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Influence of phosphorus supplements upon cellulose digestion by ruman microorganisms and ration digestibility by sheep

Clarence D. Gaddy

To the Graduate Council:

I am submitting herewith a thesis written by Clarence D. Gaddy entitled "Influence of phosphorus supplements upon cellulose digestion by ruman microorganisms and ration digestibility by sheep." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

O. Glen Hall, Major Professor

We have read this thesis and recommend its acceptance:

C. S. Hobbs, R. L. Murphree

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 16, 1960

To the Graduate Council:

I am submitting herewith a thesis written by Clarence D. Gaddy, Jr. entitled "Influence of Phosphorus Supplements upon Cellulose Digestion by Rumen Microorganisms and Ration Digestibility by Sheep." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

O. Glen Hall
Major Professor

We have read this thesis
and recommend its acceptance:

Charles A. Hobbs

R. L. Murphree

Accepted for the Council:

Dale Wantling
Dean of the Graduate School

INFLUENCE OF PHOSPHORUS SUPPLEMENTS UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS AND RATION DIGESTIBILITY BY SHEEP

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Clarence D. Gaddy, Jr.

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Clarence D. Gaddy, Jr.

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CHAPTER I

INTRODUCTION

Efficient feed utilization is a major factor to consider for profitable livestock production. The presence, amounts and ratios of nutrients all contribute to efficient feed utilization.

It has been demonstrated that phosphorus is one of the elements which is essential in the animal body. Many different types of phosphatic feed supplements exist today and the question as to which can best be utilized by livestock naturally arises.

Numerous experiments have been conducted on the availability of various sources of phosphorus with cattle, sheep, swine, poultry and laboratory animals. Many of these experiments show great differences in the utilization of these phosphates. The majority of these studies have been conducted using feeding and metabolism trials. Feed intake, rate of gain, lactation, reproduction, blood serum phosphate level and bone formations were the primary criteria used to evaluate different phosphorus sources in these experiments.

The disadvantage of the large number of animals and the duration of time required to complete an experiment have necessitated the need for a method of evaluating nutrients which would be less expensive and have a shorter period of experimental time. In the last few years, a method called the artificial rumen technique has been developed which uses microbes in the rumen of cud-chewing animals to evaluate nutrients.

Microorganisms have long been known to be of primary importance for the utilization of roughage by ruminants. Maximum rumen microbial

activity which is essential for efficient feed utilization in cattle and sheep, depend upon the presence and availability of various nutrients in the ration fed. Experiments have shown that a source of nitrogen, readily available energy, minerals, fatty acids and other factors are required by the bacteria. Since phosphorus has been found to be of vital importance for rumen bacteria and since there are a large number of phosphorus supplements available for feed use, this study was undertaken for the following purposes:

1. To determine the effects of phosphorus from dicalcium phosphate (furnace process), dicalcium phosphate (wet acid process), Curaco rock phosphate and a defluorinated phosphate upon cellulose digestion by rumen microorganisms in vitro.
2. To compare the relative availability of these four sources of phosphorus.
3. To compare the relative availability of the four sources of phosphorus to rumen microorganisms collected from a steer on pasture and to rumen microorganism from a steer on dry feed.
4. To determine the effect of an adaption period on the availability of a phosphorus supplement to rumen microbes.
5. To compare the relative availability of three defluorinated phosphates obtained from three different sources.
6. To determine the effect of phosphorus from a dicalcium phosphate supplement and a defluorinated phosphate supplement upon cellulose digestion by sheep.

CHAPTER II

REVIEW OF LITERATURE

The importance of phosphorus to livestock was demonstrated by two reports published in the 1920's by Hart et al. (1927) and Meigs et al. (1921).

Ten years prior to 1927 a mysterious disease was reported among some of the dairy herds of Door County, Wisconsin. The symptoms of this disease were emaciation, stiffness in the front and rear quarters, swollen joints, hardness of coat, dull eyes, unthrifty conditions, perverted appetite and decreased milk production. It was also reported that the legs and ribs were easily broken. It was assumed that a dietary deficiency existed when the cattle were observed chewing and eating raw bones.

Six of these diseased cattle were obtained by the Wisconsin experiment station and fed rations containing wheat bran and steamed bone meal by Hart et al. (1927). They reported that in three months' feeding of the above ration, all animals were back to normal condition. The stiffness disappeared, the coat became smoother and milk production increased. It was concluded that the cattle were not diseased but lacked phosphorus in the diet.

Meigs et al. (1921) also found that cattle which had been pastured according to accepted standards had a decreased milk yield. This condition was corrected by feeding a legume hay and grain rich in protein and phosphorus for two months prior to calving. The milk yield doubled in the next lactation in some cases. It was found that feeding low producing dairy cattle on an alternate ration with phosphate during the dry

period greatly increased yield.

The deficiency symptoms of cattle receiving an inadequate level of phosphorus were further reported by Eckles et al. (1927). In this study cattle fed phosphorus deficient rations had irregular estrus cycles, usually being exhibited once or twice at normal intervals following parturition. Cattle that were mated after parturition conceived normally, however, cows failing to conceive at this time did not show estrus again until dry or until phosphorus was provided. In most cases cattle on low levels of phosphorus had a tendency to produce only one calf every two years.

Kleiber et al. (1936) compared the performance of beef heifers on a phosphorus deficient diet and on a normal diet. Heifers fed low phosphorus rations showed normal gains for six months but then ceased to gain. After one and one-half years on the depleted ration, these cattle began to decline in weight. Heifers showed a decrease in appetite after six months on the phosphorus deficient ration and developed bone chewing and coprophagea. The inorganic phosphorus content in their blood dropped from 9.0 to 3.9 milligrams of phosphorus per 100 milliliters of blood serum while the controls remained at 9 milligrams.

