of .06 to .17% for 35 kg lambs and with mature steers (509 kg) at dietary phosphorus levels below .12%. The linear portion of the regression between dietary phosphorus intake and ruminal phosphorus concentration for steers was employed to estimate the ruminal availability of the four phosphorus sources. Compared to sodium phosphate, assumed to have a ruminal phosphorus availability of 100%, mono-dical, dical and defluorinated rock phosphate were found to be 59, 42 and 28% as available in the rumen.

(Key Words: Phosphorus, Ruminal Availability.)

Introduction

The biological availability of phosphorus from various commercial phosphorus sources is important for determining the amount of phosphorus needed to meet the animal's phosphorus requirement. Dicalcium phosphate and defluorinated rock phosphate are the commercial phosphorus sources most often added to livestock rations (Thompson, 1980). When compared to standard phosphorus sources, dicalcium phosphate and defluorinated rock phosphate appear to be slightly less available than the standard (Wise et al., 1961; Hemingway and Fishwick, 1975; Ammerman et al., 1957; O'Donovan et al., 1965; Arrington et al., 1962). Defluorinated rock phosphate has been found to be only about half as useful as dicalcium phosphate as a source of phosphorus for lambs (Ammerman et al., 1957). Overall, dicalcium phosphate has been found to be a consistently available source of phosphorus. Results with defluorinated rock phosphate have been more variable, but have generally exhibited similar availability to dicalcium phosphate. However, past workers have not examined ruminal phosphorus availabilities which could be important in meeting the phosphorus requirement of ruminal microorganisms to insure maximum efficiency of digestion. Ruminal fluid phosphorus concentrations have been found to change in proportion to dietary phosphorus levels (Preston and Pfander, 1964; Evans and Davis, 1966; Tomas et al., 1967). Thus, if a linear relationship between ruminal fluid phosphorus and dietary phosphorus existed for animals

supplemented with a reference phosphorus standard, the relative ruminal availability of various commercial phosphorus sources could be determined by comparison to this standard curve. The objectives of these trials were 1) to determine if ruminal phosphorus concentration was linearly related to phosphorus intake, so that it might be employed to evaluate the ruminal availability of phosphorus from various phosphorus sources, and 2) to estimate the ruminal availability for mature cattle of two mono-dicalcium phosphates and defluorinated rock phosphate as compared to sodium phosphate, a reference standard.

Experimental Procedure

Trial 1. Lamb Standard Curve. Sixteen ram lambs (35 kg) housed in metabolism stalls were individually fed 911 g daily of a basal ration (table 1) with a standard orthophosphate source, $Na_2HPO_4 \cdot 7H_2O$, hereinafter called sodium phosphate (table 2), added to provide dietary levels of phosphorus of .064, .101, .131 and .166%. To deplete phosphorus stores, lambs were fed the low phosphorus basal ration for 3 weeks prior to the initiation of the trial. The treatment diets were fed twice daily during a 7 day adaptation period, with lambs being fed every 6 hr for the 24 hr period prior to rumen sampling. Ruminal fluid samples were collected via stomach tube 3 hr postprandially. For a second period, lambs were rerandomized to the four treatment groups, and similar adaptation and sampling procedures were followed. After

TABLE 1. RATION COMPOSITION

Ingredient	Lamb std. curve	Steer std. curve	Steer availability
		%	
Cotton hulls (IFN 1-10-599)	91.9	97.0	97.0
Sugarcane, molasses (IFN 4-04-696)	3.9	.....	.....
Urea	2.46	1.6	1.6
Salt, trace mineralized	.61	.5	.5
Calcium carbonate (IFN 6-02-632)	.26	.05	.05
Phosphorus source + sodium bicarbonate[a]	.87	.85	.85
Vitamins A & D	+	+	+

[a]See table 2.

TABLE 2. PHOSPHORUS SUPPLEMENT COMPOSITION

Phosphorus source	Lamb std. curve				Steer std. curve			Steer availability			
	%										
Sodium phosphate	0	.29	.58	.87	0	.43	.85	.85	...	...	...
Mono-dical	...	...	...	...	...	...	...	...	.47	...	...
Dical (IFN 6-01-080)	...	...	...	...	...	...	...	...	...	.54	...
Defluorinated rock phosphate (IFN 6-01-780)	...	...	...	...	...	...	...	...	...	...	.55
Sodium bicarbonate	.87	.58	.29	0	.85	.42	0	0	.38	.31	.30

collection of ruminal fluid in each period, samples were immediately centrifuged at 1640 g for 15 min and the supernatent fluid analyzed for inorganic phosphorus by the colorimetric phosphomolybdate reaction (Fiske and Subbarow, 1925). Data were analyzed as a randomized block design, with periods serving as blocks (Snedecor and Cochran, 1967). Ruminal phosphorus concentrations were regressed on ration phosphorus levels to determine the slope and range over which ruminal phosphorus concentrations might be used to assess the supply of phosphorus soluble in the rumen and thereby readily available to ruminal microorganisms.

Trial 2. Steer Standard Curve. Six mature ruminally cannulated Hereford steers (509 kg) were individually fed 6470 g daily of a basal ration (table 1) with phosphorus from sodium phosphate (table 2) added to provide ration phosphorus concentrations of .068, .123 and .173%. Steers were fed twice daily for a 5 day adaptation period. On day 6, steers were fed at 8 hr intervals for 24 hr. After the third feeding a ruminal fluid sample was collected via ruminal cannula 4 hr postprandially. Within 30 sec after collection, the samples were strained through eight layers of cheesecloth and centrifuged at 17000 g for 10 min. Inorganic phosphorus concentrations of the supernatent were determined by the same procedure as with ruminal fluid from lambs. Data were analyzed as a completely randomized design with six steers, three periods and three phosphorus intakes (Snedecor and Cochran, 1967). Ruminal phosphorus concentration was

regressed on ration phosphorus concentration to determine the slope and range over which ruminal phosphorus levels in cattle would be linearly related to phosphorus intake. This regression line was employed in the subsequent trial to estimate the relative ruminal availability of various phosphorus sources.

Trial 3. Steer Ruminal Availability of Phosphorus Sources. Steers used above were individually fed 6300 g daily of cottonseed hulls (table 1) with 196 g of supplement containing equal amounts of phosphorus from one of the following: sodium phosphate, a standard phosphorus source (table 2); a mono-dicalcium phosphate mixture containing 21% phosphorus, mono-dical; a mono-dicalcium phosphate mixture containing 18.5% phosphorus, dical; and a defluorinated rock phosphate (table 3). Feed was provided twice daily for a 5 day adaptation period. On day 6, steers were fed every 8 hr for 24 hr. Ruminal fluid was collected 4 hr after the final feeding via ruminal cannula. Samples were handled and analyzed as in the steer standard curve trial. Rumen pH level was determined for each steer at time of sample collection. Steers received each treatment in a partially balanced incomplete block design with four treatments, four weeks and six steers (Cochran and Cox, 1957). The error term included the three-way interactions plus the period effect. Relative ruminal availability of the various sources of phosphorus was calculated by relating ruminal phosphorus concentrations to the standard curve for sodium phosphate generated in the previous steer trial.

TABLE 3. CHEMICAL ANALYSIS OF PHOSPHORUS SOURCES STUDIED

Phosphorus source	Minimum phosphorus	Minimum calcium	Maximum calcium	Maximum fluorine
	- - - - - - - - - - - - - - - - % - - - - - - - - - - - - - - - -			
Sodium phosphate[a]	11.6	....	....	....
Mono-dical[b]	21.0	15.0	18.0	.21
Dical[c]	18.5	20.0	24.0	.185
Defluorinated rock phosphate[d]	18.0	31.0	34.0	.18

[a]Analytical reagent, Mallinckrodt, Inc., St. Louis, MO.

