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PART III. PHOSPHORUS UTILIZATION BY LAMBS AS DETERMINED WITH THE RADIOACTIVE ISOTOPE P^{32}

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PART I. COMPARATIVE AVAILABILITY OF PHOSPHORUS IN NATIVE GRASS HAY PRODUCED IN SOUTHEASTERN AND NORTH CENTRAL OKLAHOMA

INTRODUCTION

Range livestock producers have been cognizant of the problems arising from inadequate mineral nutrition for many years in many areas of the United States and other countries. During recent years, many of these so-called "sick areas" have been found to be either deficient in, or over-abundantly supplied with, one or more of the mineral elements which are known to affect growth, reproduction and lactation in range livestock. The relationships which exist between the soils, plants, and animals are a field of nutrition which needs additional study.

The failure of forage in certain regions of Oklahoma to promote optimum growth and reproduction in cattle and sheep has led to research on the content and availability of certain minerals in native forage. Many soils in central Oklahoma are known to border on phosphorus deficiency, while many of those in southern Oklahoma are definitely deficient in phosphorus. During recent years, low levels of soil fertility have been associated with poor plant growth and inefficient livestock production. Thus adequate phosphorus nutrition and the factors which affect the utilization of phosphorus are of vital concern to the stockman.

Experimental work with beef cattle at the Oklahoma Agricultural Experiment Station has indicated that there may be marked differences in the availability of phosphorus in forages produced in Oklahoma on soils that differ in their content of available phosphorus (Ross, et al. 1949). Cows and heifers showed typical symptoms of phosphorus deficiency when fed prairie hay containing approximately .06 per cent phosphorus, with corn gluten meal as a protein supplement. The hay fed was produced near Wilburton, in southeastern Oklahoma, on soil very low in available phosphorus. None of these symptoms was shown by cows and heifers fed prairie hay which contained about the same percentage of phosphorus, but which was grown near Stillwater on a soil higher in available phosphorus. Since there was no great difference in the total phosphorus content of these forages, differences in the availability of the phosphorus for beef cattle seemed likely. The need for information concerning the availability of the phosphorus in these two hays provides the basis of this study. In addition, hay from a meadow near the Lake Carl Blackwell area which had been fertilized with 200 pounds of superphosphate per acre was included in the study in an effort to determine possible effects of such fertilizer treatment on phosphorus availability.

REVIEW OF LITERATURE

Certain plants are able to grow on soils that are very infertile and low in available phosphorus. This is particularly true of many native pasture plants of low economic value, such as the Aristida species, commonly known as the poverty grasses,and Andropogon virginicus or broom sedge. Among the various harvested crops, there are also wide differences in the ability of the crops to grow on soils low in phosphorus. Rye and oats, for example, have been considered, generally, to be able to grow well on soils where barley suffers from a lack of available phosphorus. Many studies have been conducted in attempts to learn why certain crops can obtain more phosphorus from the soils than others, and can make more growth on soils low in phosphorus.

Sommer (1936) found that in solution cultures, wheat and buck wheat, which have large root systems and a large absorbing surface, made a better growth on low concentrations of phosphorus than cotton which has a relatively poor root system. Pierre (1938) has shown that the solubility of compounds of phosphorus in the soil has a great influence upon the availability of phosphorus in the soil to plants. The following groups of soil phosphorus compounds are listed in what he believes to be the order of decreasing availability for plant nutrition: (a) compounds of calcium and phosphorus or magnesium and phosphorus, (b) combinations of phosphorus with organic matter, (c) compounds of iron and aluminum with phosphorus and (d) compounds of phosphorus present in the form of the parent rock from which the soils are formed.

There are numerous references in the literature in general agreement concerning the effect of soil reaction on plant nutrient availability. Whitson and Stoddart (1927) studied the response of soils to phosphate fertilization and found that acid soils gave a much greater response to phosphorus fertilization than non-acid soils. They suggested that in acid soils the phosphorus was largely present as iron and aluminum phosphates instead of the more available calcium phosphate.

Truog (1947) has illustrated graphically the general trend of the relation of pH and accompanying soil conditions to the availability of plant nutrient elements. His work indicated that soil phosphorus is most readily available to plants when the pH range is between 6.5 and 7.5. Harper (1947) reported the results of a study in which over 3000 Oklahoma soil samples were classified according to pH value and available phosphorus content. This study showed that a soil whose parent rock material was low in available phosphorus was also very likely to be low in available phosphorus. Under average Oklahoma conditions, the availability of inorganic phosphorus to plants was found to decline with increasing soil acidity. According to Browne (1938), although plants are dependent upon the soil for their mineral nutrients, climatic conditions so affect respiration, assimilation, photosynthesis, metabolism and other physiological processes, that the composition of both the mineral and organic matter of crops may be greatly modified even though they are grown on identical soils.

Beeson (1941) reviewed the work of Kunze in Germany who found no significant differences in the phosphorus content of wheat grain, but about half as much phosphorus was found in the straw and chaff produced in dry years as compared to years of average rainfall. Daniel and Harper (1935) reported the results of a study concerning the relation between effective rainfall in Oklahoma and total calcium and phosphorus in alfalfa and prairie hay which support these findings. Although the mathematical correlation between the actual amount of rain and the phosphorus content of alfalfa was not high, their data show that phosphorus content fluctuated with the rainfall. McCreary (1939) at the Wyoming Station reported that pastures in the Laramie Plains clipped in April during a season of sufficient moisture for maximum growth contained 0.73 per cent P_2O_5 and 18.61 per cent crude protein on the dry basis, while forage clipped from the same area on July 23 (after a period of low precipitation) contained 0.47 per cent P_2O_5 and 9.3 per cent crude protein. Another sample from the same area representing the growth of forage between August 16 and September 3, after a period of high precipitation and more than sufficient moisture, contained 0.74 per cent P_2O_5 and 15.97 per cent crude protein. Schrieber (1948) in findings concerning phosphate fertilization in Germany, stated that in unusually dry years, beet crops have proved low in phosphoric acid because insufficient rainfall caused a great reduction in the solubility of phosphate fertilizers. On the other hand, normal rainfall produced crops of abundant phosphorus content.

Numerous references in the literature cite the effect of lime and fertilizer applications in increasing plant nutrient availability as shown by changes in chemical composition of pasture forage. Fertilizer treatment of soils affects the composition of forage in two ways: (1) by favoring in a mixed forage the growth of certain species of plants in preference to others, and (2) by directly promoting an increased absorption of the nutrient or nutrients added. Pierre and Robinson (1937) found in West Virginia that forage from an unproductive soil at the Morganstown Station was only 60 per cent as high in phosphorus as the forage from the untreated plots of the more fertile soil at the Morfield Station. Liberal phosphate fertilization increased the phosphorus content of the forage to about the same level on the two areas. The percentage increases were 29 per cent at the Morfield Station and 101 per cent at the Morganstown Station. Phosphorus fertilization increased the phosphorus content of white clover an average of 10 per cent as compared with an average of 40 per cent for Kentucky blue grass. These workers also reported that the percentage increase in the phosphorus content of poverty grass from phosphate fertilization was approximately twice as high as that of the blue grass.

O'Brien and Obenshain (1942) found that on low fertility soils, phosphate fertilization increased the weighted average percentage of nitrogen, phosphoric acid and calcium oxide in the forage. On similar soils, these workers reported that di-calcium, tri-calcium and superphosphate treatments all increased the average phosphoric acid content of the forage by 50 per cent over the respective check plots.

Brown (1932) found substantial increases in the percentage of clover in pasture forage after the application of phosphate and still a greater increase after the addition of phosphate and lime. From a low value of 11.7 per cent protein in forage from unfertilized plots, which contained little clover, phosphate alone increased the clover to 14 per cent and the protein of the mixture to 14 per cent. Phosphate and lime increased the clover to 41 per cent and the protein to 21.1 per cent. Phosphate, lime and potash together increased the clover to 55 per cent and the protein to 20.4 per cent.

Sullivan and Garber (1947) in studies at the Northeastern Pasture Research Laboratory reported that ammonium nitrate furnishing 0, 50, 150 and 300 pounds of nitrogen per acre was applied to blue grass plots, resulting in 16.7, 19.9, 24.9 and 23.2 per cent crude protein respectively for the first cuttings. The effect was not so pronounced for later cuttings.

Sheldon, et al. (1948) reported studies on the amino acid content of lespedeza produced on different soil types and of alfalfa produced on soils of varying fertilizer treatments. The quality of protein from the alfalfa was modified by soil treatment and a wide diversity manifested itself in the case of each amino acid. The methionine content, however, varied most widely.

Martens and associates (1949) however, in a study of the effects of phosphate fertilization on the composition and nutritive value of forage for sheep, found that phosphate fertilization produced no significant change in the chemical composition, including the calcium and phosphorus content. Feeding trials and digestibility studies with lambs gave no significant differences either in weight gains or apparent digestibility values between feeds from phosphated and non-phosphated plots.

Fudge and Fraps (1944) in a study of 1140 forage samples from Gulf Coast prairie pastures in Texas found that phosphoric acid was deficient (.17-.32%) in 65 per cent of samples of little blue stem and 39 per cent of samples of bermuda grass; in 50 per cent of all young samples and in 96 per cent of all mature samples. Of the 68 individual soils from which the forage samples were collected, 64 were deficient in active phosphoric acid. (Soluble in 0.2 N nitric acid.)

There are numerous accounts in the literature concerning the response in livestock production to the supplemental feeding of mineral elements.

Considerable data have been published regarding minimum and optimum levels of phosphorus for beef cattle and sheep.

Black and associates (1943) found that the feeding of 6.9 grams of phosphorus to dry cows and 14.3 grams to lactating cows proved beneficial under range conditions in southern Texas. For the 2-year period covered by this study, the control cows weaned a 58 per cent calf crop, whereas, the supplemented cows weaned an 81 per cent calf crop. Moreover, the average weaning weight of the calves produced by the supplemented cows was 69 pounds greater than the average weaning weight of calves produced by cows in the control lot.

DuToit and co-workers (1929) fed bone meal to breeding cows grazing phosphorus deficient pasture. The supplemented lot of cows received 5 ounces of bone meal daily over a 3-year period. The bone meal fed cows produced an average calf crop of 87.3 per cent as compared to 56.3 per cent for the control lot. In the bone meal lot, 66 per cent of the cows produced 3 calves in 3 years, while not a single cow in the control lot produced a calf each year. These workers concluded that under conditions of this study in the Union of South Africa, the feeding of bone meal hastened maturity and favored regular breeding.

Ross and associates (1949) observed that the feeding of 26 pounds of di-calcium phosphate (about 4 grams of phosphorus per head daily) per head during a period of approximately 21 months at the Wilburton Station in Oklahoma produced heifers that were 166 pounds heavier than a similar lot receiving no mineral supplement. The difference in apparent general health and vigor between the supplemented and control lots was reported to be more noticeable than the difference in weight.

Mitchell and McClure (1937) have set forth the approximate clacium and phosphorus requirements for growing and fattening beef steers. These workers concluded that a 600 pound growing steer requires a daily intake of 12.4 grams of phosphorus and a fattening steer of the same weight requires 16.4 grams of phosphorus.

Beeson and associates (1941) found that the phosphorus requirements of growing and fattening steers in Idaho were met by feeding rations containing 0.18 per cent of phosphorus or a daily intake of 2 grams of phosphorus per 100 pounds of body weight. These investigators suggest that the minimum phosphorus requirement is nearer 1.8 grams per 100 pounds of body weight than 2 grams. Mitchell (1947) concluded that for body weights ranging from 150 to 1200 pounds, the phosphorus requirement of Holstein heifers drops from 0.52 per cent on a dry matter basis to 0.13 per cent, the calcium requirement drops from 0.85 per cent to 0.18 per cent and the calcium-phosphorus ratio from 1.63 to 1.38:1. For later stages of growth, the calcium requirement continued to decrease, while the phosphorus requirement remained approximately constant. The optimum calcium phosphorus ratio at maturity was found by these workers to be about 0.5 or 0.6:1. Beeson, <u>et al</u>. (1944) found that lambs receiving rations with a phosphorus content ranging from 0.15 to 0.23 per cent were adequately supplied with phosphorus. On the basis of phosphorus intake per unit of live weight, these percentages were equivalent to 2.17 to 3.8 grams daily per 100 pounds of live weight.

Gallup and Briggs (1950) conducted phosphorus balance studies in an effort to determine the minimum phosphorus requirements for lambs weighing from 55 to 70 pounds. These workers reported negative phosphorus balances with rations which supplied 1.4 to 1.7 grams of phosphorus daily per 100 pounds of live weight, about an equal number of positive and negative balances with those that supplied from 1.9 to 2.1 grams of phosphorus and positive balances in every case with those rations that supplied from 2.4 to 2.9 grams of phosphorus per head daily.

Reviews of the literature dealing with the minimum and optimum phosphorus requirements of range beef cattle are presented by Robertson (1948), Albert (1949) and Nance (1952).

The chief phosphorus-containing constituent of cereals and legumes and their by-products is phytin, the calcium-magnesium salt of inositolhexaphosphoric acid. Thus, the question of the availability of phytin phosphorus is an important one in nutrition, worthy of consideration.

According to McCance and Widdowson (1935) phytin phosphorus represents 46.4 per cent of the phosphorus of wheat, 52 per cent of oats, 63 per cent of barley, 58 per cent of yellow corn and 57.5 per cent of peanuts.

Hay (1942) reported that in wheat bran, the phytin phosphorus represents 90 to 100 per cent of the total phosphorus and that the phytin phosphorus content of wheat by-products is directly proportional to their fiber content.

Numerous experiments have shown that phosphorus utilization is impaired in rations containing phosphorus largely in the form of phytin. There are controversial theories which attempt to explain if and why such is the case. Lowe, Steenbock and Krieger (1939) found that the addition of phytin to a low phosphorus basal ration, thereby raising the phosphorus content to 0.68 per cent, resulted in no significant improvement in bone formation in chicks as revealed by bone ash determinations. On the other hand, the addition of phosphorus as di-sodium phosphate equivalent to the phytin phosphorus increased the bone ash approximately 10 per cent. The results were obtained in both the absence of vitamin D and the presence of suboptimal levels.

Heuser, Norris, McGinnis and Scott (1943) made a study of the effect on bone formation in chicks of omitting supplementary phosphorus in the form of bone meal from two rations which contained no animal protein feedstuffs. One of these rations contained 0.38 per cent phosphorus and the other 0.58 per cent. The omission of the bone meal from the rations caused a marked reduction in the bone ash at 8 weeks of age.