The desire to learn more about phosphorus sources led to several experiments designed to compare various phosphorus supplements in livestock rations.

One of the earliest experiments was reported by Hart et al. (1909). Swine were fed a basal ration of rice, wheat gluten and washed bran. Organic and inorganic phosphorus were added to the basal ration at different levels to compare the two sources in availability and level

required for proper body maintenance.

Animals fed a low phosphorus ration supplemented with inorganic phosphate grew as well as animals receiving their phosphorus supply wholly in an organic form. Precipitated calcium phosphate, a mixture of di- and tricalcium phosphate, equaled a crude tricalcium phosphate. It was determined that pigs receiving a phosphorus deficient ration had 50 per cent less ash in their bones than pigs receiving a normal ration. It was also recommended in this report that the phosphorus supply for a 50 pound growing pig be at least three grams daily.

Eckles et al. (1926) reported a condition found in parts of Minnesota where inadequate amounts of phosphorus in roughages fed to cattle were causing poor appetite, poor feed efficiency, stunted growth, reduced milk production and inhibition of estrus. Finely ground bone meal fed as a supplement cured these deficiency symptoms. In these experiments cattle utilized inorganic phosphorus compounds such as tricalcium phosphate and mono-basic sodium phosphate when these were added to a ration deficient in phosphorus. It was also concluded that the feeding of bone meal or spent bone black and the application of phosphate fertilizer to the pastures and hay lands were economical methods for the cure and prevention of phosphorus deficiencies in cattle.

Theiler et al. (1928) also reported on a feeding experiment using ground bone meal as a phosphorus supplement. Cattle were divided into groups of 50 head each. One group received a supplement of bone meal to rectify the phosphorus deficiency of the natural pastures; the other received no phosphorus source. Of the cows receiving the bone meal ration,

80 per cent calved normally whereas the group receiving no bone meal had a 51 per cent calf crop.

Steamed bone meal, Curaco Island phosphate and dicalcium phosphate were compared in a beef cattle feeding experiment by Long et al. (1957). In this trial heifers were individually fed rations containing 0.07 per cent and 0.09 per cent phosphorus and rations supplemented with NaH_2PO_4 to provide from 0.11 per cent to 0.17 per cent total phosphorus. Feed intake, rate of gain and plasma phosphorus increased with increased amounts of the supplemental phosphorus in rations over 0.07, 0.11, 0.15 and 0.19 per cent total phosphorus.

Steamed bone meal, Curaco Island phosphate and dicalcium phosphate were compared as sources of phosphorus by the above workers. Heifers on the 0.07 per cent phosphorus ration had less feed intake, lost weight and had lower plasma phosphorus levels. The heifers on the supplemental rations performed normally and the differences among the different sources were not statistically significant. The results indicated equal availability of phosphorus in the three supplements.

During the years 1955-56, reports of Chapman et al. (1955), Long et al. (1956) and Gobble et al. (1956) were published which compared colloidal clay with other sources of phosphorus as a phosphorus supplement in rations. The first of these reported by Chapman et al. (1955), showed that when colloidal clay was fed to swine as the only phosphorus source the result was a significant decrease in rate of gain and feed efficiency as compared to steamed bone meal and dicalcium phosphate.

Long et al. (1956) used three groups of beef heifers to compare the value of colloidal clay to that of dicalcium phosphate as phosphorus

supplements. One group was fed a basal ration containing 0.09 per cent phosphorus. The other two groups were fed the basal ration plus 0.05 per cent of dicalcium phosphate or colloidal clay supplement. The differences in response were significantly in favor of the dicalcium phosphate-fed group.

In a swine experiment in which growth, feed utilization and bone composition were used as criteria for evaluation, Gobble et al. (1956) compared soft phosphate with colloidal clay and dicalcium phosphate. In all criteria used for evaluation, the pigs fed soft phosphate with colloidal clay did not differ significantly from the pigs fed dicalcium phosphate. It was concluded in this trial that the two sources of phosphorus were approximately equal in biological availability to swine.

Several experiments have been conducted where a variety of different phosphorus sources have been compared in availability. Gillis et al. (1954) studied the biological value of inorganic phosphates. In this experiment, a purified diet containing 0.03 to 0.05 per cent phosphorus was fed to chicks and supplements of different sources of phosphorus were used to bring the phosphorus level up to 0.25, 0.30 and 0.35 per cent. When the phosphorus supplied in supplementary form was kept at low levels the chicks response was proportional to the amount of phosphorus added to the diet. When the amount of phosphorus added to the diet was optimun, growth and calcification were excellent, indicating that the diet was adequate in other respects for the chick.

There were wide differences in the availability of phosphorus from the different untreated raw rock phosphates. Curaco Island phosphate showed a satisfactory degree of availability for the chick; phosphorus in

Florida pebble rock was appreciably more available than that in the brown Tennessee rock. It was concluded that equivalent amounts of phosphorus from different sources are not necessarily of equal nutritional value. The availability of the phosphorus, rather than the total phosphorus, determines the usefulness of the supplement.

The effectiveness of six defluorinated superphosphates and defluorinated phosphate rock, phosphate slag, calcium pyrophosphate, vitreous calcium metaphosphate, tricalcium phosphate and bone meal were compared by Bird et al. (1945). Bone formation, per cent of bone ash and growth on chicks were used as criteria for evaluation. One source of the superphosphate was completely unavailable; the other five superphosphates were less available than bone meal and tricalcium phosphate; defluorinated phosphate rock, phosphate slag and vitreous calcium metaphosphate were intermediate in availability. Bone meal and tricalcium phosphate were the most available. Calcium pyrophosphate was found totally unavailable or nearly so.