[b]Mono-Dical, Occidental Chemical Co., Houston, TX.

[c]Dynaphos, International Minerals and Chemical Corp., Mundelein, IL.

[d]CDP, Borden Chemical/Borden, Inc., Norfolk, VA.

Results and Discussion

Trial 1. Lamb Standard Curve. Feeding a low phosphorus ration supplemented with sodium phosphate to levels of .064, .101, .131 and .166% phosphorus in the ration produced ruminal phosphorus concentrations of 201, 330, 481 and 619 mg phosphorus per liter of ruminal fluid, respectively (figure 1). The phosphorus requirement for growing lambs is .18% phosphorus (NRC, 1968). All rations fed in this trial were below this level. The relationship between phosphorus intake and phosphorus concentration in ruminal fluid was linear over the range of phosphorus concentrations tested in this trial. Each additional level of phosphorus in the ration increased ($P<.01$) the concentration of phosphorus in ruminal fluid. At higher phosphorus intakes, ruminal phosphorus concentrations would not be expected to continue to increase linearly ad infinitum based on the results of Evans and Davis (1966). Nevertheless, since the relationship was linear over the range tested, ruminal phosphorus concentrations in this region could be employed to estimate ruminal availability of various phosphorus sources.

Trial 2. Steer Standard Curve. Cattle fed a basal diet with sodium phosphate added to provide .068, .123 and .173% phosphorus had ruminal phosphorus concentrations of 264, 406 and 434 mg phosphorus per liter of ruminal fluid (figure 2). All treatment rations were below the .18% phosphorus level required to maintain mature steers (NRC, 1976). At the highest level of phosphorus feeding, ruminal phosphorus

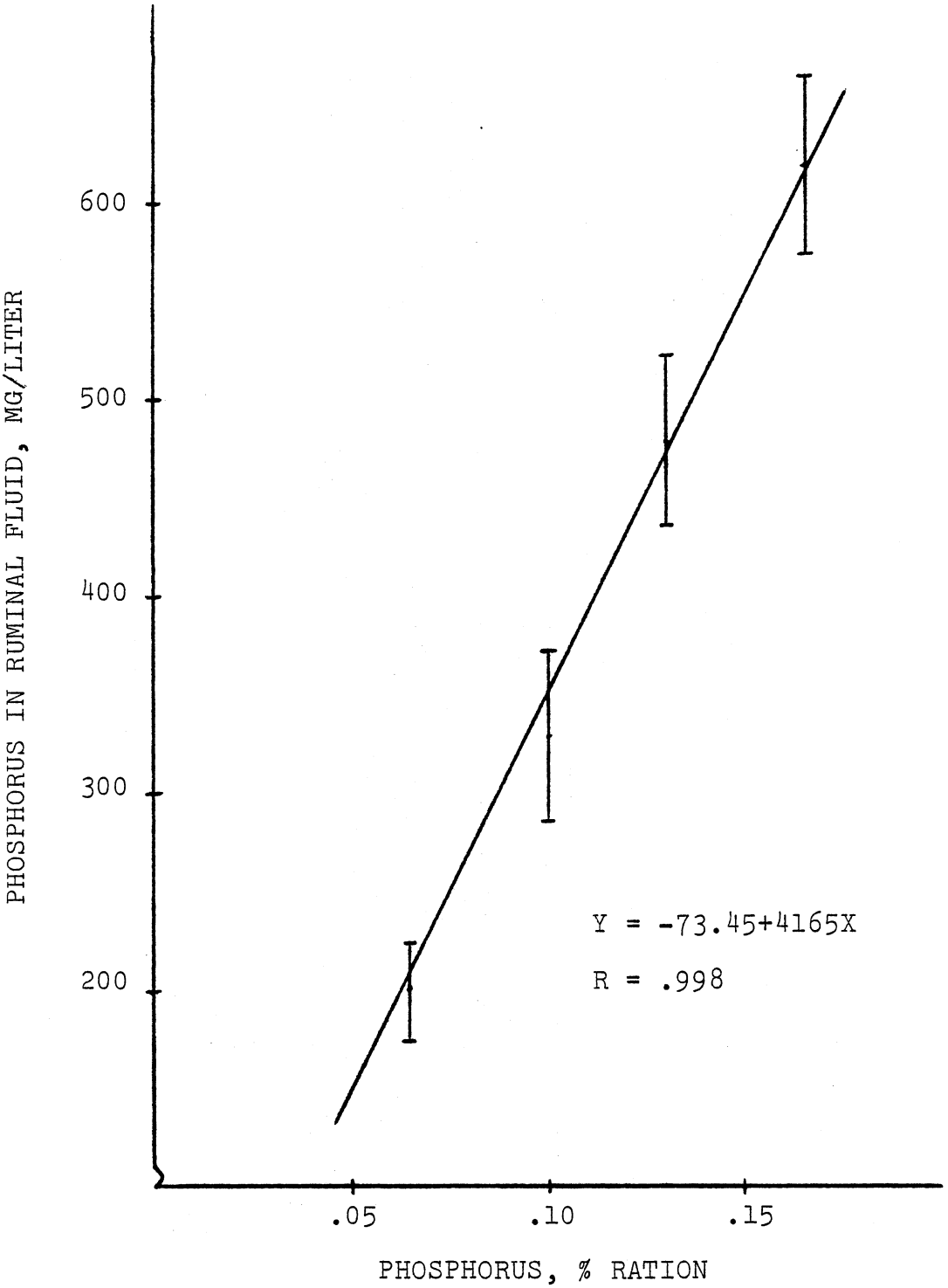

Figure 1. Effect of phosphorus intake on ruminal fluid phosphorus concentrations in lambs.