McGinnis (1946) has shown that the chick is unable to use the phosphorus of phytin as effectively as inorganic phosphorus for bone formation.

Hart and associates (1927) observed that cattle in a phosphorus deficient area of Wisconsin were completely protected from rickets by the addition to the ration of either wheat bran or bone meal. These cattle also had access to direct sunlight during the experimental period. Experimental evidence during recent years has indicated that in the absence of vitamin D, the phosphorus of phytic acid is poorly available to the rat. Krieger and associates (1940) have demonstrated that in the presence of vitamin D there is a marked improvement in the utilization of phytic acid phosphorus by the rat.

In studies with chicks, Lowe, Steenbock and Krieger (1939) and McGinnis, Norris and Heuser (1944) showed that the phosphorus of cereals and legumes was poorly available in the absence of vitamin D. The latter group found phytin phosphorus to be nearly as available as inorganic phosphorus in the presence of 160 A.O.A.C. units of vitamin D per 100 grams of diet.

It has been suggested by Hart, et al. (1909), Lowe and Steenbock (1936) and Singsen and Mitchell (1944) that the ingestion of the enzyme phytase with the food may be necessary to the utilization of phytin phosphorus. The observation of Krieger and associates (1940) that the phosphorus of isolated phytic acid fed in a purified diet is poorly available is thought by these workers to be explained by a lack of a dietary source of the enzyme. Singsen and Mitchell (1944) found that the efficiency of the chick in utilizing the phytic acid phosphorus of soybeans was satisfactory when field cured alfalfa meal was included in the ration as a source of phytase, whereas, commercially prepared dehydrated meal was not effective. It was postulated by these workers that the phytase of the commercially prepared meal had been destroyed during processing.

Spitzer and Phillips (1945) concluded that a dietary source of phytase was not essential for the utilization of phytin phosphorus of soybean meal by the rat. Boutwell and associates (1946), in an effort to determine the effect of dietary phytase and vitamin D on the availability of the phosphorus of wheat bran to albino rats, found that in the absence of vitamin D the phosphorus of wheat bran was poorly utilized. An adequate intake of this vitamin increased the utilization nearly equal to that of inorganic phosphorus, as measured by bone ash. In this work the presence or absence of phosphorus splitting enzymes in the diets did not alter the availability of phytin phosphorus.

Little information is available with specific emphasis on the availability of phosphorus and calcium in hay and pasture forage for beef cattle and sheep.

Williams, et al. (1940) reported the results of a study on the availability to the rat of phosphorus in lespedeza and alfalfa hay. In this study it was found that when the experimental diets contained phosphorus at a minimal level of adequacy and all other nutrients were at optimal levels, (the same for all diets), the phosphorus of a low-phosphorus lespedeza or alfalfa hay was much less available to the rat for growth and bone development than was the phosphorus of a high phosphorus hay. These workers also reported (1942) similar results with red clover hay. Their work indicated that the ratio of iron and aluminum to phosphorus in the hays was the disturbing factor in the utilization of phosphorus. Insoluble phosphates of iron and aluminum were found in the hay which decreased the amount of total phosphorus available to the animal.

Screenivasan (1939) studied the differences in availability of phosphorus in two varieties of rice and found that rice containing 1.12 per cent P_2O_5 promoted greater growth than a rice containing 0.61 per cent P_2O_5.

Cox and associates (1931) fed guinea pigs a ration containing soluble aluminum salts in excess of the total phosphorus of the diet, and obtained a marked lowering of the inorganic phosphorus of the blood to a level of 15 per cent of normal. The ash, calcium and phosphorus contents of the bones were reduced to 70 per cent of the normal in a 10 week period. Ferric salts produced a similar but less marked effect. The addition of monosodium phosphate equivalent to the aluminum or iron content of the diet entirely prevented the occurrence of the symptoms of aphosphorosis. These workers fed rabbits the same ration and observed that the inorganic blood phosphorus dropped rapidly when the iron or the aluminum content of the diet exceeded its phosphorus content. Within 9 days after the beginning of this work, it was noted that the excretion of phosphorus in the urine practically ceased. These observers postulated that the inability of experimental animals in this study to utilize phosphorus was due to the precipitation of the alimentary phosphorus as ferric and aluminum phosphates.

Street (1942) fed rats a purified diet containing various levels of phosphorus, aluminum sulfate and aluminum hydroxide. He concluded that when a soluble form of aluminum such as aluminum sulfate was fed to rats in an amount equal to the phosphorus content of the ration (equimolar), there was almost complete precipitation of the phosphorus in the intestinal tract. This conclusion was based on growth rates and inorganic phosphorus levels in the blood plasma. Aluminum sulfate was more effective in precipitating the phosphorus than aluminum hydroxide.

Krieger and Steenbock (1940) in a study of the effect of calcium and vitamin D on the availability of phosphorus, found in feeding experiments with rats that the availability of phytic acid phosphorus was markedly affected by the calcium and vitamin D content of the diet. With an optimal intake of phosphorus and a calcium-phosphorus ratio of 1:1, phytic acid phosphorus was almost as readily utilized as the inorganic form. However, when the calcium-phosphorus ratio was increased to 2:1, the availability of phytic acid phosphorus was markedly decreased. Vitamin D was found to improve the utilization of both the organic and inorganic forms of phosphorus. In the presence of vitamin D, phytic acid phosphorus was not as readily available as the inorganic forms.

The effect of citrates upon the availability of phosphorus has been a topic of considerable study. Langford (1942) found that the administration of a mixture of citric acid and potassium citrates in approximately the proportions and quantities which would be supplied with 10 c.c. of fresh orange juice daily increased the rate of growth and the total calcium retention of rats on a wheat and milk diet. Day and McCullum (1939) on the other hand, observed that when rats were fed a purified ration high in calcium and low in phosphorus, the feeding of citrates did not promote calcification. Neither was it effective when the diet contained moderate levels of calcium or phosphorus, or low calcium and high phosphorus, irrespective of whether a cereal ration or a purified ration was used. When a purified ration containing phytin was fed to rats, citrates caused an increase in calcification when the calcium:phosphorus ratio was high, but there was no effect when the calcium:phosphorus ratio was about 2:1.

Hathaway and Meyer (1939) fed rats a rachitogenic diet and added lactic acid and citric acid or the salts of these acids to the diet. Each of these acids increased the average bone ash values. Chaney and Blunt (1925) found that both calcium and phosphorus retention was increased when orange juice was fed to growing girls. More than three times as much phosphorus was assimilated when orange juice was added to the diet than when none was added.

Jones (1942) used the rat to study the relation of pH of the intestinal contents to calcium and phosphorus utilization. This worker found that when given in addition to a rachitogenic diet, lard, oleic acid and vitamin D all increased the acidity of the lower portion of the ileum and less consistently increased the acidity of the cecum and colon. The same substances also showed definite antirachitogenic action, which was much greater in the case of vitamin D than either lard or oleic acid. The addition of aluminum sulfate to a stock diet resulted in severe rickets and a definite increase in the pH throughout the greater part of the intestinal tract of the rat.

Shohl (1937) demonstrated with rats that the feeding of acid producing salts such as NH_4Cl intensified rickets, and had a definite but limited effect on production of rickets. When citric acid-sodium citrate mixtures were added to rachitogenic diets they no longer produced rickets in rats.

Bunkfelt and Steenbock (1943) found that when cottonseed oil was included in a low-phosphorus, cereal-free, rachitogenic ration for rats, calcification was uniformly decreased in proportion to the amount of cottonseed oil fed. When adequate phosphorus was provided, however, the feeding of cottonseed oil resulted in increased calcification above that without cottonseed oil.

From this review, the major factors which affect phosphorus utilization include (1) the Ca:P ratio, (2) vitamin D, (3) the chemical nature of the phosphorus containing constituents in feedstuffs, (4) the pH of the intestinal contents and (5) the presence or absence of certain soluble salts in feedstuffs.

EXPERIMENTAL OBJECTIVES

This experiment was designed to study the relative utilization of phosphorus in prairie hays produced in two different areas of Oklahoma.

EXPERIMENTAL PROCEDURE

A. Experimental Rations

Trials 1, 2, 3 and 4.

Rations were formulated in these trials to test the availability of calcium and phosphorus in three prairie hays. One hay was produced on an unfertilized plot near Wilburton, (Ration 1); another was produced near Stillwater on soil which had received 200 pounds of superphosphate per acre, (Ration 2); and a third one was produced on an unfertilized plot near Stillwater, (Ration 3). Analyses of the soil of the two general areas appear in Table 1. Proximate analyses of the feeds used in this study appear in Table 2.

The hays were incorporated in maintenance rations. The content of phosphorus, protein and energy was approximately equalized among the rations. About 50 per cent of the total phosphorus in each ration was supplied by the hay. This required adjustments in the amount of hay in each ration. The other ration ingredients were dried molasses beet pulp, corn gluten meal, starch, salt and wood cellulose.[1] The wood cellulose was added for bulk in rations 2 and 3, which contained the smaller amounts of hay. The Ca:P ratios for the rations in this experiment were approximately 6.3:1, 3.6:1 and 4.8:1 for lots 1, 2 and 3 respectively. No attempt was made in these trials to equalize the calcium intake among lots. The phosphorus intake per 100 pounds of body weight was about 0.80 grams. The phosphorus intake was kept at a

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[1]Solka-Floc, purchased from The Brown Company, Berlin, New Hampshire.

low level, because it was thought that if differences in phosphorus availability did exist between Stillwater and Wilburton hays, these differences might be more obvious at a low level of phosphorus intake. The composition of the daily rations is shown in Table 4.

Trials 5 and 6.

The rations used in these trials were the same as those in trials 1, 2, 3, 4 with calcium equalized by the addition of $CaCO_3$. Inasmuch as calcium and phosphorus are closely related in many metabolic processes, it was thought that further study should be made with rations in which both the amounts of calcium and phosphorus were equalized in all rations. In trials 5 and 6, sufficient calcium was added as $CaCO_3$ (Merck's Reagent grade) to make the total intake of calcium nearly equal in all lots. The Ca:P ratio was approximately 6.3:1 in these trials.

Trials 7 and 8.

Ration 2 containing the prairie hay from the fertilized plot near Stillwater was not fed in these trials. Rations 1 and 3 were compounded similar to those of previous trials except that about 1,000 I.U. and 300 I.U. of vitamins A and D, respectively, per 100 pounds of body weight, were added in the form of cod liver oil. The composition of feeds fed in these trials is presented in Table 5. New supplies of hay were used and it will be noted that the Wilburton prairie hay was slightly higher in phosphorus than the Stillwater hay. The composition of the rations is presented in Table 6.

Trials 9 and 10.

Steers were used as experimental animals in these trials. The rations were the same as those used in trials 7 and 8 except that for reasons of palatability, the cellulose was omitted from the Wilburton hay ration. Also, different corn gluten meal having the following percentage composition was used: dry matter, 93.54; protein, 46.22; calcium, 0.18; and phosphorus, 0.33. The composition of rations is presented in Table 8.

Trial 11.

It was felt that inasmuch as the data obtained in trials 1-10 with normal lambs did not show great differences in the utilization of phosphorus contained in Wilburton and Stillwater prairie hays, perhaps a further refinement in technique was necessary. This trial was designed to simulate the conditions of beef cattle in the Wilburton area by conducting balance trials with lambs low in body reserves of phosphorus.

To deplete the lambs in phosphorus preceding the balance trial, a ration was fed which was composed of corn gluten meal, dried beet pulp, cottonseed hulls and a vitamin A and D supplement of high potency cod liver oil. This ration was fed for a period of seven months. At the end of this period, the lambs were divided into two groups. One group of lambs was fed the Wilburton hay which contained 0.052 per cent phosphorus, and the other group the Stillwater hay which contained 0.058 per cent phosphorus. The remainder of the ration consisted of corn gluten meal, dried beet pulp, cod liver oil and sufficient calcium carbonate to equalize calcium intake. The composition of the ration is shown by Table 7. This ration with a Ca:P ratio of 3.8:1 supplied 1.16 grams of phosphorus per 100 pounds of live weight. Approximately fifty per cent of the phosphorus in each ration was supplied by the hay. The rations were nearly equal in protein, phosphorus, energy and bulk.

B. Experimental Animals

Trials 1, 2, 3 and 4.

Western feeder wether lambs weighing between 60 and 75 pounds were used in these trials. Two weeks prior to being placed on their respective rations, the lambs were drenched with a phenothiazine preparation in order to minimize the effects of parasitic infestation upon the physiological response of the lambs. The lambs were weighed on three consecutive days and eight lambs were randomly assigned to each treatment by weight. Blood samples were taken at intervals throughout these trials for the determination of calcium and phosphorus.

Trials 5 and 6.

Western type wether lambs weighing from 65 to 70 pounds were used in these trials. The details described in the previous trial were followed here except that blood samples were not taken for analysis.

Trials 7 and 8.

The animals which were used in the previous trials (5 and 6) were used during these trials and the same details were followed in reference to the care of the animals.

Trials 9 and 10.

Four yearling grade Hereford steers weighing 600 to 630 pounds were used in these trials in an effort to determine if there is a difference in the way in which steers metabolize phosphorus from Stillwater and Wilburton prairie hays.

Trial 11.

Western wether lambs weighing between 70 and 80 pounds were used in this study. These lambs were placed on the low phosphorus ration at about six months of age and were about thirteen months of age at the start of the balance study. As is indicated for previous trials, the lambs were treated for internal parasitism in order to diminish its possible effect upon the physiological response of the lambs. Assignment to treatment was at random in pairs of lambs near the same weight.

C. Procedure During Metabolism Periods

Trials 1, 2, 3 and 4.

The term "triad", as used in this writing, applies to three lambs of approximately the same weight, each fed a ration in which about fifty per cent of the phosphorus was supplied by one of the different prairie hays under investigation. To keep lambs within each triad on the same level of phosphorus intake, the amounts of rations fed were adjusted in each triad to the intake of the lamb eating the least.

Previous to collection periods the lambs were fed their respective rations at the barn in individual stanchions, and when not eating, were allowed the freedom of a large box stall. As soon as all lambs were on feed, two triads were placed in metabolism stalls at the Animal Husbandry Arena. The metabolism stalls were similar to those described by Briggs and Gallup (1949). The lambs were fed only that amount of feed which they would clean up in a reasonable length of time. Feed refusals were left in the feed box for consumption during the following feeding period. The metabolism period consisted of a preliminary period of ten days and a collection period of twenty days, feces and urine being collected during the latter period. At the conclusion of the collection period, the two triads were returned to the barn and two more triads were placed in the metabolism stalls. Lambs at the barn were continued on their respective rations and individually fed until all twenty-four lambs had been on collection.