Shrewsbury et al. (1945) conducted four experiments using yellow corn, ground oats and soybean oil meal as a basal ration with steamed bone meal, defluorinated phosphate, rock phosphate and superphosphate as phosphorus supplements. Steamed bone meal was found superior to either defluorinated phosphate or rock phosphate but equal to superphosphate when this ration was fed to growing pigs.

In another experiment where swine were used as experimental animals, Plumlee et al. (1955) compared the utilization of dicalcium phosphate, steamed bone meal, defluorinated rock phosphate, commercial monocalcium phosphate, imported rock phosphate and soft phosphate with

colloidal clay. The pigs were fed a cerelose-casein basal ration containing 0.18 per cent phosphorus supplemented with all known nutrients required for swine. Each supplement was added to raise the total phosphorus to the desired level. Monocalcium phosphate and imported rock phosphate gave the best results in growth rate and feed efficiency at the 0.30 per cent level. Soft phosphate at the 0.45 per cent and 0.60 per cent levels decreased growth rate of the growing pigs.

Wilcox et al. (1954) studied the availability of the phosphorus in 14 different phosphorus supplements to turkey poults. The poults were fed a purified diet supplemented with dried buttermilk, dried brewer's yeast and forage juice and supplemented with the different phosphates to furnish the major source of phosphorus in the diet. Ratings of the phosphorus supplements were determined by body weight, bone ash and mortality.

Dibasic and monobasic calcium phosphate and two defluorinated phosphates were found to be the most available of the phosphates tested. Tribasic calcium phosphate, steamed bone meal, beta-tricalcium phosphate, commercial dicalcium phosphate and a defluorinated phosphate appeared to be intermediate in availability while imported rock phosphate and colloidal phosphate were completely unavailable to the poults.

Lambs were used by Ammerman et al. (1955) to study the utilization of inorganic sources of phosphorus. Phosphorus supplements of dicalcium phosphate, Curaco Island phosphate, soft phosphate with colloidal clay and defluorinated rock phosphate were fed after the lambs had been on a 0.03 per cent phosphorus ration for four weeks. Blood serum phosphate levels, expressed in milligrams per cent, fell during

the depletion period from 10 to 6 and rose for the supplements as listed above by the average amounts of 2.4, 2.6, 1.3 and 1.3, respectively. These averages were not found to be significantly different.

In an earlier experiment conducted with steers, Ammerman et al. (1954) compared the utilization of five different sources of inorganic phosphorus. In this study, 550 pound yearling steers were fed a basal ration supplying 50 per cent of the phosphorus requirement of the animal. The phosphorus level was brought up to suboptimal levels for good gains by adding the following supplements: two dicalcium phosphates; bone meal; defluorinated rock phosphate; imported rock phosphate; and colloidal phosphate. Feces and blood plasma were analyzed for phosphorus. The supplements compared were not determined significantly different in utilization.

Bone formation in the rat was used by Ellis et al. (1945) to determine the availability of calcium and phosphorus in commercial and experimental defluorinated phosphate. Defluorinated phosphate rock, prepared by the fusion process, compared favorably with bone meal and calcium phosphate. Phosphate slag was rated good to fair in availability. Commercial defluorinated superphosphate showed considerable variation in availability ranging from reasonably good to poor.

The form of calcium phosphate had a bearing on its availability. The beta form, the beta-pyrophosphate and gamma-pyrophosphate were relatively unavailable forms, while the alpha, beta and ortho forms of tricalcium phosphate were highly available. The vitreous calcium metaphosphate was intermediate in availability.

Three sources of defluorinated phosphate were compared by Barrentine et al. (1944) based on bone formation in rats. One source of defluorinated phosphate was significantly less efficient than calcium phosphate or bone meal. This source inhibited the growth of rats. Another defluorinated phosphate used in this experiment when used at twice the level of calcium phosphate produced equal bone formation as the calcium phosphate. These two defluorinated phosphates were manufactured by treating rock phosphate with sulfuric acid and excess acid and fluorine driven off by heat.

A rock phosphate manufactured by the fusion process or the addition of silicia resulted in equal bone formation when fed at a 0.5 per cent higher level than calcium phosphate. It was concluded from this experiment that the low availability of the defluorinated superphosphates may be due to the presence of large amounts of poorly utilized calcium metaphosphate.

In an extensive study on phosphorus sources using a biological assay method for determining the availability of phosphates, Gillis (1957) found most samples of domestic steamed bone meal to be very good sources of phosphorus. It was also determined in this study that a wide range of values was obtained for various defluorinated phosphates. Most of the defluorinated phosphates were satisfactory nutritionally but some, however, had very low availability. Curaco Island rock phosphate was found to vary in its availability depending upon the geographical location the rock was mined on the island but in general was not as available as some of the other phosphates. Colloidal phosphate was found to be so poorly available that it could not be evaluated. Monocalcium phosphate and

phosphoric acid were found to have a very high biological value.

In four recent studies conducted by Tillman et al. (1958a, 1958b, 1958c, 1959), various criteria were used in determining the availability of phosphorus from different sources. Eighteen grade Hereford steers were used in a comparative procedure for measuring the phosphorus requirements of cattle in one study (Tillman et al., 1959). The steers were divided into three groups after a 47-day feeding of a basal ration containing 0.12 per cent phosphorus. Group one received the basal ration plus enough supplemental phosphorus to supply 1.5 grams of phosphorus per 100 pounds body weight daily. Steers in groups two and three received 2.0 and 2.5 grams of phosphorus in the basal rations per 100 pounds of body weight, respectively. The supplemental phosphorus was provided by a mixture of reagent grade dibasic calcium phosphate and calcium carbonate. These supplied 3.75 parts of calcium to one part of phosphorus, the ratio of these elements in the basal rations.