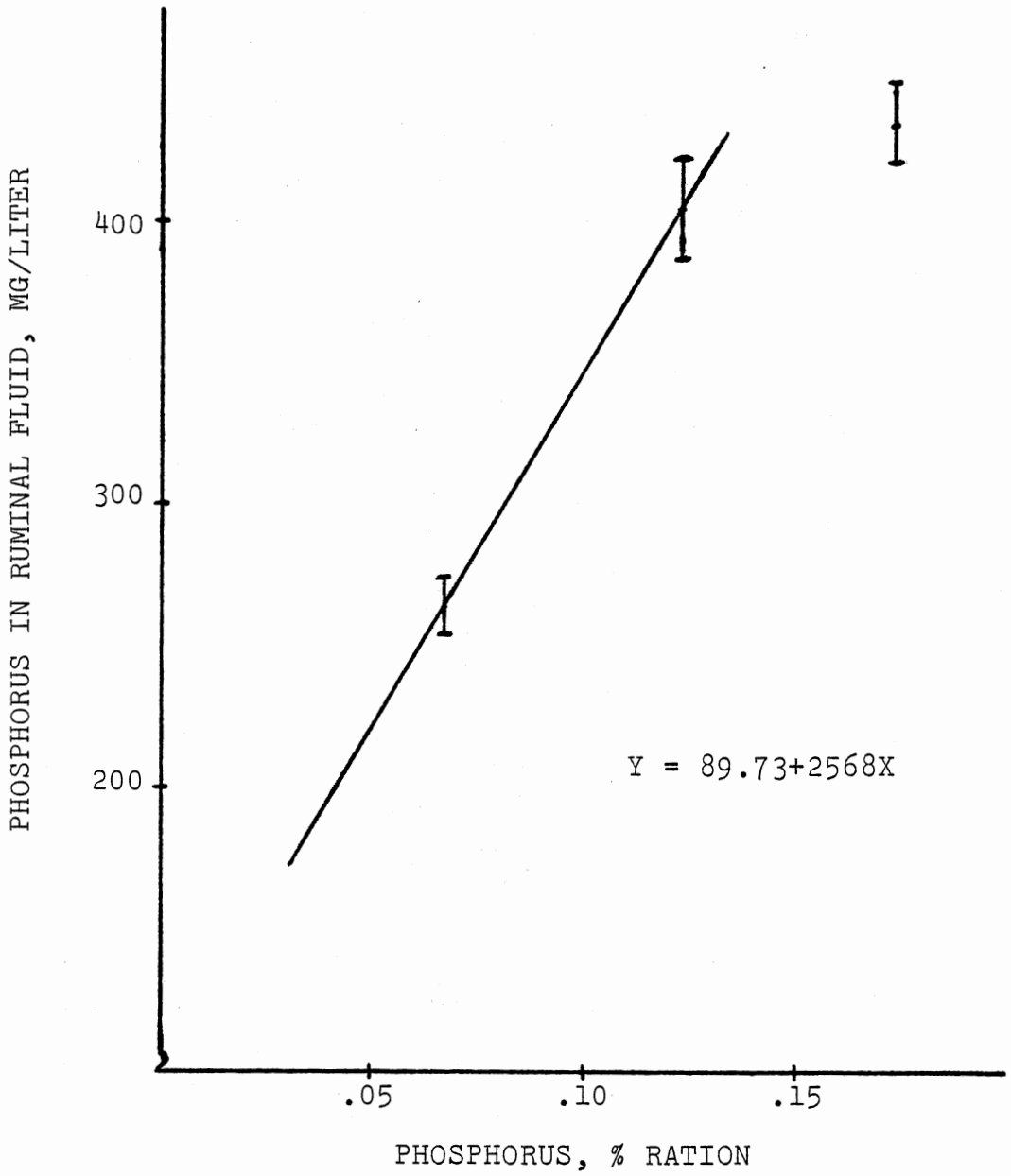

Figure 2. Effect of phosphorus intake on ruminal fluid phosphorus concentrations in steers.

concentrations had not increased greatly but appeared to plateau. The ruminal phosphorus concentrations in this study closely matched results of Evans and Davis (1966) who found ruminal phosphorus concentrations of 198, 417 and 543 mg phosphorus per liter of ruminal fluid with phosphorus intakes of .04, .16 and .54% of the ration for cattle.

Comparison with the standard curve developed for lambs reveals a higher basal level and an earlier plateau of ruminal phosphorus for the steers. This could suggest more active recycling of phosphorus to the rumen for cattle than sheep.

Trial 3. Steer Ruminal Availability of Phosphorus Sources. Ruminal phosphorus concentrations were determined with sodium phosphate, mono-dical, dical, and defluorinated rock phosphate. Each phosphorus source was added to a basal ration (.067% phosphorus) to increase the phosphorus level to approximately .17%. Ruminal fluid phosphorus concentrations of the three commercial phosphorus sources were compared with the sodium phosphate standard curve for steers (figure 2). Estimates for ruminal phosphorus availability relative to sodium phosphate given an arbitrary value of 100% based on its high solubility were 59.3% for mono-dical, 42.2% for dical and 28.4% for defluorinated rock phosphate. Ration and ruminal fluid phosphorus concentrations for the three phosphorus sources are presented in table 4. Use of curvilinear regression only reduced the calculated ruminal availability values slightly. Ruminal pH was not altered by phosphorus source, with all steers being similar at pH 6.8$\pm$.02.

TABLE 4. CALCULATION OF RUMINAL AVAILABILITY OF PHOSPHORUS SOURCES

Phosphorus source	Phosphorus		Ruminal phosphorus	Ruminal availability[a]	Ruminal availability[b]
	Basal ration	Added			
	%	%	mg/l	%	%
Sodium phosphate	.067	.0560	406 ± 26	100	100
Sodium phosphate	.067	.1060	434 ± 14	100	100
Mono-dical	.067	.0885	397 ± 16	59.3	57.1
Dical	.067	.1038	375 ± 25	42.2	37.6
Defluorinated rock phosphate	.067	.1000	336 ± 14	28.4	23.0
None	.067	.0000	264 ± 15	....	....

[a]Calculated from linear standard curve as follows:

$$\text{Availability} = 100 \times \frac{\text{Available P}}{\text{Added P \%}} = 100 \times \frac{(\text{Ruminal P} - 264)/142 \times .056}{\text{Added P \%}}$$

[b]Calculated from curvilinear standard curve.

Biological availability estimates for these phosphorus sources using the criterion of the percentage bone ash of chicks has found a similar order of availability (IMC, 1978) for these materials. It should be noted that this study evaluated only the ruminal availability of these phosphorus sources. Gastric or intestinal digestion may increase the total phosphorus availability of these sources; although, the capacity to recycle intestinally absorbed phosphorus to the rumen, especially with high concentrate diets, may be limited.

CHAPTER IV

SOLUBILITY OF PHOSPHORUS SOURCES IN VITRO

Summary

Solubilities of sodium phosphate, a mono-dicalcium phosphate mixture containing 21% phosphorus (mono-dical), a mono-dicalcium phosphate mixture containing 18.5% phosphorus (dical), and defluorinated rock phosphate were determined in a ruminal buffer adjusted to pH levels of 5, 6 and 7, and in abomasal fluid at pH 2.5. Phosphorus sources exhibited slightly greater solubility in ruminal buffer at the lower pH. Solubility in ruminal buffer differed ($P<.01$) among the four phosphorus sources. Compared to sodium phosphate, phosphorus from mono-dical, dical and defluorinated rock phosphate was 46.4%, 28.8% and 2.5% as soluble, respectively. Phosphorus sources differed ($P<.05$) in solubility in abomasal fluid. Maximum solubility for all phosphorus sources in the acidic abomasal fluid was obtained after 1 hr. Relative solubility of the phosphorus sources at 1 hr, compared to sodium phosphate at 100%, were 71.6%, 41.3% and 29.7% for mono-dical, dical and defluorinated rock phosphate, respectively. Thus solubility of all phosphorus sources relative to sodium phosphate was greater

in abomasal fluid than in ruminal buffer. This suggests that despite low solubility of some sources of phosphorus in the rumen, phosphorus can be solubilized and become available postruminally.

(Key Words: In Vitro Phosphorus Solubility, Ruminal Buffer, Abomasal Fluid.)

Introduction

The availability of phosphorus from different commercial sources is an important consideration when formulating rations to meet the phosphorus requirement of ruminants. Phosphorus is necessary for maintenance of normal body functions, bone structure and ruminal microflora. Adequate phosphorus levels in the diet are critical particularly for young growing animals and lactating females. Relative ruminal availabilities of 59.3% for a mono-dicalcium phosphate mixture containing 21% phosphorus (mono-dical), 42.2% for a mono-dicalcium phosphate mixture containing 18.5% phosphorus (dical), and 28.4% for defluorinated rock phosphate (Chapter III, Witt and Owens) indicate that drastic differences exist in the usable phosphorus supplied in rations. With ruminants consuming a wide range of diets, from the high concentrates fed to rapidly growing feedlot steers to the low energy, high forage intakes of wintering range cows, the relative availability of phosphorus from various sources could differ even further. Since ruminal pH values can be as low as 5 with a high concentrate ration to about 7 with a high roughage

ration, the type of diet consumed could alter the ruminal pH, and thus alter the availability of the phosphorus source. Therefore, an in vitro study was designed to determine the relative solubility of mono-dical, dical and defluorinated rock phosphate compared to the standard orthophosphate, sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) in a ruminal buffer at pH levels covering the physiological range normally found in the rumen. Length of time that the phosphorus source was incubated was also examined to determine rate of solubility. A second in vitro trial examined the solubility of the four phosphorus sources in abomasal fluid to observe whether the lower pH in the abomasum would enhance the solubility, and, presumably, availability of phosphorus for use by the animal. Overall these trials were conducted to determine if in vitro solubilities could be used as indicators of the relative in vivo availability of phosphorus for ruminants.