Feces and urine were collected in the usual manner once daily, measured and aliquoted for analysis. The samples were acidified with HCl and kept in tightly sealed jars under refrigeration at about 5° C. At the close of the trials, the lambs were slaughtered in an Oklahoma City packinghouse and samples of bone taken for photographing and ash determinations. A one-inch section near the center of the front cannon bone was taken from the carcass of each lamb. The bones were cleaned of tendon and lean tissue and extracted in hot anhydrous ether for 48 hours. The samples were dried in an oven at 100° C for 48 hours and ashed in a muffle furnace at 450° C.

Trials 5 and 6.

The triad feeding technique described for the first four trials was continued in the same manner in these trials. Other details of the metabolism periods were the same as those for earlier trials.

Trials 7 and 8.

Inasmuch as ration 2 was discontinued at the close of trial 6, the triad feeding technique was also discontinued and a paired feeding technique substituted. Further, the collection period was divided into two ten-day periods. At the conclusion of trial 7, lambs within pairs were reversed and placed on the ration containing the other test hay. Details of the collections were the same as for earlier trials. Blood and bone samples were not taken for analysis.

Trials 9 and 10.

As soon as the steers were on feed, they were placed in metabolism stalls similar to those used for lambs which provided for the separate collection of urine and feces. Rubber funnels were used for the collection of urine. These trials were of 30 days duration, one ten-day preliminary preceding two successive ten-day collection periods. Urine and feces were measured and aliquoted once daily, and representative samples tightly sealed in jars and stored at about 5° C. At the end of two ten-day collection periods, the steers were rotated and placed on the ration containing the other test hay. Two steers in trial 9-A went off feed and the trial was discontinued.

Trial 11.

Balance trials were conducted with depleted lambs involving a ten-day preliminary period followed by a twenty-day collection period. Three lambs were fed each test ration and the trial was concluded at the end of the twenty-day collection period. Details of procedure for the metabolism periods were the same as those for earlier trials.

D. Chemical Analysis of Feeds, Blood and Excreta

Feeds were analyzed for calcium, phosphorus and other constituents according to procedure described by the A.O.A.C. (1945). Feces and urine were analyzed for calcium and phosphorus by similar procedures as modified in the Department of Agricultural Chemistry Research.

Blood plasma calcium determinations were made according to the method of Clark and Collip (1925). Blood plasma phosphorus determinations were made according to the method of Fiske and Subbarow (1925).

RESULTS AND DISCUSSION

Trials 1, 2, 3 and 4.

The chemical analyses of hays show that on a dry matter basis the percentage of calcium was 0.65, 0.67, 0.56 and the phosphorus 0.056, 0.113, 0.066 for the Wilburton, Stillwater fertilized and Stillwater unfertilized hays, respectively.

The phosphorus and calcium intake of the lambs on the different rations are reported in Table 9. The average intakes of phosphorus in grams for lambs fed rations 1, 2 and 3 were 15.57, 16.78 and 15.68, respectively. Table 16 presents a summary of these data. In every case, the total phosphorus intake for the collection period was greatest for those lambs fed rations containing the fertilized Stillwater hay. This higher phosphorus intake is accounted for by the phosphorus present in the starch and cellulose included in this ration. By the same token, the total phosphorus intakes for lambs of lot 3 was somewhat higher than that of lot 1.

The average phosphorus retention in grams for the twenty-day collection period was -0.54, +2.49 and -0.09 for lots 1, 2 and 3, respectively, as presented in Table 16. The negative phosphorus balances which occurred during trial 4 are difficult to explain, aside from the fact that the lambs were nearly six months older and their maintenance phosphorus requirements were possibly higher than those at the time trial 1 was initiated. Practically all of the phosphorus excreted was present in the feces. In trial 4 the combination feces and urine samples were found to contain 0.223, 0.328, 0.260, 0.333 and 0.275 per cent phosphorus on dry matter basis for lambs 1 through 6, respectively, while the feces analyzed alone contained

0.213, 0.319, 0.244, 0.220, 0.334 and 0.266 per cent phosphorus on dry matter basis. The retention of phosphorus by lambs fed prairie hay produced on soil fertilized with superphosphate was significantly greater than that by lambs fed similar hay produced on unfertilized soil. When subjected to an analysis of variance as outlined by Snedecor (1948) the mean differences were significant at the five per cent level of probability. Differences in phosphorus retention by lambs fed the unfertilized Stillwater and Wilburton hays were small; however, the trend was for slightly higher retention values for lambs fed the Stillwater hay.

The average calcium retention in grams for the twenty-day collection period was -6.04, +2.32 and -9.04 for lots 1, 2 and 3 respectively. No explanation for the rather large negative calcium balances for lots 1 and 3 or the positive calcium balance for lot 2 is offered.

Blood plasma phosphorus and calcium levels at various intervals throughout trials 1, 2, 3 and 4 are presented in Table 10. The average plasma levels of phosphorus were within normal limits in all cases except for lot 1 when bled on March 3rd, March 25th and May 25th. Beginning with the January 11th bleeding, it is noteworthy that lot 2 had the highest average plasma phosphorus level throughout the remainder of the experiment. Further, in all cases except the June 27th bleeding, the lambs in lot 3 had consistently higher average plasma phosphorus levels than those in lot 1. This would tend to indicate that the lambs were making the most efficient use of the phosphorus from the fertilized hay and that under the conditions of this experiment, blood plasma phosphorus levels might suggest that lambs fed Stillwater hay made more efficient use of the hay phosphorus than did those fed Wilburton hay.

Photographs of the bone samples were made and accompany this discussion. (Figures 1 and 2) It appears quite obvious upon examination of the photographs that lambs in lot 2 fed the fertilized hay ration had bones with thicker, heavier walls than those of either lots 1 or 3. There seem to be no great differences between the bones of lots 1 and 3.

The percentage of ash is presented in Table 11. Bone ash determinations were very uniform both within and between lots. Average percentage ash in bone sections from lots 1, 2 and 3 were 67.08, 67.39 and 67.26 respectively.

Trials 5 and 6.

Phosphorus and calcium retention data for lambs in these trials are presented in Table 12. It will be noted that the average phosphorus retention values were positive only for lambs fed the fertilized Stillwater hay ration. The average phosphorus retention in grams for the twenty-day collection period was -1.76, +0.73 and -.58 for lots 1, 2 and 3 respectively. As in previous trials, negative calcium balances occurred, the averages being -10.21, -3.67 and -12.18 for lots 1, 2 and 3 respectively. As was also true in previous trials, the retention of phosphorus by lambs fed prairie hay produced on soil fertilized with superphosphate was greater than that by lambs fed similar hay produced on unfertilized soil at both Wilburton and Stillwater. For this reason, the results of the six trials were pooled. The pooled retention results of the six trials revealed statistically significant differences between phosphorus retention values for lambs fed prairie hay grown on fertilized and unfertilized soil. The following is the analysis of variance table for these pooled retention results:

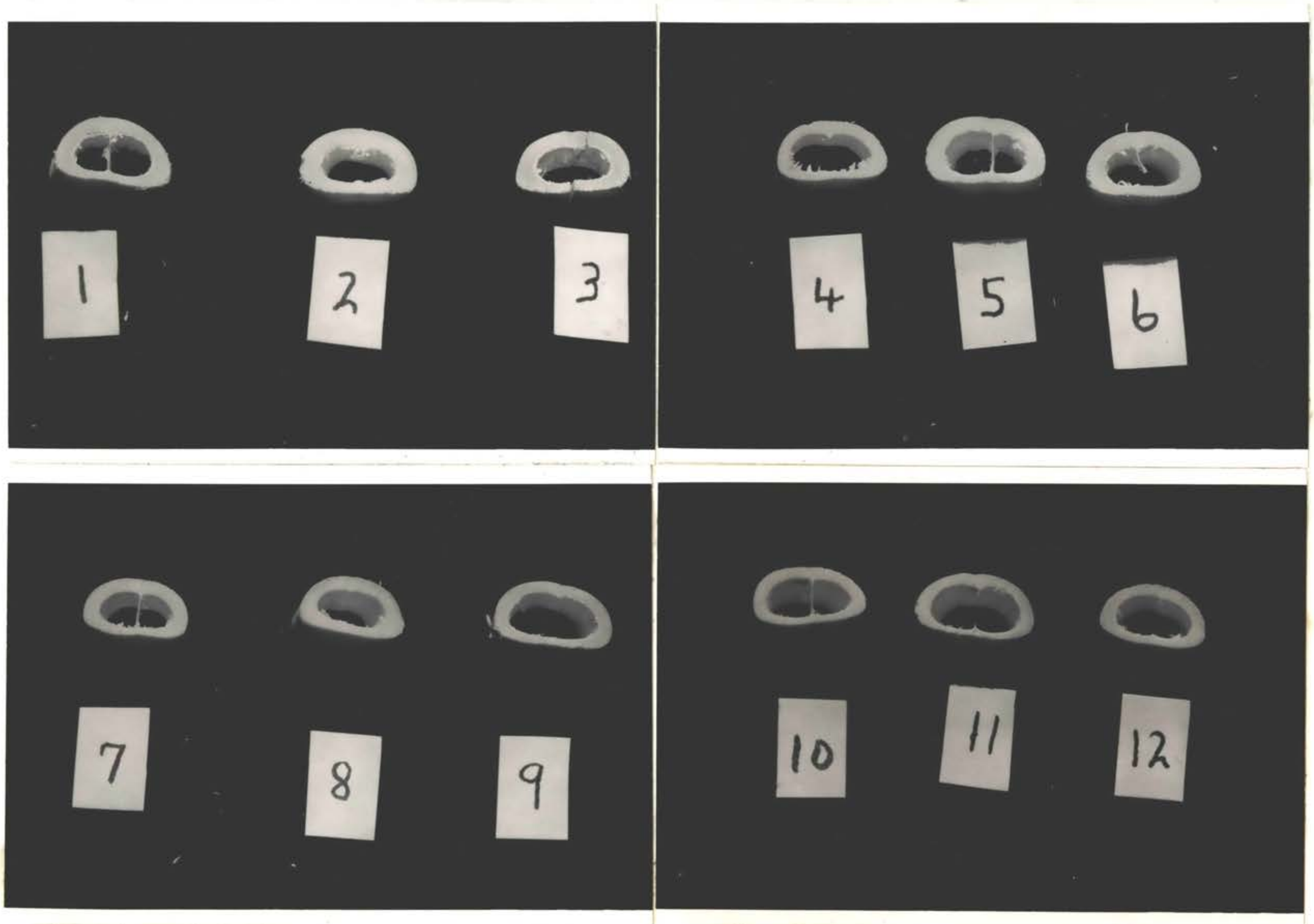

Figure 1. Sections of cannon bones from lambs in trials 1, 2, 3 and 4. Bones 1-4-7-10 from lambs fed Wilburton hay; 2-5-8-11 from lambs fed fertilized Stillwater hay; 3-6-9-12 from lambs fed unfertilized Stillwater hay.

Source	D.F.	S.S.	M.S.
Total	17	576.98	
Exp.	5	444.48	
Ration	2	107.83	53.91
Ration x Exp.	10	24.67	2.47

$F = 21.8^{**}$

Trials 7 and 8.

The phosphorus and calcium retention data for lambs in trials 7 and 8 are presented in Table 13. It appears that vitamin A and D supplementation had a pronounced effect upon calcium retention. Contrary to findings in earlier trials, calcium balances were positive with average calcium retentions in ten-day collection periods of +6.33 and +4.57 grams for lambs fed unfertilized Wilburton and Stillwater hay rations, respectively. Phosphorus retention values, on the other hand, were slightly negative with -0.63 and -0.48 grams phosphorus balance in a ten-day collection period for lambs fed rations of Wilburton and Stillwater hay, respectively.

Trials 9 and 10.

The phosphorus and calcium retention data for steers in trials 9 and 10 are presented in Table 14. These data are characterized by a greater predominance of positive phosphorus and calcium balances than was true with lambs in trials 7 and 8. The average phosphorus and calcium retention in grams during the ten-day collection periods for steers in these trials were as follows:

Trial No.	No. of Steers	Hay Ration	Average P Intake (gms.)	P Retained (gms.)	Average Ca Intake (gms.)	Ca Retained (gms.)
9-B	2	Wilburton	32.50	+1.90	186.8	+58.57
9-B	2	Stillwater	32.50	+4.77	186.8	+45.34
10-A	2	Wilburton	44.64	-0.67	233.5	+50.61
10-A	2	Stillwater	44.64	+6.21	233.5	+56.84
10-B	2	Wilburton	46.52	-5.86	233.5	+31.13
10-B	2	Stillwater	46.52	+0.82	233.5	+41.72

The mean phosphorus retention in grams was -1.53 and +3.93 for steers fed the Wilburton and Stillwater hay rations, respectively. Although the numbers of animals involved in these trials were too small and the variation in phosphorus retentions too great to reveal statistically significant differences, it would appear from these data that these steers utilized the phosphorus from the Stillwater hay more efficiently than that of the Wilburton hay. Relatively small differences were observed in average calcium balances between lots. Blood plasma calcium and phosphorus levels for these steers were within what is considered as normal limits. The blood plasma calcium and phosphorus values expressed as milligrams per cent for steers at the completion of the experiment were:

Steer No.	Calcium	Phosphorus
1	11.55	5.60
2	12.18	6.38
3	9.16	4.80
4	10.55	6.04

Trial 11.

Daily phosphorus balances for lambs in this trial are presented in Table 15. In a comparison of the daily amounts of phosphorus retained by lambs, it can be seen that in every case there was a slight advantage for the Stillwater hay ration. This advantage was small, however, and the average daily phosphorus retention in grams was +0.09 and +0.11 for lambs fed Wilburton and Stillwater hays, respectively. The average daily phosphorus intake was 0.72 grams for lambs fed the Wilburton hay ration and 0.72 grams for lambs fed the Stillwater hay ration. Thus, again only a trend in favor of the Stillwater hay is established with respect to phosphorus utilization by lambs.

SUMMARY

Trials were conducted with lambs and steers to determine the relative retention of phosphorus in prairie hays grown on soils of different phosphorus content.