When response criteria were weight gains, feed consumption, efficiency of feed utilization, percentage digestibility of phosphorus or percentage net retention of phosphorus, 2.0 grams of phosphorus per 100 pounds body weight did not meet the phosphorus requirements of the animals. Based on bone growth measured by autoradiographs and plasma inorganic phosphorus levels, 2.0 grams of phosphorus per 100 pounds body weight were adequate.

In an experiment comparing the utilization of sodium meta-ortho- and pyrophosphate to sheep the same criteria as described in the previous experiment were used by Tillman et al. (1958). All phosphorus supplements supplied 66.1 per cent of phosphorus in a ration supplying 2.0 grams of

phosphorus per 100 pounds of live weight. Acid sodium pyrophosphate was found to be equally available as monosodium phosphate. Vitreous sodium metaphosphate caused increased fecal phosphorus excretions. The true digestibility of the three phosphates was not different. This study indicates that although the phosphorus in vitreous sodium metaphosphate was absorbed, it was inefficiently utilized.

Apparent digestibilities, net retention, fecal endogenous excretions and true digestibilities were used as criteria in two other experiments published in 1958 (Tillman et al., 1958a, 1958b). When these criteria were used to compare the true digestibility of dicalcium phosphate and phosphoric acid, no treatment differences were determined statistically significant, indicating the availability of phosphorus of phosphoric acid and dicalcium phosphate to be equal for beef cattle. Monocalcium phosphate and calcium phytate were found to be equally available sources of phosphorus for sheep by Tillman et al. (1958).

An artificial rumen technique employing washed suspension of rumen microorganisms was used by Anderson et al. (1956) in measuring phosphorus availability of feed supplements fed to ruminants. It was found that composite dicalcium phosphate was equally as available as a standard sodium-potassium phosphate mixture. An acidulated phosphate product and steamed bone meal were intermediate in phosphorus availability while Curaco rock phosphate and colloidal clay were less available.

The artificial rumen technique was also employed by Baxter (1957) in the study of the effect of ortho, meta, pyro and phytin phosphates upon cellulose digestion by rumen microorganisms. Cellulose digestion

by rumen microbes in vitro was used as an indication of the availability of sodium orthophosphate, sodium pyrophosphate, sodium hexa-metaphosphate and calcium phytate. Graded amounts of phosphorus from the various sources were added to 75 milliliter digestion tubes at the rate of 0,20, 40 and 60 micrograms per milliliter of basal medium. The pyro-form was significantly more available than the meta-form at the 20 microgram level. The phytate was less available than the other sources at the 20-40 microgram levels. No significant difference was found at the 60 microgram level among the four sources.

CHAPTER III

EXPERIMENTAL

Washed Suspension Technique

The technique used in these studies was similar to that described by Cheng *et al.* (1955), Anderson *et al.* (1956) and Hall (1955) with slight modifications. Rumen contents were obtained from a fistulated steer grazing a bluegrass-clover pasture and from a fistulated steer maintained on mixed grass hay and three pounds of concentrates daily. Both animals received a mineral mixture containing equal parts of dicalcium phosphate and common salt *ad libitum*. The rumen liquid collected was strained through four layers of cheese cloth into thermal-neutral containers.

In the laboratory, 1200 milliliters of rumen liquid were centrifuged at 1,000 r.p.m. for one minute to separate the protozoa and feed particles from the liquid. This heavy material was thrown to the bottom of the tube during centrifugation and was discarded. The supernatant was then centrifuged at 5,000 r.p.m. for 20 minutes to separate the bacteria from the liquid. The supernatant was decanted and the bacterial layer was suspended in 360 milliliters of one per cent sodium bicarbonate solution which had been bubbled with carbon dioxide gas. The bacteria were dispersed in the washing solution by the use of a Waring Blendor. The washing solution was centrifuged at 5,000 r.p.m. for 20 minutes to wash away any nutrients which might be clinging to the bacteria. This process was repeated and the final sediment suspended in 600 milliliters

of nutrient solution prepared according to a formula described by Anderson et al. (1956) and shown in Table I.

Three grams of purified wood cellulose and nine milliliters of specially hydrolyzed feather meal made by a process reported by Hall et al. (1954) was added to the suspension. The solution was bubbled with carbon dioxide gas for ten minutes and the pH adjusted to 7.0 with a saturated solution of sodium carbonate. The suspension was incubated for 24 hours in a one-liter Erlenmeyer flask which was placed in a water bath thermostatically controlled at 39° Centigrade. Anaerobic conditions were maintained by bubbling carbon dioxide gas continuously through the liquid. The 24 hour preliminary digestion period allowed the bacteria to deplete any phosphorus which was not removed in the washing process or which might have been in the basal medium.

At the end of the preliminary fermentation period one-half of the contents of the flask was discarded and 300 milliliters of phosphorus deficient basal medium and three grams of cellulose were added to the remaining liquid. The suspension was again bubbled with carbon dioxide gas and the pH adjusted to 7.0 with sodium carbonate. Aliquots of 20 milliliters each were pipetted into 75 milliliter pyrex centrifuge tubes containing 0.3 milliliter of hydrolyzed feather meal and graded amounts of phosphorus from various sources. The tubes were incubated at 39° Centigrade for 20 hours. Carbon dioxide was bubbled into each tube to establish and maintain anaerobic conditions as well as to agitate the fermenting suspension. Each tube was fitted with a rubber stopper and inlet and outlet glass tubing for bubbling the solution with carbon dioxide.