Experimental Procedure

Trial 1. In Vitro Solubility of Phosphorus Sources in Ruminal Buffer. Each of four phosphorus sources (sodium phosphate, a standard orthophosphate source; a mono-dicalcium phosphate mixture containing 21% phosphorus, mono-dical; a mono-dicalcium phosphate mixture containing 18.5% phosphorus, dical; and defluorinated rock phosphate) were weighed into 125 ml Erlenmeyer flasks to provide 25 mg of phosphorus. Fifty ml of ruminal buffer (Johnson, 1969) providing 25.6 mg of phosphorus was added to each flask. Duplicate flasks for

each phosphorus source were adjusted to a pH of 5, 6 or 7 using .1 N HCl. The ruminal buffer solution alone was similarly adjusted in pH to serve as a blank. A sample of solution was removed from the flask immediately after pH adjustment and 30 min, 1, 2 and 3 hr later. Flasks were incubated in a water bath at 39 C. Samples were analyzed for inorganic phosphorus (Fiske and Subbarow, 1925), with the phosphorus level of the blanks being subtracted from sample readings to estimate solubility. Data were statistically analyzed as a 3 x 4 x 5 factorial arrangement of treatments with three pH levels, four phosphorus sources and five time periods of incubation (Snedecor and Cochran, 1967). Solubilities relative to the standard, sodium phosphate, were calculated.

Trial 2. In Vitro Solubility of Phosphorus Sources in Abomasal Fluid. Abomasal fluid was collected from a high roughage fed Hereford steer (430 kg) fitted with a permanent abomasal cannula. Immediately after collection, the abomasal fluid was strained through eight layers of cheesecloth and determined to have a pH of 2.5. Inorganic phosphorus analysis (Fiske and Subbarow, 1925) of the abomasal fluid found it to contain .5664 mg of phosphorus per ml of fluid. Thirty ml of abomasal fluid was placed in 50 ml test tubes to which 17 mg of phosphorus from each of the four phosphorus sources described above was added. This provided an equal amount of phosphorus from the supplement as was found in the medium. Duplicate tubes were incubated in a 39 C water bath and

agitated before each sample collection. Samples were taken at 0, 15, 30, 60 and 120 min and analyzed for inorganic phosphorus with phosphorus concentration in the abomasal fluid at each incubation time being subtracted from sample readings to estimate solubility. Data were statistically analyzed as a completely randomized block design with time periods serving as blocks (Snedecor and Cochran, 1967). Solubilities relative to the standard, sodium phosphate, were calculated.

Results and Discussion

Trial 1. In Vitro Solubility of Phosphorus Sources in Ruminal Buffer. Solubilities in ruminal buffer differed ($P<.01$) among the four phosphorus sources examined (table 5). The standard orthophosphate, sodium phosphate, was virtually all soluble in the buffer solution. Mono-dical at 46.4% solubility was more soluble than dical at 28.8%, while defluorinated rock phosphate was practically insoluble with only 2.5% of the added phosphorus being in solution.

A phosphorus source by pH interaction was detected ($P<.01$). Sodium phosphate was less soluble at pH 6 than at pH 7 (figure 3). This may have been influenced by the peak buffering capacity for phosphates occurring at a pH of 7.2 (Lehninger, 1975). Solubility of both mono-dical and dical was greater at lower pH values. The defluorinated rock phosphate had a very low solubility at all pH levels tested.

Time had no effect on solubility in ruminal buffer

TABLE 5. SOLUBILITY OF PHOSPHORUS SOURCES IN RUMINAL BUFFER

Phosphorus source	Ruminal buffer solubility
	- - - - - - % - - - - - -
Sodium phosphate	100[a]
Mono-dical	46.4[b]
Dical	28.8[c]
Defluorinated rock phosphate	2.5[d]
SEM[e]	1.3

[abcd]Means in a column with different superscripts differ (P<.05) by Duncan's multiple range.

[e]Standard error of treatment means.

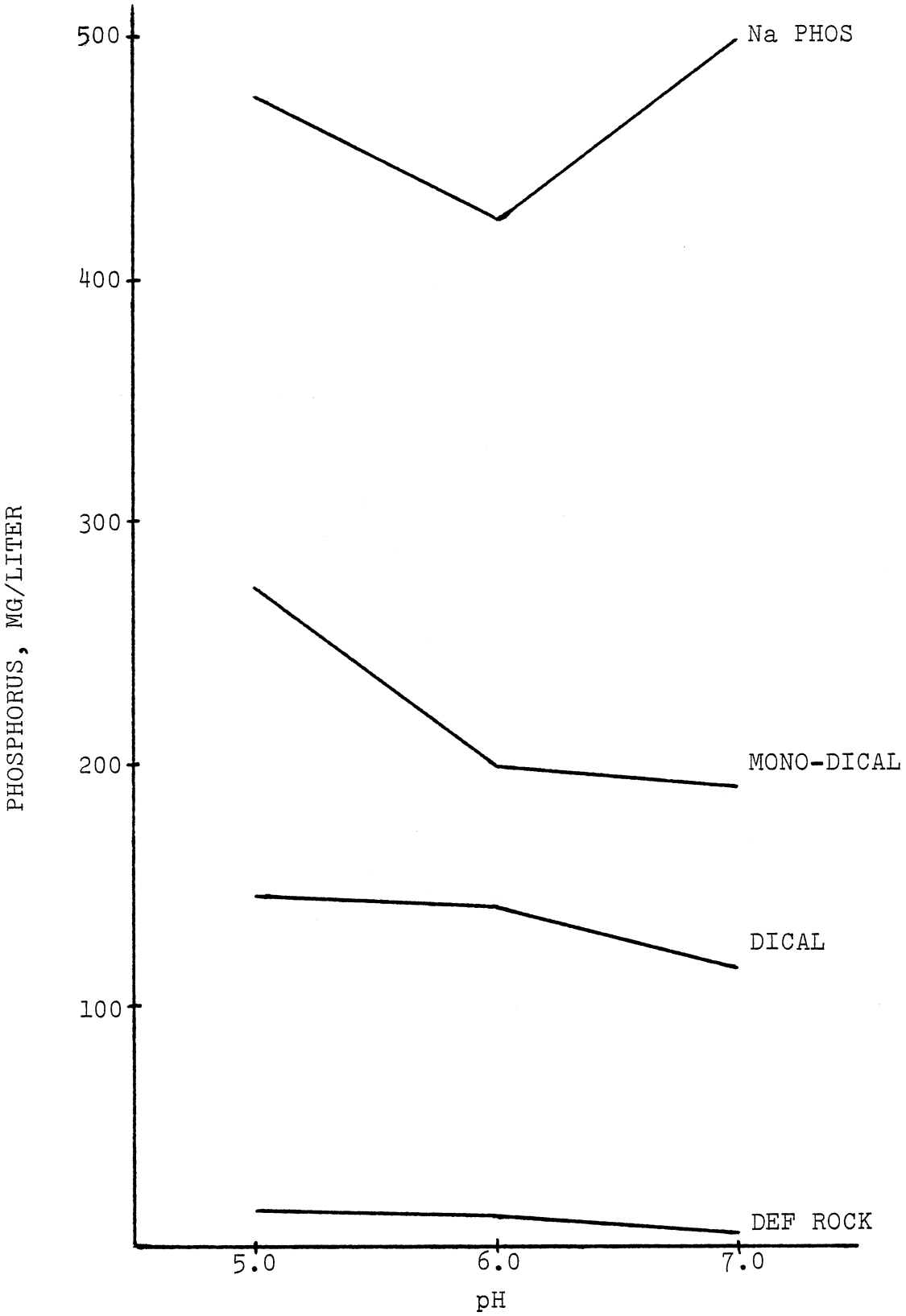

Figure 3. Effect of pH on solubility of phosphorus sources in ruminal buffer.