The retention of phosphorus by lambs fed prairie hay produced on soil fertilized with superphosphate was greater than that by lambs fed similar hay produced on unfertilized soil. The difference was statistically significant. Although statistically significant differences in phosphorus retention were not obtained when the hays from untreated soils were compared, phosphorus retention was slightly greater for those lambs fed prairie hay grown on soil higher in available phosphorus. There was no difference in the per cent ash in bones from lambs fed the three test hays. From a gross examination, however, it appeared that the bones from lambs fed the fertilized hay had thicker and more dense walls than those from lambs fed the unfertilized hays. There were no obvious differences in the physical structure of the bones from lambs fed Wilburton and Stillwater unfertilized hays.

The addition of a vitamin A and D supplement to rations in trials 7 and 8 appeared to have a pronounced effect upon calcium retention. Vitamin A and D supplementation increased the utilization of calcium in all rations as indicated by much higher calcium retention values than were obtained in trials 5 and 6. As in previous trials, phosphorus retention was slightly greater by those lambs fed the unfertilized hay from Stillwater than by those fed the unfertilized hay from Wilburton.

Hereford steers were fed Stillwater and Wilburton hay rations to determine comparative differences in the hays in phosphorus availability. It appears from the results with steers, that the phosphorus from the Stillwater hay was utilized more efficiently than that from the Wilburton hay.

In trial 11, lambs which had been on a low phosphorus diet were fed rations of Stillwater and Wilburton hay. It was found that under these conditions the lambs receiving the Stillwater hay stored slightly more phosphorus than those receiving the Wilburton hay.

Table 1

Soil Analyses

Description	Soil Type	Soil Reaction	Organic Matter %	Available Phosphorus ppm.	Available Potash ppm.
Wilburton (Southeast Okla.)					
Depth 0"-8"	gray very fine sandy loam	strongly acid	2.9	1+	56.2
8"-12"	gray very fine sandy loam	strongly acid	2.0	1-	83.4
Stillwater (North Central Okla.) No fertilizer treatment					
Depth 0"-8"	brown sandy loam	moderately acid	3.5	2+	128.8
8"-12"	brown sandy loam	moderately acid	2.7	2	96.0

Table 2

Proximate Analyses of Feeds

	Dry Matter %	Composition of Dry Matter							
		Ash %	Protein %	Ether Extract %	Crude Fiber %	N.F.E. %	Ca %	P %	Lignin %
Wilburton*	92.11	6.16	4.24	2.44	31.75	55.41	0.651	0.056	9.33
Stillwater**	92.45	7.04	6.79	3.02	29.70	53.45	0.670	0.113	9.94
Stillwater†	92.43	6.65	6.52	2.62	30.51	53.70	0.562	0.066	13.20
Corn Gluten Meal	91.72	2.52	47.15	2.75	2.80	44.78	0.188	0.395	
Molasses Beet Pulp	93.06	6.52	9.50	0.77	13.79	69.42	0.457	0.056	
Corn Starch	87.62	--	--	--	--	--	0.002	0.022	
Cellulose	92.17	--	--	--	--	--	0.0002	0.0058	
Salt	99.59	--	--	--	--	--	0.092	0.0064	

*Wilburton hay from unfertilized land.

**Stillwater hay from land fertilized with 200 lbs. of superphosphate per acre.

†Stillwater hay from unfertilized land.

Table 3

Average Daily Ration Fed Lambs in Trials 1, 2, 3, 4.

Trial No.	Lot No.	Lamb No.	Corn Gluten Meal	Dried Molasses Beet Pulp	Hay[1] No.1	Hay[2] No.2	Hay[3] No.3	Starch	Cellulose	Salt
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	1[1]	16	95.30	152.50	686.25					18.0
1	2[2]	17	95.30	152.50		367.7		114.37	76.02	18.0
1	3[3]	18	95.30	152.50			582.17	38.12	38.12	18.0
1	1	19	97.18	155.50	699.50					18.0
1	2	20	97.18	155.50		374.94		116.64	77.75	18.0
1	3	21	97.18	155.50			593.62	38.87	38.87	18.0
2	1	1	97.50	156.00	702.00					18.0
2	2	2	97.50	156.00		376.16		119.00	78.00	18.0
2	3	3	97.50	156.00			595.56	39.00	39.00	18.0
2	1	22	95.00	152.00	684.00					18.0
2	2	23	95.00	152.00		366.51		116.00	76.00	18.0
2	3	24	95.00	152.00			580.29	38.00	38.00	18.0
3	1	10	106.90	171.50	771.75					18.0
3	2	11	106.90	171.50		411.60		128.05	88.00	18.0
3	3	12	106.90	171.50			654.85	42.30	42.30	18.0
3	1	13	92.15	148.50	668.25					18.0
3	2	14	92.15	148.50		356.40		110.90	72.25	18.0
3	3	15	92.15	148.50			566.35	36.65	36.65	18.0
4	1	1	89.85	145.50	654.75					18.0
4	2	2	89.85	145.50		349.20		108.40	72.75	18.0
4	3	3	89.85	145.50			554.90	35.65	35.65	18.0
4	1	4	85.25	138.50	623.25					18.0
4	2	5	85.25	138.50		332.60		103.10	69.25	18.0
4	3	6	85.25	138.50			528.30	38.85	38.85	18.0

[1] Wilburton Hay.

[2] Fertilized Stillwater Hay.

[3] Unfertilized Stillwater Hay.

Table 4

Composition of Rations — Trials 1, 2, 3, 4.

Lot No.	Feed	Amount	Protein	Estimated Net Energy	Ca	P
		gm.	gm.	therms	gm.	gm.
1	Corn gluten meal	125	58.93		.230	.487
	Molasses beet pulp	200	19.00		.926	.114
	Wilburton hay	900	38.16	.744	5.859	.504
	Salt	18				
	Total	1243	116.09	.744	7.015	1.105
2	Corn gluten meal	125	58.93		.230	.487
	Molasses beet pulp	200	19.00		.926	.114
	No. 2 Hay (Stillwater Fertilized)	482.3	32.74	.391	3.231	.504
	Starch	150.0	--	.325	--	--
	Cellulose	100.0	--	.022	--	--
	Salt	18				
	Total	1075.3	110.67	.738	4.387	1.105
3	Corn gluten meal	125.0	58.93		.230	.487
	Molasses beet pulp	200.0	19.00		.926	.114
	No. 3 Hay (Stillwater Unfertilized)	763.6	49.78	.625	4.291	.504
	Starch	50.0	--	.108	--	--
	Cellulose	50.0	--	.011	--	--
	Salt	18				
	Total	1206.6	127.71	.744	5.447	1.105

Table 5

Proximate Analyses of Feeds -- Trials 5 and 6.

Feed	Dry Matter	Composition of Dry Matter Protein	Ca	P
	%	%	%	%
Wilburton Hay*	93.62	4.95	0.69	0.60
Stillwater Hay**	94.11	7.91	0.57	0.83
Stillwater Hay†	94.00	5.41	0.40	0.52
Corn gluten meal	93.22	44.79	0.12	0.86
Dried molasses beet pulp	92.55	7.61	0.81	0.08

*Wilburton hay from unfertilized land.

**Stillwater hay from land fertilized with 200 lbs. of superphosphate per acre.

†Stillwater hay from unfertilized land.

Table 6

Composition of Rations -- Trials 7 and 8.

Feed	Amount	Protein	Estimated Net Energy	Ca	P
	gm.	gm.	therms	gm.	gm.
Ration 1					
Corn gluten meal	136	58.39		0.234	0.4200
Dried molasses beet pulp	120	8.45		0.930	0.0900
Wilburton hay	540	25.01	0.447	3.510	0.3024
Starch	24	---	0.052	---	---
Cellulose	60	---	0.013	---	---
Salt	18	---	---	---	---
Total	898	91.85	0.512	4.674	0.8124
Ration 3					
Corn gluten meal	136.00	58.39		0.234	0.4200
Dried molasses beet pulp	120.00	8.45		0.930	0.0900
Stillwater hay	616.00	31.35	0.506	2.346	0.3024
Calcium carbonate	2.91	---	---	1.164	---
Salt	18.00	---	---	---	---
Total	892.91	98.19	0.506	4.674	0.8124

Table 7

Composition of Rations -- Trial 11.

Feed	Amount	Protein	Ca	P
	gm.	gm.	gm.	gm.
Ration 1				
Corn gluten meal	105	47.02	0.126	0.556
Dried beet pulp	160	12.16	1.296	0.090
Wilburton hay	840	41.58	2.688	0.436
Salt	10	----	----	----
Total	1115	100.76	4.110	1.082
Ration 3				
Corn gluten meal	105	47.02	0.126	0.556
Dried beet pulp	160	12.16	1.296	0.090
Stillwater hay	752.8	40.72	2.408	0.436
$CaCO_3$	0.9	----	.360	----
Salt	10	----	----	----
Total	1028.7	99.90	4.190	1.082

Cod liver oil to supply about 300 I.U. of vitamin D and 1000 I.U. of vitamin A per 100 pounds of body weight were added to each ration.

Table 8

Composition of Rations Fed to Steers -- Trials 9 and 10.

Feed	Amount	Protein	Estimated Net Energy	Ca	P
	gm.	gm.	therms	gm.	gm.
Wilburton hay ration:	(Ration 1)				
Corn gluten meal	540	233.54		0.92	1.68
Molasses beet pulp	480	33.80		3.72	.36
Wilburton hay	2160	100.00	1.786	14.04	1.21
Starch	96	---	.206	---	---
Salt	70				
Total	3296	367.34	1.992	18.68	3.25

Cod liver oil to supply about 300 I.U. of vitamin D and 1000 I.U. of vitamin A per 100 pounds of body weight.

Feed	Amount	Protein	Estimated Net Energy	Ca	P
Stillwater hay ration:	(Ration 2)				
Corn gluten meal	540	233.54		0.92	1.68
Molasses beet pulp	480	33.80		3.72	.36
Stillwater hay	2468	125.40	2.026	9.38	1.21
$CaCO_3$	11.64			4.66	
Salt	70				
Total	3569.64	392.74	2.026	18.68	3.25

Cod liver oil to supply about 300 I.U. of vitamin D and 1000 I.U. of vitamin A per 100 pounds of body weight.

Table 9

Phosphorus and Calcium Retention Data -- Trials 1, 2, 3, 4.

Trial No.	Ration No.	Lamb No.	Total P Intake	Total P Excreted	Total P Retained	Daily P Retention	Total Ca Intake	Total Ca Excreted	Total Ca Retained	Daily Ca Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	1*	16	15.61	12.72	2.89	.14	98.84	91.87	6.97	.35
"	2**	17	16.73	13.22	3.51	.18	62.13	56.11	6.02	.30
"	3***	18	15.82	13.49	2.33	.12	77.03	69.73	7.30	.37
"	1	19	15.91	14.22	1.69	.08	100.79	94.49	6.30	.32
"	2	20	17.80	11.71	6.09	.30	63.36	50.94	12.42	.62
"	3	21	15.31	14.17	1.14	.05	71.60	73.45	-1.85	-.09
2	1	1	15.96	15.37	.59	.03	101.11	102.30	-1.19	-.06
"	2	2	17.12	14.79	2.33	.12	63.56	60.54	3.02	.15
"	3	3	16.18	14.73	1.45	.07	78.80	91.07	-12.27	-.61
"	1	22	15.57	14.34	1.23	.06	98.52	103.12	-4.60	-.23
"	2	23	16.70	12.81	3.89	.19	61.93	60.09	1.84	.09
"	3	24	15.78	15.29	.49	.02	76.79	82.06	-5.27	-.26
3	1	10	17.53	16.22	1.31	.07	110.10	124.74	-14.64	-.73
"	2	11	18.77	16.37	2.40	.12	69.58	70.17	-.59	-.03
"	3	12	17.76	15.84	1.92	.10	86.59	91.93	-5.34	-.27
"	1	13	15.15	16.60	-1.45	-.07	96.24	118.22	-21.98	-1.10
"	2	14	16.22	13.35	2.87	.14	60.29	62.68	-2.39	-.12
"	3	15	15.35	14.37	.98	.05	74.95	80.94	-5.99	-.30
4	1	1	14.81	18.50	-3.69	-.18	94.29	99.54	-5.25	-.26
"	2	2	15.86	15.10	.76	.04	59.07	59.36	-.29	-.01
"	3	3	15.01	17.50	-2.49	-.12	73.43	100.32	-26.89	-1.34
"	1	4	14.09	20.90	-6.81	-.34	89.46	103.39	-13.93	-.70
"	2	5	15.08	16.99	-1.91	-.10	56.25	57.71	-1.46	-.07
"	3	6	14.27	20.83	-6.56	-.33	69.88	91.82	-21.94	-1.10

*Lot 1 - Unfertilized Wilburton Hay
**Lot 2 - Fertilized Stillwater Hay
***Lot 3 - Unfertilized Stillwater Hay

Table 10

Average Blood Plasma Phosphorus and Calcium Levels -- Trials 1, 2, 3, 4.

Lot No.	Nov. 6	Dec. 12	Jan. 11	Feb. 11	Mar. 3	Mar. 25	Apr. 25	May 25	June 27
				(mg. phosphorus per 100 ml. of plasma)					
1	4.84	3.55	4.64	3.48	2.74	2.78	3.52	2.78	4.07
2	4.70	4.70	5.53	5.18	3.28	3.92	5.74	3.93	5.02
3	5.08	4.78	4.64	3.94	2.93	3.06	4.77	3.07	3.79
				(mg. calcium per 100 ml. of plasma)					
1	12.21	10.68	--	11.43	11.44	11.19	11.30	11.30	11.67
2	11.19	11.54	--	11.12	11.09	11.32	11.40	11.40	11.39
3	11.80	10.59	--	11.68	11.50	11.57	11.65	11.65	11.97

Table 11

Bone Ash Data (Ether Extracted Sections) -- Trials 1, 2, 3, 4.

Lot 1 Lamb Bone No.	Ash	Lot 2 Lamb Bone No.	Ash	Lot 3 Lamb Bone No.	Ash
	%		%		%
1	65.99	2	68.57	3	66.52
4	66.88	5	69.02	6	67.94
7	67.55	8	67.87	9	67.17
10	67.88	11	69.34	12	67.48
13	68.42	14	67.03	15	67.15
16	66.50	17	69.06	18	66.57
19	66.12	20	64.98	21	67.33
22	67.31	23	67.28	24	67.94
Average	67.08	Average	67.89	Average	67.26

Table 12

Phosphorus and Calcium Retention Data -- Trials 5 and 6.