TABLE I

COMPOSITION OF NUTRIENT SOLUTION

Constituent	Amount Gram/liter
$NaHCO_3$	1.750
KCl	0.375
NaCl	0.375
$COCl_2 \cdot 6H_2O$	0.001
$FeSO_4 \cdot 7H_2O$	0.0375
$MgSO_4$	0.075
$CuSO_4 \cdot 5H_2O$	0.001
$MnSO_4$	0.0002
$ZnSO_4$	0.00004
Urea	1.000

Zero control tubes were prepared by stopping the microbial activity before the second incubation period by adding 12.5 milliliters of glacial acetic acid. The tubes were then set aside for chemical cellulose determinations later. At the end of the 20 hour fermentation period, chemical determination for cellulose was made on the tubes by the method described by Crampton and Maynard (1938) with slight modifications. An average of the cellulose content of the zero control tubes represented the initial cellulose in the digestion tubes. Digestion coefficients were calculated for each tube. A series of twelve experiments was first conducted to determine the individual response curves to phosphorus from dicalcium phosphate (wet acid process), dicalcium phosphate (furnace process), Curaco rock phosphate and a defluorinated phosphate. In each experiment phosphorus from one source was added at levels of 0, 10, 20, 30, 40, 60, 80, and 100 micrograms per milliliter of nutrient solution. Each experiment was repeated three times before the next phosphorus supplement was tested. Each treatment within a given experiment was also replicated three times.

All the above phosphorus sources were also compared simultaneously at levels of 0, 20, 40 and 60 micrograms per milliliter of nutrient solution. Triplicate tubes were used at each level and the experiment was repeated three times with bacteria obtained from a steer on pasture and three additional times with bacteria from a steer fed hay and concentrates. When it was shown in earlier experiments that the defluorinated phosphate was unavailable to rumen microorganisms, an experiment was conducted to compare its relative availability with two other sources of defluorinated phosphates. This was done to determine the relative

availability of the three sources of defluorinated phosphates. This experiment was repeated three times.

An experiment was conducted to determine if the rumen microbes could adapt themselves to the low available source of defluorinated phosphate. A fistulated steer was fed the low available source of defluorinated phosphate for 30 days and cellulose digestion trials were conducted to determine the availability of defluorinated phosphate to rumen microbes after the adaptation period.

Analysis of variance technique (Snedecor, 1957) and the Multiple Range Test (Duncan, 1955) were used in the statistical analysis of the data.

Digestion Trial

Twelve of the most thrifty lambs averaging 70 pounds in weight were selected from a group of 17 animals and fed a low quality grass hay for 14 days. At the end of this two-week period the lambs were weighed and assigned to one of four outcome groups according to body weight and put into metabolism stalls. Each lamb within an outcome group was then randomly assigned one of the three rations tested. One group of four lambs was fed the basal ration shown in Table II, containing 0.05 per cent phosphorus. The second group of lambs received the basal ration and a defluorinated phosphate to bring the phosphorus content of the ration up to 0.34 per cent. Earlier experiments had determined this defluorinated phosphate to be unavailable to rumen microorganisms. The third group of lambs received the basal ration and a dicalcium phosphate.

TABLE II

BASAL RATION

Constituent	Amount Per cent
Cottonseed hulls	65.0
Cerelose	5.0
Corn starch	16.5
Molasses	3.0
Lard	3.0
Urea	4.0
Alfalfa meal	3.0
Trace mineral salt*	0.5
Vitamin A (grams/100 lbs.)**	20.0
Vitamin D (grams/100 lbs.)***	7.0

*Salt 97 per cent, maganese 0.25 per cent, iron 0.10 per cent, sulphate sulphur 0.10 per cent, copper 0.033 per cent, cobalt 0.015 per cent, iodine 0.007 per cent and zinc 0.005 per cent.

**Vitamin A supplement containing 10,000 I. U. per gram.

***Vitamin D_2 supplement containing 3,000 I. U. per gram.

The total phosphorus content of this ration was also 0.34 per cent. This source of phosphorus had been shown to be readily available to rumen microorganisms in earlier experiments.

The calcium content of the ration containing no phosphorus supplement was brought up to equal the other two rations by the addition of calcium carbonate. The rations of all lambs were supplemented daily with 20 milliliters of specially hydrolyzed feather meal prepared by a process reported by Hall et al. (1954). Lambs in heavy outcome groups received 1,000 grams of feed daily while those in lighter outcome groups received 900 grams daily. The rations were fed seven days in metabolism stalls prior to a seven-day collection period. The feed not eaten was weighed to determine the amount consumed and feces and urine were collected and weighed each day. The lambs were allowed access to water for six hours daily. The per cent of cellulose and air dry matter excreted and the per cent of cellulose and air dry matter digested were calculated for each lamb.

Analysis of variance technique (Snedecor, 1957) and the Multiple Range Test (Duncan, 1955) was used in the statistical analysis of the data.

CHAPTER IV

RESULTS AND DISCUSSION

Sources of Phosphorus and Cellulose Digestion

Dicalcium phosphate (wet acid process) was added to fermentation tubes to supply 0, 10, 20, 30, 40, 60, 80 and 100 micrograms of phosphorus per milliliter of nutrient solution. The results of these experiments are presented in Table III.