(figure 4). However, there was a phosphorus source by time interaction ($P<.05$). Mono-dical reached maximum solubility only after two hr, while all other phosphorus sources appeared to be solubilized immediately.

Results from this study indicate that commercial phosphorus sources may be more soluble, and thus available, at lower ruminal pH values than at higher pH values. High concentrate rations, therefore, may require lower levels of supplemental phosphorus than high roughage rations to provide similar amounts of soluble phosphorus. The limited solubility and ruminal availability of defluorinated rock phosphate should be considered when using it as a source of phosphorus for ruminant animals.

The order of solubility observed in this in vitro study for the four phosphorus sources was identical to the order of ruminal availability determined by Witt and Owens (Chapter III; figure 5). This suggests that solubility of phosphorus sources in ruminal buffer could be employed to indicate ruminal availability of the phosphorus for ruminants.

Trial 2. In Vitro Solubility of Phosphorus Sources in Abomasal Fluid. Solubility of phosphorus sources in abomasal fluid differed ($P<.05$) across all time periods examined (table 6). Figure 6 indicates the solubility of the four phosphorus sources at 0, 15, 30, 60 and 120 min. Sodium phosphate, the standard orthophosphate, exhibited the highest solubility across all time periods, and solubility tended to increase with incubation up to 1 hr. Mono-dical and dical

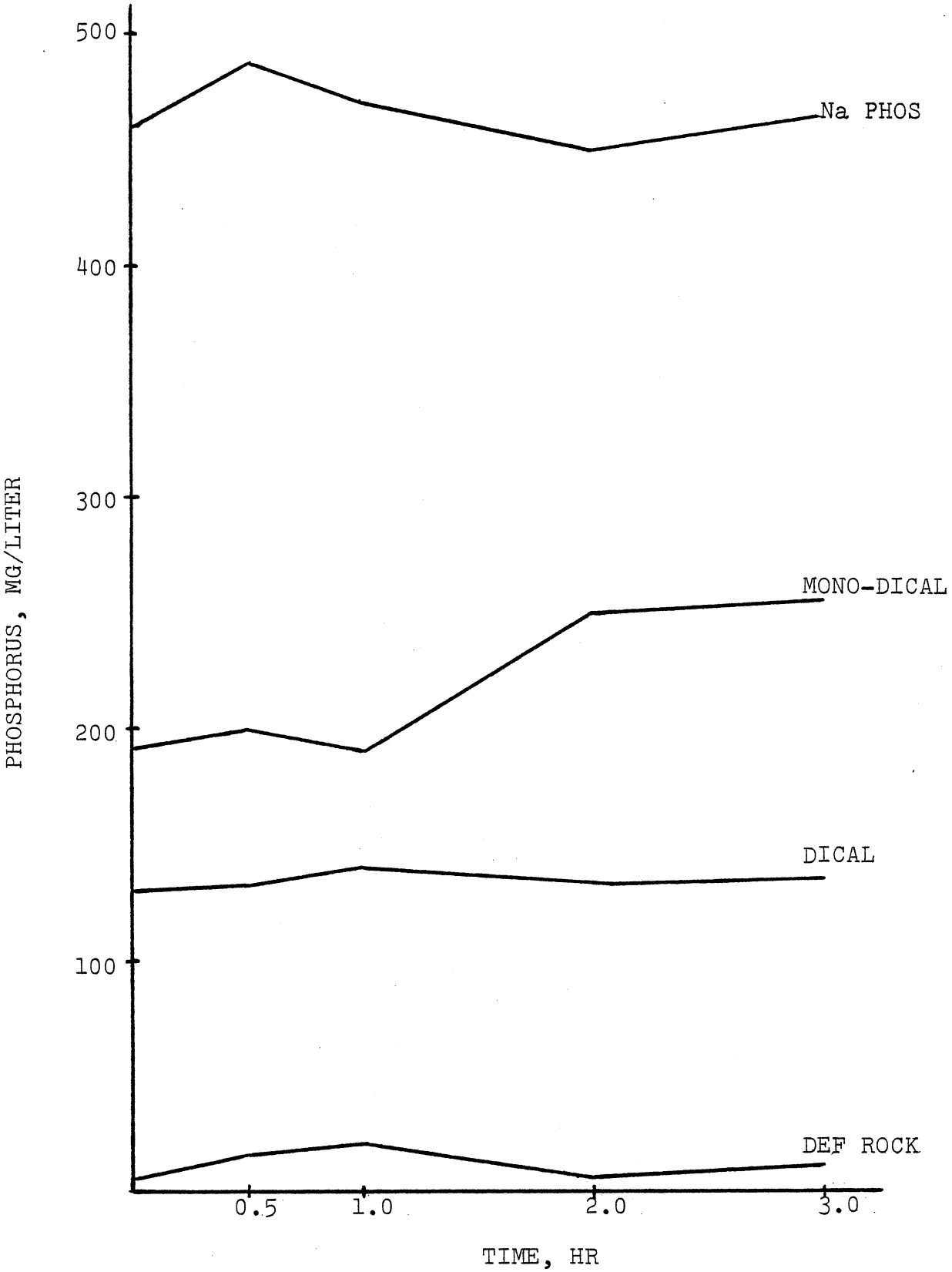

Figure 4. Effect of time on solubility of phosphorus sources in ruminal buffer.