Trial No.	Ration No.	Lamb No.	Total P Intake	Total P Excreted	Total P Retained	Daily P Retention	Total Ca Intake	Total Ca Excreted	Total Ca Retained	Daily Ca Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
5	1*	1	16.36	16.66	-.30	-.015	103.73	116.60	-12.87	-.644
"	2**	2	17.51	14.47	3.04	.152	107.05	105.59	1.46	.073
"	3***	3	16.57	17.25	-.68	-.034	105.86	134.14	-28.28	-1.414
"	1	4	16.37	17.04	-.67	-.034	103.74	118.48	-14.74	-.737
"	2	5	17.51	15.82	1.69	.084	107.05	109.95	-2.90	-.145
"	3	6	16.57	16.35	.22	.011	105.86	127.02	-21.15	-1.048
6	1	7	11.82	12.84	-1.02	-.051	87.50	83.27	-4.23	.211
"	2	8	12.11	11.71	.40	.020	83.81	91.50	-7.69	-.384
"	3	9	11.67	13.07	-1.40	-.070	83.51	85.06	-1.55	-.077
"	1	10	10.92	15.98	-5.06	-.253	75.53	92.97	-17.44	-.872
"	2	11	11.81	14.01	-2.20	-.110	82.39	87.91	-5.52	-.276
"	3	12	11.60	12.06	-.46	-.073	81.37	79.30	-2.07	-.130

*Lot 1 - Unfertilized Wilburton Hay

**Lot 2 - Fertilized Stillwater Hay

***Lot 3 - Unfertilized Stillwater Hay

Table 13
Phosphorus and Calcium Retention Data -- Trials 7 and 8.

Trial No.	Ration No.	Lamb No.	Total P Intake	Total P Excreted	Total P Retained	Daily P Retention	Total Ca Intake	Total Ca Excreted	Total Ca Retained	Daily Ca Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
7-A	1*	1	8.124	9.390	-1.266	-.1266	46.74	40.060	+6.68	+.668
"	3**	2	8.124	8.242	-0.118	-.0118	46.74	49.810	-3.07	-.307
"	1	3	7.718	8.920	-1.202	-.1202	44.403	41.448	+2.955	+.2955
"	3	4	7.718	8.586	-0.868	-.0868	44.403	42.306	+2.097	+.2097
"	1	5	7.718	9.110	-1.392	-.1392	44.403	38.468	+5.935	+.5935
"	3	6	7.718	8.761	-1.043	-.1043	44.403	44.524	-0.121	-.0121
7-B	1	1	8.124	8.843	- .719	-.0719	46.74	43.00	+3.68	+.368
"	3	2	8.124	8.893	- .769	-.0769	46.74	45.01	+1.73	+.173
"	1	3	8.124	9.360	-1.236	-.1236	46.74	44.96	+1.78	+.178
"	3	4	8.124	8.657	-0.533	-.0533	46.74	41.22	+5.52	+.552
"	1	5	8.124	9.035	-1.317	-.1317	44.403	42.538	+1.865	+.1865
"	3	6	8.124	8.900	-1.182	-.1182	44.403	45.108	-0.705	-.0705
8-A	3	1	8.124	8.03	+ .094	+.0094	46.74	40.15	+5.59	+.559
"	1	2	8.124	7.59	+ .534	+.0534	46.74	39.79	+6.95	+.695
"	3	3	8.124	8.25	- .126	-.0126	46.74	41.36	+5.38	+.538
"	1	4	8.124	9.59	-1.466	-.1466	46.74	35.80	+10.94	+1.094
"	3	5	8.124	9.05	- .926	-.0926	46.74	36.75	+9.99	+.999
"	1	6	8.124	8.36	- .236	-.0236	46.74	37.98	+8.76	+.876
8-B	3	1	8.124	7.63	+ .494	+.0494	46.74	37.05	+9.69	+.969
"	1	2	8.124	7.70	+ .424	+.0424	46.74	37.98	+8.76	+.876
"	3	3	8.124	8.19	+ .066	+.0066	46.74	36.94	+9.80	+.980
"	1	4	8.124	8.75	- .626	-.0626	46.74	40.70	+6.04	+.604
"	3	5	8.124	8.42	- .306	-.0306	46.74	35.16	+11.58	+1.158
"	1	6	8.124	8.20	- .076	-.0076	46.74	37.36	+9.38	+.938

*Lot 1 - Unfertilized Wilburton Hay

**Lot 3 - Unfertilized Stillwater Hay

Table 14

Phosphorus and Calcium Retention Data -- Trials 9 and 10.

Trial No.	Ration No.	Steer No.	Total P Intake	Total P Excreted	Total P Retained	Daily P Retention	Total Ca Intake	Total Ca Excreted	Total Ca Retained	Daily Ca Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
9-B	1*	1	32.5	29.42	+3.08	+.308	186.8	118.83	+67.97	+6.797
"	2**	2	32.5	27.59	+4.91	+.491	186.8	136.90	+49.90	+4.990
"	1	3	32.5	31.78	+0.72	+.072	186.8	137.62	+49.18	+4.918
"	2	4	32.5	27.87	+4.63	+.463	186.8	146.02	+40.78	+4.078
10-A	1	4	44.64	40.62	+4.02	+.402	233.5	184.06	+49.44	+4.944
"	2	3	44.64	42.23	+2.41	+.241	233.5	197.70	+35.80	+3.580
"	1	2	44.64	50.01	-5.37	-.537	233.5	171.72	+61.78	+6.178
"	2	1	44.64	34.62	+10.02	+1.002	233.5	155.62	+77.88	+7.788
10-B	1	4	46.52	50.27	-3.75	-.375	233.5	198.27	+35.23	+3.523
"	2	3	46.52	43.22	+3.30	+.330	233.5	188.98	+44.52	+4.452
"	1	2	46.52	54.49	-7.97	-.797	233.5	205.47	+28.03	+2.803
"	2	1	46.52	48.18	-1.66	-.166	233.5	194.57	+38.93	+3.893

*Wilburton hay ration

**Stillwater hay ration

Table 15

The Average Daily Retention of Phosphorus by Depleted Lambs Fed Wilburton and Stillwater Prairie Hay

Lot	Lamb No.	Phosphorus		
		Intake	Excretion	Retention
		gm.	gm.	gm.
1[1]	1	0.70	0.65	+0.05
1	3	0.71	0.70	+0.01
1	5	0.76	0.55	+0.21
Average		0.72	0.63	+0.09
3[2]	2	0.70	0.61	+0.09
3	4	0.71	0.69	+0.02
3	6	0.76	0.53	+0.23
Average		0.72	0.61	+0.11

[1]Wilburton hay ration

[2]Stillwater hay ration

Table 16

Summary: Average Phosphorus and Calcium Retention Data.

Trial Nos.	Ration No.	Phosphorus: Intake per period	Phosphorus: Excretion per period	Phosphorus: Retention per period	Phosphorus: Daily Retention	Calcium: Intake per period	Calcium: Excretion per period	Calcium: Retention per period	Calcium: Daily Retention
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1, 2, 3 and 4 (20 day collection periods) (Lambs)	1[1]	15.57	16.11	-0.54	-0.027	98.67	104.71	-6.04	-0.302
	2[2]	16.78	14.29	+2.49	+0.124	62.02	59.70	+2.32	+0.116
	3[3]	15.68	15.77	-0.09	-0.005	76.13	85.17	-9.04	-0.452
5 and 6 (20 day collection periods) (Lambs)	1	13.87	15.63	-1.76	-0.088	92.62	102.83	-10.21	-0.511
	2	14.73	14.00	+0.73	+0.036	95.07	98.74	-3.67	-0.183
	3	14.10	14.68	-0.58	-0.029	94.20	106.38	-12.18	-0.609
7A, 7B, 8A and 8B (10 day collection periods) (Lambs)	1	8.06	8.69	-0.63	-0.032	46.15	39.82	+6.33	+0.633
	3	8.06	8.52	-0.48	-0.024	46.15	41.47	+4.57	+0.57
9B, 10A and 10B (10 day collection periods) (Steers)	1	41.23	42.76	-1.53	-0.15	217.9	169.3	+48.6	+4.86
	3	41.23	37.30	+3.93	+0.39	217.9	169.9	+48.0	+4.80
11 (20 day collection periods)	1	14.40	12.60	+1.80	+0.09				
	3	14.40	12.20	+2.20	+0.11				

[1] Lot 1 - Approx. 50% of total phosphorus intake from prairie hay grown in southeast Oklahoma - Wilburton.

[2] Lot 2 - Approx. 50% of total phosphorus intake from prairie hay grown in central Oklahoma - Stillwater. This hay was grown on land which had previously received 200# Superphosphate per acre.

[3] Lot 3 - Approx. 50% of total phosphorus intake from unfertilized prairie hay grown in central Oklahoma - Stillwater.

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PART II. EFFECT OF MANGANESE INTAKE ON CALCIUM AND PHOSPHORUS UTILIZATION BY LAMBS

INTRODUCTION

Previous discussion has indicated the nature of differences in nutritive condition of beef cattle at the Range Cattle Minerals Station, near Wilburton, and those at the Lake Carl Blackwell Range, near Stillwater, thought to be due to differences in nutritive value of the range forage at these two areas. Beef cattle at Wilburton displayed symptoms typical of aphosphorosis but remained unthrifty even when phosphorus was supplied. Chemical analysis showed that forage from the two areas contained similar amounts of the major nutrients but that the Wilburton forage was somewhat lower in phosphorus and was unusually high in manganese. The hays collected during one year were found to have the following mineral composition, expressed as percentages:

	Ca	P	Si	Fe	Mn	Mg	K	Na	Al
Wilburton Hay	0.524	.053	4.04	.0095	.0253	.516	.450	.144	.0152
Stillwater Hay	0.422	.061	5.63	.0091	.0075	.159	.688	.161	.0148

From these data, it can be seen that the prairie hay grown in the Wilburton area contained many times as much manganese and magnesium as the hay grown in the Stillwater area. Smaller differences were found in other mineral constituents. In other reports, Tidwell (1951) found that prairie hay produced near Wilburton contained from 150 to 270 ppm. of manganese, whereas prairie hay produced in the Stillwater area contained only 25 to 75 ppm. The grass species which made up the botanical composition were similar in the two hays. The Wilburton hay contained a greater proportion of weeds.

It was suggested therefore that manganese, or factors associated with it in the Wilburton forage, possibly interfere with the utilization of nutrients, including phosphorus. Numerous references have indicated a possible interference of manganese, especially at high levels of intake, with the proper absorption and assimilation of other mineral elements. Evidence of somewhat low phosphorus availability in Wilburton prairie hay is presented in Part I of this thesis.

The purpose of this investigation was to determine the effect on phosphorus and calcium retention, of manganese added in increasing amounts to a ration of natural feeds for growing lambs.

REVIEW OF LITERATURE

The essentiality of manganese as a dietary element has been demonstrated. Waddell (1930) reported that whole cows' milk, as the sole diet of animals, failed to support growth and well being. Young rats, for instance, when fed such a diet, developed a severe anemia in the course of a few weeks' time. To the diet of one group of female rats, this worker added manganese as a solution of manganese chloride at the level of 0.5 milligrams of manganese per 100 c.c. of milk; to another, iodine as a solution of potassium iodide at the level of 0.2 milligrams of iodine per 100 c.c. was added; while the third female rat group received both manganese and iodine in the above forms and concentrations. Immediately following the addition of either mineral or the combination, the female rats began to ovulate at regular intervals.

Daniels and Emerson (1935) in a study of the relation of manganese to congenital debility reported that the death of young rats reared on milk diets modified with copper and iron was the result of too little manganese in the diet of the mother. Young born of these milk-fed females were found to contain 65 per cent less manganese than the young from females receiving a smilar ration with added manganese.

Becker and McCullum (1938) fed diets containing the following levels of manganese chloride to rats: 0.18, 0.36, 0.9, 1.8 and 3.6 per cent. Animals grew well on all diets, except the one containing 3.6 per cent manganese chloride. The diets contained 0.75 per cent phosphorus. These workers postulated that the high level of phosphorus effectively prevented symptoms of toxicity by reducing the amount of absorbed manganese, and further stated that the condition in the intestine which influences the absorption of manganese constitutes the controlling factor in determining the level at which manganese is toxic.

Blumberg and co-workers (1938) demonstrated that manganese compounds which are easily soluble in the gastro-intestinal tract are capable of producing rickets in rats. The amounts of manganese fed, however, were far greater than those which would be found in natural foodstuffs. These workers were of the opinion that the essential factor in the production of manganese rickets is the formation of relatively insoluble and poorly absorbable manganese phosphate in the intestinal tract, with a consequent decrease in the amount of phosphorus available for bone formation.

Chornock and co-workers (1942) studied the effects of various amounts of manganese in the diets of young growing rats, with special reference to the influence of this element on calcium and phosphorus metabolism in the presence and absence of vitamin D. They found that the growth of rats on high manganese intakes (1.12 and 1.73 per cent manganese in the diet) was retarded in proportion to the amount of manganese in the diet. The manganese was fed as manganese carbonate. High manganese intakes (0.66 to 1.12 per cent) in connection with high-calcium and low-phosphorus diets increased the excretion of phosphorus in all cases, and of calcium at the highest manganese intake. They reported that phosphorus retention was affected more than calcium retention. A more favorable calcium:phosphorus ratio in the diet, produced by increasing the phosphorus content, tended to improve retention of these elements even in the presence of manganese. Animals fed large amounts of manganese developed severe rickets as evidenced by low blood plasma phosphorus levels and by the condition of the bones. Vitamin D administration resulted in an improved condition.

Blumberg and associates (1938) reported that manganese rickets in rats was produced by the substitution of an equimolar amount of manganese carbonate for 2.5 per cent of calcium carbonate in a high-calcium and low-phosphorus rachitogenic diet, and also by the addition of 2.9 per cent of manganese carbonate to a stock diet of optimal calcium and phosphorus content. These workers reported that a soluble manganese salt, manganese chloride, was rachitogenic, but that a less soluble salt, manganese dioxide, was not. In these studies, vitamin D was found to be effective in curing manganese rickets when added to a diet optimal in calcium and phosphorus, but only slightly effective when added to a diet low in both calcium and phosphorus.

Smith, et al. (1944) reported that manganese deficiency in the rabbit seriously interfered with normal bone development. These workers reported a decrease in breaking strength, weight, density, length and ash content of the humeri of deficient animals. A microscopic study of the humeri revealed extensive deviations from the normal which was interpreted as suppressed osteogenesis. There was a significant decrease in growth and a decreased content of manganese in the liver of manganese deficient rabbits as compared to the controls. Testicular degeneration was found in the deficient animals. Although the ovaries and uteri of the female rabbits were found to be significantly smaller than the controls, microscopic studies did not reveal any significant alterations in structure.