Rate of cellulose digestion increased as the amount of phosphorus from this source was added up to 40 micrograms per milliliter of nutrient solution. Higher levels of phosphorus did not result in any significant increase in cellulose digestion. This would indicate that a level of 40 micrograms of phosphorus per milliliter of nutrient solution was adequate for maximum cellulose digestion under the conditions of these experiments. The importance of phosphorus for cellulose digestion is demonstrated by the fact that only 19 per cent of the cellulose was digested in tubes with no added phosphorus as contrasted to approximately 70 per cent in tubes containing 40-100 micrograms phosphorus per milliliter of nutrient solution.

Similar experiments as those described above were conducted using dicalcium phosphate (furnace process) as the source of phosphorus. Results of these experiments are presented in Table IV. Marked increases ($P < 0.05$) in cellulose digestion resulted from additions of phosphorus from this source. Only 33.6 per cent of the cellulose was digested at

TABLE III

EFFECTS OF PHOSPHORUS FROM DICALCIUM PHOSPHATE (WET ACID PROCESS) UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN VITRO

Level of added phosphorus	Cellulose digested**			
	Experiment I	Experiment II	Experiment III	Average*
Mcg./ml.	Per cent	Per cent	Per cent	Per cent
0	21.6	22.1	13.6	19.1 a
10	32.1	46.2	28.1	35.5 b
20	53.1	59.5	41.5	51.4 c
30	58.8	65.4	54.8	59.7 d f
40	66.7	66.2	54.9	62.6 e f
60	69.8	67.3	63.1	66.7 e f
80	71.0	65.8	66.6	67.8 e f
100	73.4	67.0	70.2	70.2 e

*Values not having the same superscript are significantly different at the 5 per cent level.

**Values in each experiment are an average of three observations.

TABLE IV

EFFECTS OF PHOSPHORUS FROM DICALCIUM PHOSPHATE (FURNACE PROCESS) UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN VITRO

Level of added phosphorus	Cellulose digested**			
	Experiment I	Experiment II	Experiment III	Average*
Mcg./ml.	Per cent	Per cent	Per cent	Per cent
0	48.4	14.8	37.7	33.6 [a]
10	70.4	37.4	63.2	57.0 [b]
20	83.3	35.9	66.7	61.9 [b c]
30	83.0	38.1	71.8	64.3 [c d]
40	84.4	44.7	69.0	65.9 [c d]
60	89.1	43.8	73.3	68.7 [c]
80	86.0	39.6	73.4	66.3 [c d]
100	86.7	40.2	70.9	65.9 [c d]

*Values not having the same superscript are significantly different at the 5 per cent level.

**Values in each experiment are an average of three observations.

the 0 level as compared to 68.7 per cent at the 60 micrograms per milliliter level. Generally, the cellulolytic response to this source of phosphorus was similar to that obtained from the wet acid processed-product. Results of both series of experiments agree with those of Anderson et al. (1956) who found a composite dicalcium phosphate supplement to be a good source of phosphorus for rumen microbes.

Phosphorus additions from Curaco phosphate and from a defluorinated phosphate failed to beneficially influence cellulose digestion by rumen microorganisms as shown by the data in Tables V and VI. No significant increases in cellulose digestion resulted in either experiment by the addition of these two phosphates to the fermentation tubes. Both Anderson et al. (1956) and Gillis (1957), reported these types of phosphates to be relatively poor sources of phosphorus.

Relative Availability of Phosphorus

A second series of six experiments was conducted to rank the phosphorus from each of the four supplements described above as to relative availability. Levels of 0, 20, 40 and 80 micrograms of phosphorus per milliliter of nutrient solution from each of the four supplements were compared in the same experiment. All phosphorus sources were tested at the same time and under identical conditions. The experiment was repeated three times by using rumen bacteria obtained from a steer on pasture and three additional times by using microbes obtained from a steer fed hay and concentrates in a barn. Phosphorus from both dicalcium phosphates supplements proved to be highly available to the rumen microbes as evidenced by percentages of cellulose digested. Phosphorus

TABLE V

EFFECTS OF PHOSPHORUS FROM CURACO PHOSPHATE UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN VITRO

Level of added phosphorus	Cellulose digested**			
	Experiment I	Experiment II	Experiment III	Average*
Mcg./ml.	Per cent	Per cent	Per cent	Per cent
0	22.5	32.6	32.6	29.2
10	27.5	32.7	31.8	30.7
20	24.8	35.3	33.1	31.1
30	22.6	35.4	33.3	30.4
40	23.5	31.3	35.9	30.2
60	23.0	34.0	33.6	30.2
80	25.9	33.1	33.3	30.8
100	26.4	31.5	30.6	29.5

*No significant difference found among treatment at the 5 per cent level.

**Values in each experiment are averages of three observations.

TABLE VI

EFFECTS OF PHOSPHORUS FROM DEFLUORINATED PHOSPHATE UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS IN VITRO

Level of added phosphorus	Cellulose digested**			
	Experiment I	Experiment II	Experiment III	Average*
Mcg./ml.	Per cent	Per cent	Per cent	Per cent
0	30.9	48.4	31.0	36.8
10	35.6	47.7	29.9	37.7
20	36.3	48.6	34.2	39.7
30	37.5	47.6	30.2	38.4
40	36.6	51.7	30.2	39.5
60	35.9	49.8	32.5	39.4
80	35.8	46.4	32.9	38.4
100	37.0	48.3	31.9	39.1

*No significant difference was found among treatments at the 5 per cent level.