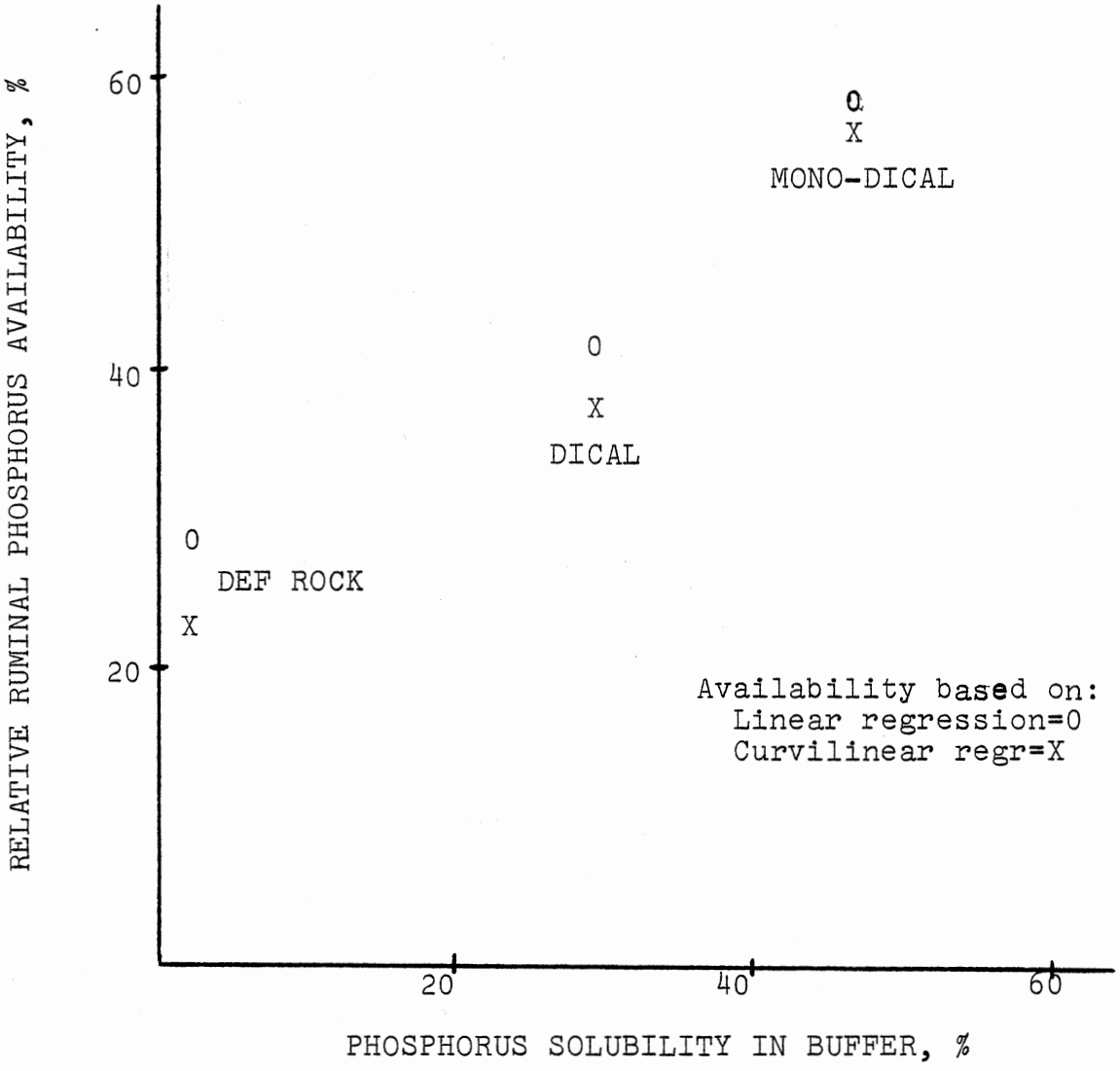

Figure 5. Ruminal phosphorus availability vs buffer solubility.

TABLE 6. EFFECT OF TIME ON SOLUBILITY OF PHOSPHORUS SOURCES IN ABOMASAL FLUID

Phosphorus source	Time, min				
	0	15	30	60	120
	- - - - - - - - - - - - solubility, % - - - - - - - - - - - -				
Sodium phosphate	33.6[a]	67.7[a]	89.4[a]	109.0[a]	101.3[a]
Mono-dical	7.0[c]	7.4[b]	53.1[b]	78.0[b]	77.7[b]
Dical	7.7[c]	21.6[b]	35.0[bc]	45.0[c]	44.5[c]
Defluorinated rock phosphate	19.5[b]	21.7[b]	25.3[c]	32.4[d]	36.0[c]
SEM[e]	2.3	10.2	5.7	2.9	2.7

[abcd]Means in a column with different superscripts differ ($P<.05$) by Duncan's multiple range.

[e]Standard error of treatment means.

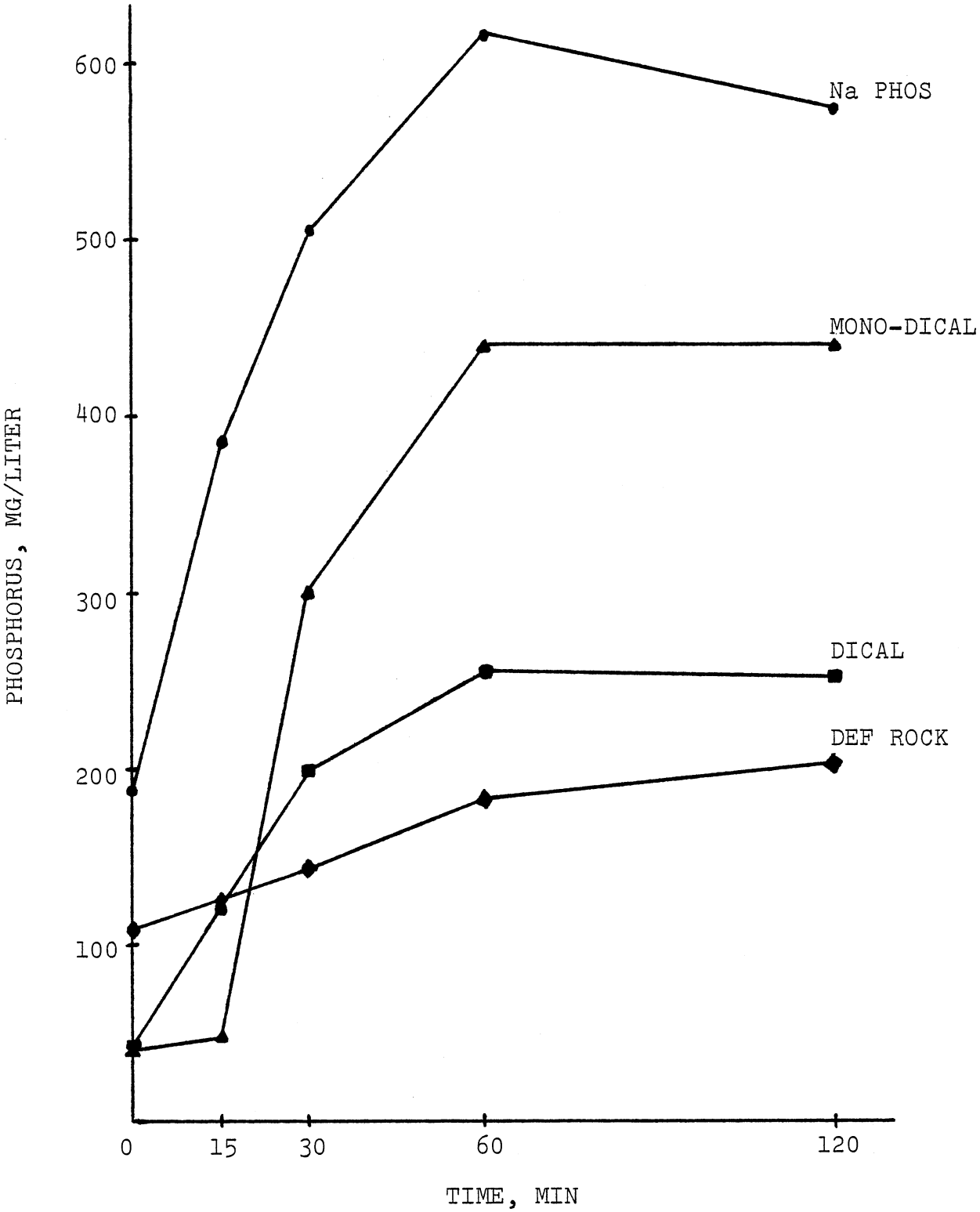

Figure 6. Effect of time on solubility of phosphorus sources in abomasal fluid.

were intermediate in solubility, but reached a maximum only after 1 hr. Defluorinated rock phosphate had the lowest solubility overall, though the small amount of phosphorus present was rapidly solubilized. Abomasal fluid solubility data suggests that mono-dical and dical require over 15 min to solubilize to the extent of defluorinated rock phosphate in this acidic (pH=2.5) medium. Whether all phosphorus would be solubilized during abomasal and duodenal passage is questionable.