Ellis and co-workers (1947) studied manganese deficiency in the rabbit using the paired-feeding technique with a basal diet of milk. For rabbits on the unsupplemented diet, these workers found a lowered arginase activity in the liver and a lower alkaline phosphatese activity of the ulna.

The fresh weight, percentage ash, total ash, density and length of the humurus was significantly lower in the manganese deficient animals.

Carratala, et al. (1935) reported that chronic poisoning developed in rabbits after feeding 0.7 - 0.9 milligrams of manganese sulfate daily or 0.10 - 0.94 milligrams of manganese chloride, daily for 20 - 25 days. Liver tissue showed the greatest damage.

Grummer, et al. (1950) fed a low manganese (12-13 ppm.) basal ration consisting of corn, soybean oil meal, corn gluten meal, ground alfalfa, salt and limestone to 4 lots of pigs. The basal ration was supplemented with 3 levels of manganese, 40, 80 and 160 parts per million. The pigs fed 40 parts per million of manganese made the most rapid daily gain and ranked highest in efficiency of gain in each of 4 trials. The ration containing 80 parts per million produced slightly faster gains than the basal ration, while the ration containing 160 parts per million produced gains which were approximately equal to that of the basal. When sows were fed rations containing 40, 80 and 160 parts per million of manganese, fertility was slightly improved, but there was no improvement in milking ability. When 500 ppm. of manganese was added to the basal ration, pigs averaged 0.97 pound daily gain and required 453 pounds of feed per cwt. gain as compared to 1.21 pounds gain and 428 pounds of feed for the unsupplemented group. According to these workers, 500 ppm. definitely retarded appetite and growth, especially during the latter part of the trial. Pigs fed the ration containing the supplemental manganese developed a stiffness of limbs and a stilted gait toward the end of the experiment.

Keith and associates (1942) reported stiffness in 80 to 100-lb. pigs on diets high in ash (6 to 9 per cent) and low in manganese (11-14 ppm.).

Manganese sulfate was then added to the rations in amounts to supply 50 to 60 parts per million of manganese. As a result, the 15 pigs which received manganese supplemented rations showed no signs of stiffness. Fifty per cent of the litter mates, however, fed the unsupplemented ration, became stiff. These workers reported that the addition of manganese to the ration was ineffective in curing stiffness in pigs after the stiffness had developed.

Johnston (1943) found that pigs grew normally on a ration containing less than 0.5 ppm. of manganese which produced high incidence of perosis in chicks. Reproduction in swine on this ration was unsuccessful. This worker reported (1944) that pigs can be grown at satisfactory rates from weaning to slaughter weights on rations of natural feedstuffs containing 7 to 10 parts per million of manganese. When the total ash content of the ration exceeded 10 per cent, there appeared to be a slight beneficial effect from the addition of manganese. From these results, it was concluded that manganese deficiency will not occur under conditions of normal swine management practices where pigs have access to pasture or manganese carrying feeds such as oats, bran or shorts.

Reid and associates (1947) studied the effect of manganese and other trace elements on the metabolism of calcium and phosphorus during early lactation in the dairy cow. During the first 5 months of lactation of 8 Holstein and 4 Guernsey cows, calcium equilibrium was maintained most frequently in the group of cows fed Mico (a mineral supplement of the following percentage composition: calcium, 33; magnesium, 2; manganese, 0.20; iron, 0.20; iodine, 0.045; copper, 0.025; zinc, 0.01 and cobalt, 0.002) as a supplement to a basal ration of grain, corn silage, and timothy hay. Supplementation of the basal ration with manganese sulfate in addition to calcium carbonate resulted in negative calcium balances in every case, whereas several positive balances occurred when the basal ration was supplemented with calcium carbonate alone. The marked depression of calcium metabolism appeared to be affected by the manganese sulfate supplementation. Phosphorus metabolism was not appreciably affected.

Caskey, Gallup and Norris (1939) reported that a deficiency of manganese in the diet of chicks resulted in a significant shortening of bones of the legs and wings as well as a shortening of the spinal column. It was also found in these studies that the ash content of the bones was significantly lower in chicks fed a low manganese diet (5.5 parts per million) than in those fed a diet adequate in manganese (100 parts per million).

Amdur, et al. (1945) have shown the need for manganese in bone development of the rat. They reported that the length, density, breaking strength, and phosphatase content of the bones of manganese deficient rats were low as compared to pairmates of the same weight receiving adequate manganese. There was no difference between the two groups in percentage of ash, calcium and phosphorus in the fat-free dry bone.

Lardy and associates (1942) found that bull calves fed to the age of 18 months on low-manganese rations were able to produce only poor quality sperm. Other bulls on the same ration supplemented with manganese sulfate produced normal sperm.

EXPERIMENTAL PROCEDURE

A. Experimental Rations

Experiment 1.

The rations fed to lambs in this experiment were composed of prairie hay, 46.9; yellow corn, 29.3; corn gluten meal, 23.5 and calcium carbonate, 0.3 per cent. This ration on a dry matter basis contained 15.5 per cent protein, 0.32 per cent calcium and 0.25 per cent phosphorus and had a Ca:P ratio of about 1.3 to 1. It supplied about 1.5 grams of phosphorus per 100 pounds of body weight. The proximate analysis of feed constituents are presented in Table 1. Manganese sulfate (reagent grade) was added to the basal ration in amounts to supply manganese at levels of about 300, 600, 900, 1200 and 1500 ppm. in the test rations. Vitamins A and D were added in the form of cod liver oil at the rate of about 1000 I.U. and 300 I.U. per 100 pounds of body weight, respectively.

Table 1

Proximate Analyses of Feeds

		Composition of Dry Matter			
Feeds	Dry Matter	Protein	Calcium	Phosphorus	Manganese
	%	%	%	%	mg/100
Prairie hay	92.75	4.44	0.37	0.06	7.3
Yellow corn	91.64	9.41	0.01	0.31	0.6
Cottonseed meal	93.65	45.20	0.10	0.58	1.2
$CaCO_3$	100.00	——	40.00	——	——

Experiment 2.

Further investigation into the possibility of manganese interference with normal calcium and phosphorus metabolism in lambs was conducted with a basal ration composed of prairie hay, 48.6; soybean meal, 20.4; yellow corn, 28.9; salt, 1.7 and calcium carbonate, 0.4 per cent. This ration supplied 15.06 per cent protein, 0.48 per cent calcium and 0.28 per cent phosphorus (dry matter basis). Sufficient manganese sulfate was added to the basal ration to provide manganese at levels of 0, 500 and 1000 ppm. of ration. Supplements of vitamin A and D were fed in the same amounts as in experiment 1.

Experiment 3.

Manganese sulfate (the source of supplemental manganese in experiments 1 and 2) is an acid salt and tends to promote an acidic condition. It was felt that perhaps this might favor the solubility of mineral elements in the gastro-intestinal tract and thus render them more easily absorbed. Manganese carbonate is a basic salt and was used as the source of supplemental manganese in this experiment. The basal ration consisted of prairie hay, 47.6: soybean meal, 20.8; yellow corn, 29.8; salt, 1.5 and calcium carbonate, 0.3 per cent. This ration supplied 15.44 per cent protein, 0.46 per cent calcium and 0.27 per cent phosphorus (dry matter basis). The levels of manganese supplementation used in this experiment were the same as for experiment 2. Supplemental vitamins A and D were fed daily as previously described.

B. Experimental Subjects

Experiment 1.

Twelve native type wether lambs weighing between 65 and 95 pounds were used in this experiment. Two weeks prior to being placed on the test rations, the lambs were drenched with a phenothiazine preparation in order to minimize the effect of parasitic infestation upon physiological response. The lambs were weighed and randomly, by weight, assigned to each test ration. The triad feeding technique was used. The lambs in four triads were fed rations containing 0, 300 and 600 ppm. of manganese. Later, they were fed rations containing 900, 1200 and 1500 ppm. of manganese.

Experiment 2.

Twelve western wether lambs weighing between 54 and 77 pounds were used in this study. The lambs were divided, according to weights, into three uniform groups of four lambs each. They were drenched for internal parasites as in the previous experiment, and fed in triads during the experiment.

Experiment 3.

Six native type wether lambs weighing from 60 to 64 pounds were used in this experiment. The details of treatment were the same as during the second experiment.

C. Procedure for Metabolism Periods

Experiment 1.

The lambs were fed the test rations for a period of ten days prior to being placed in the metabolism stalls. During this 10-day period each lamb was fed in an individual feeding stanchion and given the freedom of a large box stall between feedings. The triad feeding technique described in Part I was used in an attempt to keep lambs in each triad on the same phosphorus intake as is shown in Table 2. After the lambs were on feed, they were placed in metabolism stalls for a ten-day preliminary and twenty-day collection period. The urine and feces were collected daily. The urine was measured and a ten per cent aliquot taken for analysis; the feces was weighed, and a five per cent aliquot taken. The daily aliquots of both the feces and urine were separately combined into composites which were held under refrigeration until analyzed for calcium and phosphorus.

Experiment 2.

Details of procedure followed during metabolism periods in this experiment were the same as for experiment 1, except that the collection period was reduced to ten days. Further, the lambs were rotated in the second and third trials in such a way that at the end of the experiment each lamb had received all three levels of manganese.

Experiment 3.

The triad feeding technique employed in the first two experiments was also used in this experiment. Upon analysis of urine and feces samples from experiment 2, it appeared that some manganese was being carried over by the lambs from one trial to another. This was especially true when the lambs receiving the 1000 ppm. level in one trial were switched to the zero level in the following trial. In an attempt to decrease this carry-over effect of manganese, the preliminary feeding period was changed to twenty days. At the conclusion of the first twenty-day preliminary and ten-day collection period, the lambs were rotated at random within triads and placed on a ration containing another level of manganese. At the end of the experiment each lamb had received all three levels of manganese.

In this experiment, water consumption was measured in order to obtain a more accurate measurement of calcium intake, since it was found that the water supply contained about 0.03 grams of calcium per liter.

D. Chemical Analysis of Feeds and Excreta

Experiments 1, 2 and 3.

Feed calcium and phosphorus determinations were made according to the procedure described by the A.O.A.C. (1945). The calcium and phosphorus in feces and urine were determined by the same procedure as modified by the Department of Agricultural Chemistry Research. The method used in determining manganese was a modification of that used by Willard and Greathouse (1917). The method of analysis for other feed constituents are described by A.O.A.C. (1945).

RESULTS AND DISCUSSION

Experiment 1

Balance trials were completed with 4 lambs on the basal ration and on each level of added manganese. Table 2 presents the calcium and phosphorus retention data for the entire experiment. Table 3 presents a summary of the average calcium and phosphorus balances in relation to the levels of manganese fed.

It is noteworthy that the rations containing 300 and 600 ppm. of manganese gave lower phosphorus retention values than the basal ration. These results are in agreement with the general observation that high levels of manganese decrease calcium and phosphorus utilization in animals. When higher levels of manganese were fed, however, there was no decrease in calcium and phosphorus retention. In fact, these latter results indicate that the higher levels of manganese (900, 1200 and 1500 ppm.) favored calcium and phosphorus retention. Because of irregularities on the part of the feeder, there is some question regarding the validity of these latter results.

Experiment 2.

The individual data for lambs in experiments 2 and 3 are presented by McOsker (1953). Table 4 presents the effect of different levels of manganese intake on the retention of calcium and phosphorus by the lambs in the second experiment. It will be noted that only three lambs were included in the 500 ppm. level. One lamb was removed from the experiment because of failure to stay on feed. Table 5 presents a summary of the results obtained in this experiment. Differences in calcium and phosphorus balances between treatments were small; however, the results tend to confirm the results of experiment 1, in which the higher levels of manganese gave slightly higher retention values for these elements.

There was no indication that the high levels of manganese intake altered the pathway of excretion of calcium or phosphorus since the fecal excretion of both minerals was essentially the same at all levels of manganese intake.

Experiment 3.

Table 6 presents the average daily calcium and phosphorus retention of lambs fed rations containing supplemental manganese as manganese carbonate. One lamb in trial 3 was removed because of failure to stay on feed. Fecal excretions of phosphorus were somewhat higher in this experiment than in experiment 2, in which manganese was supplied as manganese sulfate; however, as in experiment 2, practically all of the phosphorus was voided in the feces. The average calcium and phosphorus retention values for this experiment are presented in Table 7.

According to the average values, there is a trend toward a decreased retention of phosphorus with increased manganese intake. Lambs fed rations containing 0, 500 and 1000 ppm. of manganese had an average daily phosphorus retention of +0.13, +0.10 and +0.02 grams daily, respectively. These phosphorus retention values are somewhat lower than those for lambs fed supplemental manganese in the form of manganese sulfate. Table 8 presents a summary of the calcium and phosphorus balances for lambs in experiments 2 and 3. It can be seen from this table that lambs fed the 500 and 1000 ppm. levels of manganese in experiment 3 had lower phosphorus retention values, expressed as a percent of intake, than lambs in experiment 2.

SUMMARY

The effect of high levels of manganese intake on calcium and phosphorus retention was studied in three balance experiments with lambs.

In experiment 1 manganese sulfate was added to a basal ration at levels of 0, 300, 600, 900, 1200 and 1500 ppm. of the ration. The data from this experiment indicated that manganese levels of 300 and 600 ppm. slightly decreased phosphorus retention. When higher levels of manganese (900, 1200 and 1500 ppm.) were fed, however, there was a slight increase in calcium and phosphorus retention.

In the second experiment, manganese intakes of 500 and 1000 ppm. had no appreciable effect on the retention of either calcium or phosphorus.

In experiment 3 where supplemental manganese was supplied as manganese carbonate, there appeared to be a trend toward a small decrease in the retention of phosphorus by lambs fed rations containing manganese at levels of 500 and 1000 ppm.