**Values in each experiment are averages of three observations.

from a defluorinated phosphate and Curaco phosphate was apparently unavailable to the cellulose digesting microbes as tested in these experiments. Similar responses were obtained with bacteria from a grazing animal and with bacteria from an animal fed hay and concentrates in a barn (see Table VII).

Since phosphorus from the defluorinated phosphate supplement did not increase microbial activity in these experiments, and since only one source was tested, the question therefore arose as to the availability of phosphorus in defluorinated phosphates. To answer this question two other sources of defluorinated phosphates were obtained and tested. Levels of 0, 20, 40 and 80 micrograms of phosphorus per milliliter of nutrient solution from each of three defluorinated phosphate supplements were compared in the same experiment. As shown in Table VIII phosphorus addition from defluorinated supplements B and C resulted in statistically significant increases in cellulose digestion when compared with cellulose digestion in tubes with no added phosphorus or in tubes with phosphorus additions from defluorinated phosphate supplement A. It is interesting to note that the cellulolytic response obtained from phosphorus additions from defluorinated phosphate supplement C was equal to any obtained previously with dicalcium phosphate supplements.

The findings indicate that the availability of phosphorus in different defluorinated phosphate supplements to rumen microbes varies greatly. This is in accord with biological studies with chicks and turkeys reported by Gillis (1957). In his studies a wide range of availability values were obtained for various defluorinated phosphates.

TABLE VII

RELATIVE AVAILABILITY OF PHOSPHORUS FROM DIFFERENT SOURCES BASED ON CELLULOLYTIC RESPONSE BY RUMEN MICROBES

Source of bacteria	Level of added phosphorus Mcg./ml.	Per cent cellulose digested*			
		Defluorinated phosphate	Curaco phosphate	Dicalcium phosphate Wet acid process	Dicalcium phosphate Furnace process
Steer on pasture	0	25.3	25.3	25.3	25.3
	20	26.9	28.0	46.0	50.1
	40	26.5	27.5	59.3	60.5
	80	25.0	27.3	69.9	76.3
Steer fed concentrates and hay	0	32.9	32.9	32.9	32.9
	20	33.6	34.0	64.2	60.2
	40	32.6	33.5	71.9	69.2
	80	32.6	35.5	72.8	76.9

Values not underscored by the same line are significantly different.

*Each value is an average of nine observations.

TABLE VIII

EFFECT OF PHOSPHORUS FROM THREE DIFFERENT DEFLUORINATED PHOSPHATE SOURCES UPON CELLULOSE DIGESTION BY RUMEN MICROORGANISMS

Defluorinated phosphate supplements	Per cent cellulose digested**		
	20 mcg./ml.*	40 mcg./ml.*	80 mcg./ml.*
A	36.0 [a]	37.3 [c]	37.6 [e]
B	41.3 [a]	50.0 [c]	54.3 [f]
C	70.3 [b]	79.3 [d]	85.3 [g]

*Values not having the same superscript are significantly different at the 5 per cent level.

**Each value is an average of nine observations.

Most of the defluorinated phosphates were found to be satisfactory nutritionally but some were found to be very low in availability. Dicalcium phosphate was found to be a more available source of phosphorus than were defluorinated phosphates or Curaco rock phosphates in this work also.

Adaptation Period and Phosphorus Availability

Microorganisms are known to have a remarkable ability to adapt to changes in environment. Thus, a source of phosphorus that is not available at first might possibly become available after the microbes are exposed to it for a period of time. To test this, the defluorinated phosphate supplement found to be a poor source of phosphorus was fed to a fistulated steer for a period of 30 days. Rumen microorganisms were then obtained from this steer and phosphorus studies similar to those previously described were conducted.

As shown by the results in Table IX, the adaptation period did not alter the availability of the phosphorus in the supplements to rumen microbes. Additions of phosphorus from the defluorinated phosphate supplement failed to increase microbial cellular digestion whereas addition of phosphorus from dicalcium phosphate resulted in marked ($P < 0.01$) increases in cellulose digestion. Thus the results were similar to those obtained before the defluorinated phosphate supplement was fed.

Phosphorus and Ration Digestibility

The results of the digestion trial are summarized in Tables X, XI, and XII.

TABLE IX

INFLUENCE OF AN ADAPTATION PERIOD UPON THE AVAILABILITY OF PHOSPHORUS IN A DEFLUORINATED PHOSPHATE SUPPLEMENT TO RUMEN MICROORGANISMS IN VITRO

Levels of added phosphorus	Cellulose digested**	
	Defluorinated phosphate*	Dicalcium phosphate furnace process*
mcg./ml.	Per cent	Per cent
0	27.9 [a]	27.9 [b]
20	26.3 [a]	48.4 [b]
40	23.0 [a]	55.4 [c]
80	25.7 [a]	56.1 [c]

*Values not having the same superscript are significantly different at the 5 per cent level.

**Each value is an average of three observations.

TABLE X

FEED INTAKE AND FECAL EXCRETIONS BY LAMBS FED TWO DIFFERENT PHOSPHATE SUPPLEMENTS

Outcome group	Basal ration		Basal + defluorinated phosphate		Basal + dicalcium phosphate	
	Daily feed intake	Daily fecal excretion (wet)	Daily feed intake	Daily fecal excretion (wet)	Daily feed intake	Daily fecal excretion (wet)
	Grams	Grams	Grams	Grams	Grams	Grams
1	1000	1468	971	1318	1000	1187
2	1000	1098	966	1222	937	1627
3	993	1189	980	1360	1000	1183
4	893	908	892	1445	888	1437
Average	997	1166	952	1336	956	1359

TABLE XI

DRY MATTER AND CELLULOSE CONTENT OF FECAL EXCRETIONS FROM LAMBS FED TWO DIFFERENT PHOSPHATE SUPPLEMENTS

Outcome group	Basal ration		Basal + defluorinated phosphate		Basal + dicalcium phosphate	
	Dry matter	Cellulose (dry matter basis)	Dry matter	Cellulose (dry matter basis)	Dry matter	Cellulose (dry matter basis)
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	29.5	37.4	37.8	38.9	38.2	35.3
2	39.3	35.5	37.4	36.2	27.3	34.6
3	36.4	36.6	33.8	37.8	38.3	36.0
4	43.4	36.8	35.3	35.9	28.5	36.6
Average	34.2	36.6	36.1	37.2	33.1	35.6

Differences among treatment means are not significant at the 5 per cent level.