All four phosphorus sources were observed to achieve near maximum solubility after 1 hr. In comparison, solubility data in ruminal buffer (figure 4) for these same sources indicated that sodium phosphate, dical and defluorinated rock phosphate went into solution immediately, and mono-dical reached maximum solubility after 2 hr. The relative solubility of mono-dical, dical and defluorinated rock phosphate compared to the standard, sodium phosphate, after 1 and 2 hr of incubation (table 7) was greater in abomasal fluid across all phosphorus sources. Solubilities of mono-dical, dical and defluorinated rock phosphate were 37%, 30% and 90% greater in abomasal fluid than in ruminal buffer over the longer time periods. Relative solubility of the phosphorus sources in abomasal fluid more closely approached the biological value reported for these same phosphorus sources for poultry (IMC, 1978), than ruminal availability or buffer solubility values (table 8). Therefore, abomasal fluid solubility may be more indicative of the value of a

TABLE 7. SOLUBILITIES OF PHOSPHORUS SOURCES IN RUMINAL BUFFER AND ABOMASAL FLUID

Phosphorus source	Ruminal buffer solubility		Abomasal fluid solubility	
	1 hr	2 hr	1 hr	2 hr
	- - - - - - - - - - - - - - - - % - - - - - - - - - - - - - - - -			
Sodium phosphate	100[a]	100[a]	100[a]	100[a]
Mono-dical	38.0[b]	55.9[b]	71.6[b]	76.6[b]
Dical	29.7[b]	29.7[c]	41.3[c]	43.9[c]
Defluorinated rock phosphate	4.6[c]	1.3[d]	29.7[d]	35.5[c]
SEM[e]	2.9	3.1	2.7	2.7

[abcd]Means in a column with different superscripts differ ($P<.05$) by Duncan's multiple range.

[e]Standard error of treatment means.

TABLE 8. RUMINAL AVAILABILITY, IN VITRO SOLUBILITY AND BIOLOGICAL VALUE OF PHOSPHORUS SOURCES

Phosphorus source	Ruminal availability	Ruminal buffer solubility[a]	Abomasal fluid solubility[a]	Biological value[b]
		%		
Sodium phosphate	100	100[c]	100[c]	115-125
Mono-dical	59.3	55.9[d]	76.6[d]	105-115
Dical	42.2	29.7[e]	43.9[e]	105-115
Defluorinated rock phosphate	28.4	1.3[f]	35.5[e]	95-100
SEM[g]		3.1	2.7	

[a]Relative solubility at 2 hr.

[b]Chick assay, % bone ash (IMC, 1978).

[cdef]Means in a column with different superscripts differ (P<.05) by Duncan's multiple range.

[g]Standard error of treatment means.

phosphorus source for ruminants than phosphorus solubility at ruminal pH levels, since phosphorus solubilized in the abomasum should permit absorption of phosphorus for use by the animal and for recycling to the rumen. Phosphorus recycling to the rumen may be necessary for microbial growth and digestion of feed when phosphorus sources of low ruminal solubility are fed. Limitations in recycling, however, remain to be proven.

CHAPTER V

EFFECT OF PHOSPHORUS ON RUMINAL AND TOTAL TRACT DIGESTION

Summary

Low (.12%) and high (.23%) phosphorus rations were fed to 700 kg ruminally cannulated steers in a crossover design. Ruminal phosphorus concentrations with the high phosphorus ration were greater (P<.01) than with the low phosphorus ration (398 vs 208 mg/liter). Dietary phosphorus level did not effect ruminal digestion as indicated by in situ disappearance/24 hr of ground corn, cotton duck and cottonseed hulls being 42% vs 41%, 22% vs 23%, and 8.3% vs 8.2% for the low vs high phosphorus intakes, respectively. Total tract digestibilities on the low and high phosphorus rations were similar for both dry matter and organic matter (65% vs 67%), acid detergent fiber (55% vs 58%), neutral detergent fiber (62% vs 65%) and neutral detergent solubles (69% vs 70.4%), respectively. Phosphorus retention was higher (P<.01) with the high phosphorus ration (8.3 g/day) than with the low phosphorus ration (1.0 g/day). Apparently, the minimum observed ruminal phosphorus concentration of 208 mg/liter was sufficient for normal microbial cellulose digestion, but inadequate for maintaining the

animal's phosphorus stores. Results suggested ruminal phosphorus availability to be less important than total digestive tract availability for determining the long term effectiveness of dietary phosphorus supplementation for ruminants.

(Key Words: Phosphorus, Digestibiligy, Ruminants.)

Introduction

One of the primary goals in ruminant nutrition is to maximize the efficiency of feedstuff digestion by ruminants. Adequate phosphorus is necessary for bacterial growth in the rumen and thereby for cellulose digestion (Burroughs et al., 1950; Anderson et al., 1956; Hall et al., 1961; Chicco et al., 1965). Studies by these workers have all been conducted using in vitro artificial rumen techniques. These results indicate that ruminants grazing poor quality pastures and range with low phosphorus levels could exhibit decreased cellulose digestion and less efficient utilization of forage. In vitro work by Chicco et al. (1965) indicated that 60 mg or more of available phosphorus per liter of medium was required to produce maximum cellulose disappearance. This can be compared with minimal ruminal concentrations of 198 mg/liter (Evans and Davis, 1966). The lack of sufficient dietary phosphorus could inhibit the growth and reproduction of the cellulolytic bacteria since ruminal microorganisms require phosphorus. Decreased microbial digestion might be related to one of the first clinical signs of a phosphorus deficiency, anorexia.

This trial was designed to determine if the phenomenon of decreased digestion rate or extent could be demonstrated *in vivo* with low dietary phosphorus levels. Mature steers were fed a phosphorus deficient basal ration, or this ration with phosphorus added to meet the animal's phosphorus requirement, to determine if low dietary phosphorus levels reduced digestion in the rumen and the total digestive tract.

Experimental Procedure

Four Hereford-Brown Swiss and two Hereford-Angus steers (700 kg) fitted with permanent ruminal cannulas were housed in stanchion-type metabolism stalls. Steers were individually fed twice daily at a maintenance level of intake. The trial was conducted as a crossover design (Cochran and Cox, 1957) with either the deficient phosphorus basal ration containing .118% phosphorus, or the basal ration supplemented with the standard orthophosphate, Na_2HPO_4 (sodium phosphate), to achieve a phosphorus level of .227% (table 9) being fed. The high phosphorus ration contained more than enough phosphorus to meet the requirement (NRC, 1976) of the steers. The low phosphorus basal ration was fed for 2 wk prior to the initiation of the trial to deplete the readily available phosphorus stores. All rations were completely consumed by the steers.