Table 2
Effect of Manganese Intake on the Daily Retention of Calcium and Phosphorus by Lambs.
(Manganese as manganese sulfate)

Approx. Mn in ppm.	Trial	No. of lambs	Calcium Intake	Calcium Excretion	Calcium Retention	Phosphorus Intake	Phosphorus Excretion	Phosphorus Retention
			gm.	gm.	gm.	gm.	gm.	gm.
30	1	2	1.40	1.48	-0.08	1.13	1.25	-0.12
30	2	2	1.38	1.26	+0.12	1.04	0.80	+0.24
Average			1.39	1.37	+0.02	1.08	1.02	+0.06
300	1	2	1.40	1.60	-0.20	1.13	1.31	-0.18
300	2	2	1.38	1.47	-0.09	1.04	1.00	+0.04
Average			1.39	1.53	-0.14	1.08	1.15	-0.07
600	1	2	1.40	1.67	-0.27	1.13	1.43	-0.30
600	2	2	1.38	1.43	-0.05	1.04	1.02	+0.02
Average			1.39	1.55	-0.16	1.08	1.22	-0.14
900	3	2	1.77	1.52	+0.25	1.41	1.26	+0.15
900	4	2	1.28	0.91	+0.37	0.99	0.79	+0.20
Average			1.52	1.21	+0.31	1.20	1.02	+0.18
1200	3	2	1.77	1.43	+0.34	1.41	1.08	+0.33
1200	4	2	1.28	0.87	+0.41	0.99	0.91	+0.08
Average			1.52	1.15	+0.37	1.20	1.00	+0.20
1500	3	2	1.77	1.57	+0.20	1.41	1.26	+0.15
1500	4	2	1.28	1.07	+0.21	0.99	1.12	-0.13
Average			1.52	1.32	+0.20	1.20	1.19	+0.01

Table 3

Average Daily Retention of Calcium and Phosphorus (experiment 1)

Ration Mn ppm.	Calcium Retention gm.	Phosphorus Retention gm.
30	+0.02	+0.07
300	-0.14	-0.06
600	-0.16	-0.14
900	+0.31	+0.18
1200	+0.37	+0.20
1500	+0.20	+0.01

Table 4

Effect of Manganese Intake at Levels of 0, 500 and 1000 ppm. on Average Daily Retention of Calcium and Phosphorus by Lambs. (Manganese as Manganese Sulfate)

Trial	Ration ppm. Mn	No. of lambs	Calcium Intake	Calcium Feces	Calcium Urine	Calcium Retention	Phosphorus Intake	Phosphorus Feces	Phosphorus Urine	Phosphorus Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0	4	2.65	2.55	0.30	-0.20	1.64	1.60	0.02	+0.02
1	500	3	2.65	2.46	0.23	-0.04	1.64	1.46	0.01	+0.17
1	1000	4	2.65	2.60	0.53	-0.48	1.64	1.64	0.01	-0.01
2	0	4	2.86	2.42	0.45	-0.01	1.59	1.52	0.01	+0.06
2	500	4	2.86	2.47	0.23	+0.16	1.59	1.51	0.07	+0.01
2	1000	4	2.86	2.40	0.24	+0.22	1.59	1.43	0.04	+0.12
3	0	4	2.71	2.14	0.27	+0.30	1.60	1.41	0.02	+0.17
3	500	4	2.71	2.21	0.51	-0.01	1.60	1.48	0.01	+0.11
3	1000	4	2.71	2.14	0.15	+0.42	1.60	1.39	0.03	+0.18

Table 5

Average Daily Retention of Calcium and Phosphorus (experiment 2).

Ration Mn ppm.	Calcium Retention	Phosphorus Retention
	gm.	gm.
0	+0.03	+0.08
500	+0.04	+0.10
1000	+0.05	+0.10

Table 6

Effect of Manganese Intake at Levels of 0, 500 and 1000 ppm. on Average Daily Retention of Calcium and Phosphorus by Lambs. (Manganese as Manganese Carbonate)

Trial	Ration ppm. Mn	No. of lambs	Calcium Intake	Calcium Feces	Calcium Urine	Calcium Retention	Phosphorus Intake	Phosphorus Feces	Phosphorus Urine	Phosphorus Retention
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0	2	2.68	2.55	0.19	-0.06	1.60	1.59	0.02	-0.01
1	500	2	2.60	2.87	0.25	-0.52	1.54	1.84	0.01	-0.31
1	1000	2	2.67	2.56	0.22	-0.11	1.52	1.54	0.02	-0.04
2	0	2	2.30	1.92	0.05	+0.33	1.38	1.26	0.07	+0.05
2	500	2	2.42	2.31	0.06	+0.05	1.48	1.49	0.03	-0.04
2	1000	2	2.44	2.52	0.16	-0.24	1.47	1.63	0.01	-0.17
3	0	2	2.89	1.86	0.11	+0.92	1.61	1.24	0.03	+0.34
3	500	2	2.87	2.28	0.05	+0.54	1.62	1.58	0.00	+0.04
3	1000	1	2.84	1.93	0.00	+0.91	1.62	1.29	0.07	+0.26

Table 7

Average Daily Retention of Calcium and Phosphorus (experiment 3).

Ration ppm. Mn	Calcium Retention	Phosphorus Retention
	gm.	gm.
0	+0.40	+0.13
500	+0.02	-0.10
1000	+0.19	+0.02

Table 8

Summary of Calcium and Phosphorus Excretion and Retention Values.
(Experiments 2 and 3)

Experiment	Ration ppm. Mn	Calcium Intake	Calcium Excretion	Calcium Retention[1]	Phosphorus Intake	Phosphorus Excretion	Phosphorus Retention[1]
		gm.	gm.	%	gm.	gm.	%
2	0	2.74	2.71	1.1	1.61	1.53	5.0
	500	2.74	2.70	1.5	1.61	1.51	6.2
	1000	2.74	2.68	2.2	1.61	1.51	6.2
3	0	2.62	2.23	14.9	1.53	1.40	8.5
	500	2.63	2.61	0.8	1.55	1.65	-6.4
	1000	2.65	2.46	7.2	1.54	1.52	1.3

[1] expressed as a per cent of intake

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PART III. PHOSPHORUS UTILIZATION BY LAMBS AS DETERMINED WITH THE RADIOACTIVE ISOTOPE P^{32}

INTRODUCTION

Low soil fertility has been associated with poor plant growth and inefficient livestock production. The importance of adequate phosphorus nutrition of plants and factors which affect phosphorus utilization in cattle and sheep are problems which have been given special attention by many investigators in past years.

New techniques have been devised which have had a great impact upon the field of mineral investigations in animal nutrition. Among these new techniques is the use of the radioactive isotope of phosphorus (P^{32}) as a "tracer" substance. "Tracer" experiments have proven useful because they have confirmed, in many instances, behavior which had been heretofore only suspected. They have provided a basis for more rigidly controlled nutritional experiments and have opened up new avenues of approach to many problems.

The investigation reported herein was designed to study the metabolism of radioactive phosphorus by phosphorus deficient lambs fed hay of high and low manganese content. The high manganese hay was produced near Wilburton, and the low manganese hay was produced near Stillwater.

REVIEW OF LITERATURE

Chievitz and Hevesy (1935) conducted the first experiments in which radioactive phosphorus was used to study problems of phosphorus nutrition. Radioactive phosphorus as an indicator or "tracer" showed that phosphorus atoms of the mineral constituents of bone exchanged rapidly with those present in the blood plasma.

Cohn and Greenberg (1938) employed the rat in studying the pathway of radioactive phosphorus in metabolism. These workers found that the radioactive phosphorus was absorbed within 2 to 8 hours after ingestion. Twenty to thirty per cent remained unabsorbed. Of that absorbed, 20 to 30 per cent was excreted in the urine during the first 24 hours after administration. In the rat the rank of tissues with respect to their affinity for P^{32} was shown to be: bone, liver, stomach, heart, lungs, blood, muscle, skin and brain. In this work the brain tissue appeared to have a very slow turnover of phosphorus. In the rachitic rat, small amounts of P^{32} were found in the bone. When vitamin D was fed to the rachitic rat, larger amounts of P^{32} appeared in the bone. When P^{32} was administered to phosphorus-deficient rats, there was a greater retention of P^{32} in the bone than in the normal rats. The excretion of P^{32} in the urine was greatly decreased during phosphorus deficiency.

Hevesy (1948) reported that the absorption of radioactive phosphorus from the gastro-intestinal tract of the rat was increased by glucose and decreased by iron salts. The excretion of radioactive phosphorus in the urine of rats maintained on inadequate phosphorus was about one-fourth that of rats on a normal ration. Vitamin D was found to increase the rate of renal tubular phosphate absorption, thereby increasing the concentration of this ion in the plasma. Smith and co-workers (1951) studied the distribution of intravenously injected radioactive phosphate in the tissues of swine. These workers found that older animals had a slower rate of uptake of P^{32} than young animals. The rank of soft tissues with respect to their affinity for P^{32} was shown to be: bile, liver, kidney, thymus, lymph node, heart, spleen, tongue, muscle, lung, smooth stomach muscle and brain. Hevesy and associates (1940) made use of radioactive phosphorus in a study of the rate of regeneration of the rabbit and frog skeleton. Radioactive phosphorus was administered to rabbits and frogs repeatedly during the experiment to keep the radioactive plasma inorganic phosphorus at a constant activity level. Within 50 days, 29 per cent of the epiphysis of the femur was found to be regenerated, while the corresponding figure for the diaphysis was 7 per cent. Only half of the mineral constituents of the scapula were found to be unchanged.

Spinks and associates (1948) studied phosphorus utilization in four laying hens by means of radioactive phosphorus. This substance was incorporated into the laying mash as tri-calcium phosphate, replacing the bone meal in the ration. Radioactive phosphorus was absorbed and appeared in the yolk, white and shell of the egg within 24 hours after feeding. The maximum recovery of P^{32} following a single feeding was within 24 hours in the case of the shell, 48 to 72 hours in the case of the white and 124 hours for the yolk. They reported that a considerable quantity of the absorbed phosphorus was stored in the tibae 40 days after feeding the P^{32}. According to this work, the percentage uptake of phosphorus from tri-calcium phosphate rose gradually in the egg and became relatively constant in about 14 to 15 days after the first feeding of the tri-calcium phosphate.

O'Neil, et al. (1948) found that 5 per cent of the radioactive phosphorus, in tri-calcium phosphate, appeared in the egg over a 25-day period. The P^{32} was fed daily for the entire 25-day period.

Shirley, Davis and Keller (1951) fertilized an area of meadow with a super phosphate fertilizer containing radioactive phosphorus. The harvested forage was fed to a steer in a metabolism stall and the steer was sacrificed after a period of 39 hours. The contents of the various segments of the digestive tract, as well as the principal organs and tissues, were separated and assayed for radioactivity. Approximately 45 per cent of the isotope in the grass was present in the alimentary tract and 36.3 per cent in the rumen and reticulum contents. No isotope was found in the feces excreted in the first 12 hours after feeding, but 0.92 per cent of the dose was present after 24 hours. At the end of the first 12 hours 0.77 per cent of the dose was present in the urine. The wide distribution of the radioactive P in the tissues of the steer indicated that the isotope was present in the plant in a form readily assimilated by animals.

Smith and co-workers (1952) have reported on the uptake of intravenously injected radioactive phosphorus by the tissues of sheep of different ages. As previously reported for swine, bile, thymus, liver and kidney were found to have a larger uptake than lung, smooth stomach muscle and brain. They found that the rates of phosphorus uptake were generally greater for sheep tissues than was previously noted for corresponding swine tissues. They further reported that the effect of age upon the phosphorus uptake rate was less pronounced in sheep between four and ten months of age than in swine.

Lofgreen and Kleiber (1953) reasoned that if the body phosphorus of an animal were labeled with an isotope of phosphorus, it would be possible to determine the proportion of the fecal phosphorus which was originating from the body since that fraction would be labeled, while that passing through the gastro-intestinal tract would not be labeled. In experiments with lambs, these workers made use of radioactive phosphorus to determine the true digestibility of the phosphorus in alfalfa hay. They found that an average of 88 per cent of the fecal phosphorus of lambs fed alfalfa hay was of metabolic origin and 12 per cent was undigested phosphorus from the hay. While the apparent digestibility of the phosphorus of the hay averaged 22 per cent, the true digestibility was 91 per cent. They concluded that the phosphorus in alfalfa hay was highly available for absorption by lambs.

EXPERIMENTAL PROCEDURE

A. Experimental Rations

Ration 1 contained prairie hay (0.052 per cent phosphorus) from Wilburton, Oklahoma, while rations 2 and 3 contained prairie hay (0.058 per cent phosphorus) from Stillwater, Oklahoma. The remainder of each ration consisted of corn gluten meal, dried beet pulp, cod liver oil and calcium carbonate. The rations had a calcium:phosphorus ratio of 3.8:1 and supplied 1.16 grams of phosphorus per 100 pounds of live weight. Approximately fifty per cent of the phosphorus in each ration was supplied by the hays. Ration 3, a control ration, was the same as ration 2 except that steamed bone meal was supplied to each animal in amounts to supply two grams of phosphorus per 100 pounds of live weight daily.

B. Experimental Subjects and Prior Treatment

The six lambs used in Trial 11 in Part I of this thesis were also used in this experiment. At the conclusion of Trial 11, the lambs were given a six-day rest period in a large box stall and individually fed the Wilburton and Stillwater hay test rations used in the above trial. At the end of the rest period, the lambs were returned to the metabolism stalls for treatment. The three lambs receiving the control ration (ration 3) were of similar breeding and approximately the same weight as those used in trial 11. These lambs received adequate phosphorus in the ration thirty-six days prior to the beginning of the P^{32} experiment.

C. Procedure for Radioactive Phosphorus Administration

The nine lambs were given phosphoric acid containing radioactive phosphorus in a gelatin capsule during the morning feeding. A uniform dosage was used for each lamb in the group in accordance with body weight as follows:

Lamb No.	Treatment	Ration No.	Lamb Weight (lbs.)	Lambda of P^{32} per lamb
A	Control	3	79	500
B	Control	3	110	700
C	Control	3	111	700
1	Wilburton Hay	1	81	500
2	Stillwater Hay	2	78	500
3	Wilburton Hay	1	86	500
4	Stillwater Hay	2	86	500
5	Wilburton Hay	1	73	450
6	Stillwater Hay	2	82	500

Blood and saliva samples were taken at various intervals after administration of the isotope. At time intervals of 8, 13 and 49 hours after administration of the isotope, one lamb from each group was sacrificed for samples of parotid gland, cannon bone, liver, rumen solids and rumen liquids.

D. Methods of Analysis

Total phosphorus determinations were made by the same procedure as referred to in Part I.

Samples for P^{32} determinations were weighed in a beaker upon removal from the carcass and digested in nitric acid. After digestion, the material was transferred to a Syracuse type watch glass and placed under the open window of the Geiger counting device. The number of counts per minute per unit (c.c. or gm.) of sample was calculated, and are expressed as per cent of administered dose in 100 units (c.c. or gm.) in the Table 1 and figures 1 and 2.

The activity of the P^{32} administered to the lambs was determined by the use of the Geiger counter at the time the lambs were given the capsule containing the P^{32}.