TABLE XII

INFLUENCE OF PHOSPHORUS FROM TWO SOURCES UPON DIGESTIBILITY OF RATION DRY MATTER AND CELLULOSE BY LAMBS

Outcome group	Basal ration		Basal + defluorinated phosphate		Basal + dicalcium phosphate	
	Dry matter	Cellulose	Dry matter	Cellulose	Dry matter	Cellulose
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	51.9	54.2	43.1	43.5	49.3	54.4
2	52.1	56.7	47.5	51.9	47.4	53.7
3	51.6	54.9	47.9	50.0	49.7	53.9
4	51.0	54.0	49.8	54.2	47.1	51.9
Average*	51.7[b]	54.9[c]	47.1[a]	49.9[c]	48.4[a b]	53.5[c]

*Averages not having the same superscript are significantly different at the 5 per cent level.

Whereas phosphorus additions resulted in marked increases in cellulose digestion in vitro, phosphorus additions to a phosphorus deficient ration failed to improve cellulose digestion by sheep (see Table XII). Actually sheep receiving the cottonseed hull ration supplemented with the defluorinated phosphate supplement digested 49.9 per cent of the cellulose as compared to 53.5 per cent and 54.9 per cent by sheep fed the ration supplemented with dicalcium phosphate and the basal ration only, respectively. Differences among the treatment means were not statistically significant, however.

Digestibility of dry matter by lambs fed the basal ration was significantly greater than that by lambs receiving the basal ration plus the defluorinated phosphate supplement. Thus a phosphorus source which failed to increase microbial activity in vitro actually depressed dry matter digestibility by sheep. Dry matter digestibility of the group fed dicalcium phosphate was not statistically different. All lambs maintained their body weight during the digestion trial, however.

The failure of phosphorus to increase dry matter and cellulose digestion in sheep might be explained by the amount of phosphorus present in the basal ration. Even though cottonseed hulls and purified ingredients were used, the ration contained 0.05 per cent or 500 ppm of phosphorus. In vitro experiments have shown that maximum cellulose digestion occurs at levels of phosphorus approximating 40-80 ppm. Thus ample quantities of phosphorus was available in the basal ration to meet rumen microbial requirements. Differences would undoubtedly have been evident had other criteria of evaluation been used since the total requirement for phosphorus by ruminants exceeds that amount present in

the basal ration. A basis for the depressing effect upon ration digestibility by the defluorinated phosphate is not apparent from the data.

A factor that should be kept in mind in considering the digestion trial data is the possibility of worm infestation in the sheep. During the trial excreta from several sheep were noted to contain excessive amounts of moisture, mucus and some blood. More of the sheep fed the phosphorus supplements had abnormal fecal excretions than those on the basal ration. Stomach worms were found in one animal in the group from which the experimental animals were selected after the completion of the trial. It is possible that some of the experimental animals might have been infected with worms, also. However, worm counts were not made on fecal excretions from any of the experimental animals.

CHAPTER V

SUMMARY

Washed suspension of rumen microorganisms were used in a series of studies to determine the availability of various phosphorus supplements using cellulose digestion *in vitro* as the criterion of evaluation. Samples of rumen liquid were collected from a fistulated steer in thermo neutral containers. In the laboratory the rumen microorganisms were separated from feed debris and rumen liquid by a process of differential centrifugation. These microbes were washed twice with carbon dioxide-bubbled distilled water before being suspended in a phosphorus deficient basal medium. After a 24-hour preliminary fermentation period in a liter Erlenmeyer flask, 20 milliliter aliquotes were further incubated for 20 hours in 75 milliliter digestion tubes containing graded amounts of phosphorus from the various phosphorus sources. Addition of dicalcium phosphate (wet acid processed and furnace processed) of 10-100 ppm to fermentation tubes resulted in marked increased ($P<0.01$) in cellulose digestion by rumen microbes. Similar additions of Curaco phosphate and a defluorinated phosphate failed to increase *in vitro* microbial activity. Both sources of dicalcium phosphate were found to be equally highly available sources of phosphorus for rumen microbes whereas the phosphorus in Curaco and defluorinated phosphate supplements was apparently unavailable to rumen microbes as tested in these experiments. The feeding of defluorinated phosphate to a fistulated steer for 30 days before conducting availability studies failed to alter the availability of the

phosphorus in the supplement to rumen microbes. Two additional defluorinated phosphates were found to be available sources of phosphorus for rumen microbes. One such source produced a cellulolytic response that was equal to that resulting from the dicalcium phosphates tested. The other source increased ($P<0.05$) *in vitro* cellulose digestion but not to the same extent as did the dicalcium phosphates tested. Phosphorus supplements failed to increase ration dry matter and cellulose digestibility by sheep when added to a cottonseed hull ration containing only 0.05 per cent phosphorus. It was concluded that the basal ration contained ample quantities of phosphorus for cellulose digesting rumen microbes.

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