After a 7 day ration adaptation period, total feces were collected for 5 days. Fecal pH values were obtained at the

TABLE 9. RATION COMPOSITION

Ingredients	Low P	High P
	- - - - - - - % - - - - - - -	
Cotton hulls (IFN 1-01-599)	77.9	77.9
Sugarcane, molasses dehy (IFN 4-04-695)	20.5	20.1
Urea	1.1	1.1
Salt, trace mineralized	.5	.5
Sodium phosphate	....	.44
Vitamins A & D	+	+

time of collection. A subsample (2.5%) of wet feces was obtained, dried at 55 C for 48 hr and ground through a 1 mm screen in a Wiley mill for later analysis. Since almost all of the dietary phosphorus is excreted in feces (Lofgreen, 1960), urine was not collected. On the fifth day of the collection period, ruminal fluid samples were obtained 4 hr postprandially, and ruminal pH was determined. Immediately after sampling, ruminal fluid was strained through eight layers of cheesecloth and centrifuged at 17000 g for 10 min. The supernatent fluid was frozen and later analyzed for inorganic phosphorus (Fiske and Subbarow, 1925). At the time of rumen sampling on day 5 of the collection period, nylon bags containing either 3.7 g of screened cottonseed hulls or 9.0 g of corn grain (1 mm mean particle size), and 4 mm x 14 mm strips of unbleached, unsized cotton duck weighing 1.4 g (Dinius et al., 1976) were suspended in the rumen for 24 hr. Upon completion of this ruminal incubation period, bags and strips were thoroughly washed and dried for 24 hr in a 100 C oven. Bags and strips were then reweighed to estimate dry matter and cellulose disappearance rates in the rumen. Cottonseed hull and supplement samples were obtained on the fifth collection day of both periods, composited and ground for analysis. Ration and fecal samples were analyzed for dry matter, ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), and neutral detergent solubles (NDS; Goering and Van Soest, 1970), to estimate dry matter, organic matter, ADF, NDF and NDS

digestibilities, respectively. Inorganic phosphorus concentrations (Fiske and Subbarow, 1925) were determined on ration and fecal samples for use in calculating phosphorus retention by the steers.

Results and Discussion

Steers fed the low phosphorus basal ration has a phosphorus intake of 9.5 g/day which was lower ($P<.01$) than the 18.3 g daily phosphorus intake of steers consuming the high phosphorus ration. Ruminal phosphorus concentrations were higher ($P<.01$) for steers fed the high phosphorus ration than those fed the low phosphorus diet (398 vs 208 mg/liter; table 10). This range of phosphorus intakes produced the extremes of ruminal phosphorus concentrations observed in the steer standard curve for sodium phosphate (Witt and Owens, Chapter III) verifying that the two rations did simulate differences in the amount of ruminal phosphorus available for microbial use. A similar ruminal pH value of $6.5\pm.03$ was measured with both treatments. In situ ruminal disappearance of ground corn, cotton duck, and cottonseed hulls was not affected by the level of dietary phosphorus (table 10), indicating that ruminal digestion for cattle fed the two phosphorus levels was similar. This suggests that ruminal phosphorus concentrations above 210 mg/liter provided adequate amounts of phosphorus to meet the microbial needs for maximum cellulose digestion in the rumen and that an excess of phosphorus did not depress digestion. In vitro work by

TABLE 10. EFFECT OF PHOSPHORUS INTAKE ON RUMINAL AND TOTAL TRACT DIGESTION

Item	Phosphorus in ration .119%	.227%	SEM[c]
Dry matter intake, g/day	8025	8075	1.29
Phosphorus intake, g/day	9.5[a]	18.3[b]	.23
Ruminal phosphorus, mg/liter	208.1[a]	398.1[b]	20.88
Ruminal disappearance per 24 hr			
Ground corn grain, %	42.2	41.0	1.71
Cotton duck, %	22.5	23.2	1.10
Cottonseed hulls, %	8.3	8.2	.58
Dry matter digestibility, %	64.8	67.0	1.05
Organic matter digestibility, %	64.8	66.9	1.09
Acid detergent fiber digestibility, %	55.2	57.5	1.58
Neutral detergent fiber digestibility, %	62.3	65.3	1.48
Neutral detergent solubles digestibility, %	69.6	70.4	.44
Fecal output, g/day	2857.0	2693.7	89.92
Fecal phosphorus, %	.307	.369	.02
Fecal phosphorus, g/day	8.5	10.0	.65
Phosphorus retention, g/day	1.0[a]	8.3[b]	.48

[ab]Means in a row with different superscripts differ (P<.01).

[c]Standard error of treatment means.

Hubbert et al. (1958) found that cellulose digestion was not decreased by an excess of 1000 mg of phosphorus per liter of medium. A deficiency of ruminal phosphorus appears to be more detrimental than an excess of phosphorus in the rumen. In vitro studies have found cellulose digestion decreased at extremely low levels of phosphorus in the medium. A range of 20-80 mg of phosphorus per liter of medium has been suggested to be required for adequate cellulose digestion in the rumen (Anderson et al., 1956; Hall et al., 1961; Chicco et al., 1965). Feeding low (.04 to .12%) phosphorus rations to steers has not been observed to produce ruminal phosphorus concentrations below 198 mg of phosphorus per liter of ruminal fluid (Evans and Davis, 1966; Witt and Owens, Chapter III). Apparently in an effort to control phosphorus homeostasis, ruminants recycle endogenous phosphorus via saliva to the rumen and secretion through the ruminal wall to maintain ruminal phosphorus near 200 mg/liter when phosphorus intakes are deficient. Whether recycling is adequate with all types of diets, however, is unknown.

Total gastrointestinal tract digestibilities for the low and high phosphorus rations were similar, indicating that phosphorus intake did not alter the utilization of feed by the animal. Dry matter digestibility was slightly higher for the high compared to the low phosphorus ration (67 vs 65%; table 10). Organic matter followed similar trends at 65% and 67% digestibility for the low and high phosphorus intakes, respectively. Fiber digestibility (ADF and NDF) also was not

significantly influenced by phosphorus content of the ration, being 57.5% and 65.3%, respectively, on the high phosphorus diet, and 55.2% and 62.3%, respectively, for the low phosphorus diet (table 10). Slight increases in digestibility for the high phosphorus ration were observed, however, with both of these fractions. Neutral detergent solubles or cell contents digestibility (70.4 vs 69.6%) also was not altered by phosphorus intake. Therefore, a low concentration (.12%) of phosphorus in the ration appears adequate for digestion in the rumen or the total digestion of feeds for the animals tested.

Based on the fact that phosphorus absorption is a good indicator of net phosphorus retention by the animal (Lofgreen, 1960); steers consuming 9.5 g of phosphorus per day, excreted 8.5 g of phosphorus daily which resulted in an estimated phosphorus retention of 1.0 g/day. Two of the six steers exhibited a slightly negative phosphorus retention with the low phosphorus ration. This suggests that while the low phosphorus intakes and ruminal phosphorus concentration was sufficient to maintain digestion, it was not adequate to replenish the animal's body phosphorus stores. With a young growing animal this could be critical for proper bone formation. On the 18.3 g phosphorus intake with a 10.0 g daily phosphorus excretion, a phosphorus retention of 8.3 g/day was observed. The calculated phosphorus retention by steers fed the two phosphorus treatments was higher ($P<.01$) with the greater phosphorus intake (table 10). No significant

difference was observed in the amount of phosphorus excreted in the feces, or in fecal pH values which were similar for both treatments at 6.8±.04. Therefore, dietary phosphorus level was a major factor determining the amount of phosphorus retained by the animal. Overall, this data suggests that availability of phosphorus in the rumen may be less important than availability of phosphorus in the total digestive tract for determining the effectiveness of dietary phosphorus for ruminants. This implies that with a phosphorus deficiency as with many other nutrients, tissue stores are depleted before the digestibility of nutrients is reduced which would aid in survival through intermittent periods of feast and famine.

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