RESULTS AND DISCUSSION

The percentage of the administered radioactive phosphorus found at 8, 13 and 49 hours later in the various materials analyzed is presented in Table 1. Certain tissues appeared to have a very high affinity for the radioactive phosphorus. It will be noted that at eight hours, there were relatively small differences in the amount of P^{32} in the saliva, bone, liver and rumen solids from lambs fed rations 1 and 2. The lamb fed ration 3, however, contained less P^{32} in the bone, liver and parotid glands than the lambs fed rations 1 and 2.

At thirteen hours after dosage with P^{32}, there appeared greater differences in the rate of P^{32} uptake between lambs fed different rations. It will be noted from Table 1 that at this time the saliva from the lamb fed ration 1 contained more than twice the amount found in the lamb fed ration 2, and more than five times the amount of P^{32} found in the saliva of the control lamb. Further, the parotid gland, bone and liver of the lamb fed ration 1 had stored more P^{32} than corresponding tissues of the lamb fed ration 2. The content of P^{32} in the parotid gland, bone and liver from lambs fed rations 1 and 2 was appreciably higher than corresponding tissues of the control lamb.

Samples of materials taken from lambs sacrificed at the end of forty-nine hours revealed even greater differences in the content of P^{32} in certain tissues. The bone, for example, from the lamb fed ration 1 contained about twice as much P^{32} as that of either of the lambs fed rations 2 or 3. Both the saliva and parotid gland from the lamb fed ration 1 were appreciably higher in content of P^{32} than that of the lamb fed ration 2.

Figures 1 and 2 present in graphic form the relative rates of P^{32} uptake by tissues analyzed in this experiment.

The total phosphorus present in the bone, saliva and parotid is presented in Table 2. There was no difference between the three groups of lambs in the per cent phosphorus (dry matter basis) in the ether extracted bones. The average percentage of phosphorus in the bones was 12.57, 12.07 and 12.14 for lambs fed Wilburton hay rations, Stillwater hay rations and the control rations, respectively. Likewise, there was no difference in the ash content of bones attributable to treatment. There appeared to be a difference in the amount of phosphorus present in saliva due to treatment. Samples of saliva taken at three intervals following administration of P^{32} averaged 63.7, 95.2 and 72.5 mg. of phosphorus per 100 ml. for lambs 5, 6 and C, respectively.

If these trends reflect the state of nutrition of the lambs with respect to phosphorus, the lambs fed the Wilburton hay for a period of thirty-six days were in a more depleted state and therefore had a greater need for supplemental phosphorus than those fed the Stillwater hay.

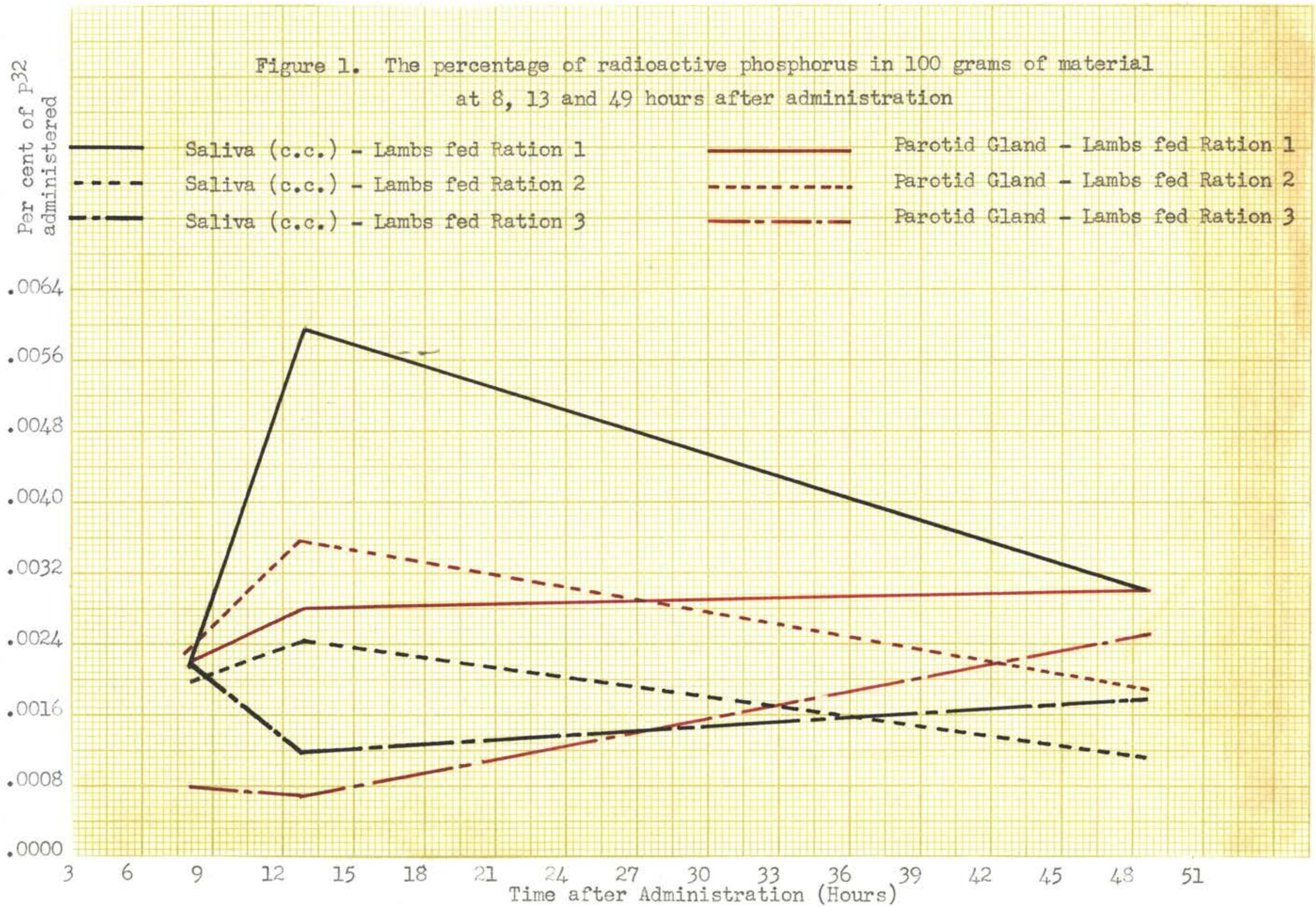

Figure 1. The percentage of radioactive phosphorus in 100 grams of material at 8, 13 and 49 hours after administration

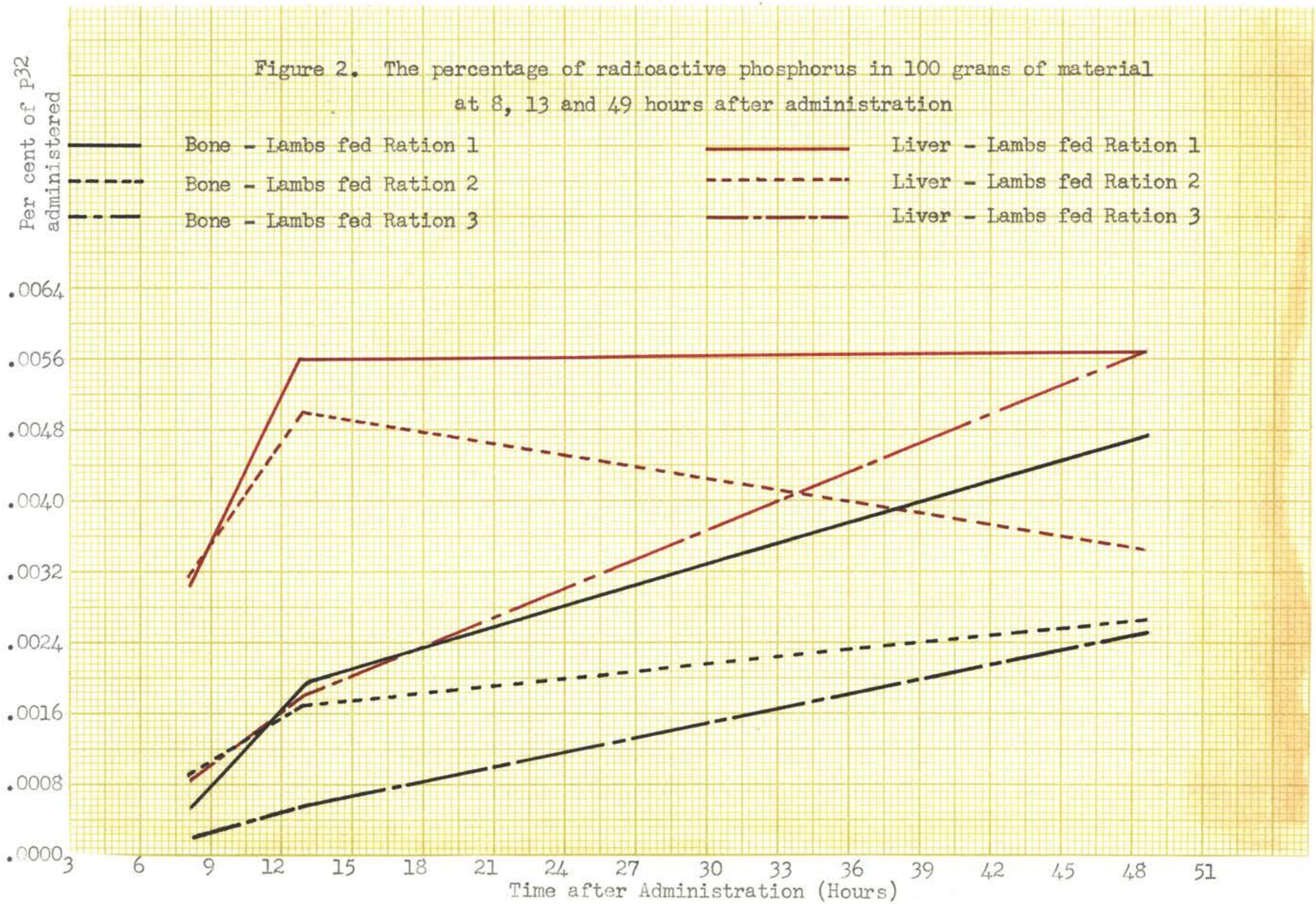
Figure 2. The percentage of radioactive phosphorus in 100 grams of material
at 8, 13 and 49 hours after administration
Bone - Lambs fed Ration 1
Bone - Lambs fed Ration 2
Bone - Lambs fed Ration 3
Liver - Lambs fed Ration 1
Liver - Lambs fed Ration 2
Liver - Lambs fed Ration 3
Per cent of P32 administered
.0064
.0056
.0048
.0040
.0032
.0024
.0016
.0008
.0000
3
6
9
12
15
18
21
24
27
30
33
36
39
42
45
48
51
Time after Administration (Hours)

SUMMARY

Radioactive phosphorus was used as a "tracer" substance to study the affinity of tissues and materials from lambs low in phosphorus and fed for 36 days on Wilburton and Stillwater hay test rations. Certain tissues and materials were found to have a high affinity for P^{32}. Saliva and bone from the lambs fed Wilburton hay contained more radioactive phosphorus than that of lambs fed Stillwater hay or the control lambs. This same trend also appeared, but was less pronounced, in the case of blood, parotid gland, and liver. These data may be interpreted as indicating that the lambs fed the Wilburton hay ration were in greater need of phosphorus then those fed the Stillwater hay.

Table 1
The Percentage of Radioactive Phosphorus in 100 Grams of Material at 8, 13 and 49 Hours after Administration

Material Analyzed	Lambs fed:	Source of Material Ration 1	Ration 2	Ration 3
At 8 Hours				
Blood		.0003	.0003	.0001
Saliva (100 c.c.)		.0021	.0019	.0021
Parotid gland		.0019	.0021	.0008
Bone		.0005	.0009	.0002
Liver		.0030	.0031	.0008
Rumen solids		.0089	.0085	.0100
Rumen liquid		——	.0053	.0097
At 13 Hours				
Blood		.0003	.0004	.0012
Saliva (100 c.c.)		.0059	.0024	.0011
Parotid gland		.0027	.0034	.0007
Bone		.0019	.0017	.0005
Liver		.0056	.0052	.0017
Rumen solids		.0083	.0105	.0072
Rumen liquid		.0050	.0067	.0053
At 49 Hours				
Blood		.0026	.0001	.0003
Saliva (100 c.c.)		.0030	.0011	.0017
Parotid gland		.0030	.0018	.0025
Bone		.0047	.0027	.0025
Liver		.0057	.0035	.0057
Rumen solids		.0044	.0029	.0050
Rumen liquid		.0026	.0016	.0026

Table 2

Chemical Analyses of Tissues and Materials

Material	Lamb No.: 1	3	5[1]	2	4	6[2]	A	B	C[3]
	Source								
	Per cent phosphorus, dry matter basis								
Bone (ether extracted)	13.34	12.20	12.19	11.96	12.14	12.11	12.05	11.92	12.55
Average		12.57			12.07			12.14	
	Milligrams phosphorus per 100 ml.								
Saliva									
6:30 p.m. (1-12-52)			53.6			97.6			65.6
10:30 p.m. (1-12-52)			81.6			109.6			67.2
9:00 a.m. (1-13-52)			56.0			78.4			84.8
Average			63.7			95.2			72.5
Saliva	38.0	26.0	44.0	38.0	44.0	56.0	50.0	48.0	32.0
Average		36.0			46.0			43.0	
	Per cent ash, dry matter basis								
Bone	67.99	67.84	67.23	68.37	67.96	66.96	67.38	68.32	68.34
Average		67.68			67.76			68.01	
	Per cent phosphorus, fresh basis								
Parotid	0.26	0.22	0.07	0.14	0.17	0.17	0.20	0.23	0.19
Average		0.18			0.16			0.21	

[1]Lambs fed Wilburton hay ration (ration 1).

[2]Lambs fed Stillwater hay ration (ration 2).

[3]Lambs fed control ration (ration 3).

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Thesis: I. COMPARATIVE AVAILABILITY OF PHOSPHORUS IN NATIVE GRASS HAY PRODUCED IN SOUTHEASTERN AND NORTH CENTRAL OKLAHOMA

II. EFFECT OF MANGANESE INTAKE ON CALCIUM AND PHOSPHORUS UTILIZATION BY LAMBS

III. PHOSPHORUS UTILIZATION BY LAMBS AS DETERMINED WITH THE RADIOACTIVE ISOTOPE P^{32}

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The content and form have been checked and approved by the author and thesis adviser. Changes or corrections in the thesis are not made by the Graduate School office or by any committee. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

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