# THE EFFECT OF HIGH MANGANESE INTAKES ON THE CALCIUM AND PHOSPHORUS METABOLISM BY SHEEP

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TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF ILLUSTRATIONS

# INTRODUCTION

The presence in feeds of manganese and other trace elements has become of nutritional importance in the feeding of farm animals during recent years. Investigations into the distribution of these elements in plants, and their metabolic role in animals, have offered a possible explanation for the unthrifty nature of cattle observed in southeastern Oklahoma, near Wilburton. This unthriftiness is characterized by poor weight gains, poor calf crops, and other submaximal physical conditions.

The forages in the Wilburton area are frequently deficient in phosphorus. Although phosphorus supplementation has some effect in alleviating the unthriftiness, the results so far obtained cannot be considered satisfactory. The forages in this area also often contain considerably larger amounts of manganese than comparable forages in other areas of Oklahoma which are considered to be normal. A study of the effect of high manganese intakes on the retention of calcium and phosphorus seemed desirable, therefore, to characterize more completely the condition existing in the Wilburton area.

## LITERATURE REVIEW

Literature concerning the effect of manganese fed at high levels is relatively limited. However, manganese in small amounts representing less than 50 ppm. of ration is known to be required for the normal development of bone in various species (11) (32). Kemmerer et al. (17) were able to show that mice, when fed on a manganese deficient ration consisting of whole cow's milk, did not reproduce normally. This difficulty was alleviated when the cow's milk was supplemented with manganese (0.01 mg. of manganese per mouse per day) in the form of manganese chloride. Other animals which are known to require manganese in small amounts are rabbits (29), cattle (18), swine (21) (16), and chickens (10). Lardy et al. (18) showed that bulls require more than 28 ppm. manganese for production of a good quality semen. Thirty-five to 50 ppm. of manganese are required for the prevention of perosis in chickens (10). Pigs fed a ration containing 12 ppm. of manganese made better gains when the ration was supplemented with manganese at levels up to, but not beyond, 55 ppm. of ration; at levels of 80, 160, and 500 ppm. of ration, gains were reduced (14) (13).

Manganese is known to function in the liver arginase enzyme system (7), and it has been suggested that it may function in others. It has also been reported that bacteria, protozoa, and other rumen organisms need manganese to function properly in the process of digestion (4). This is particularly true for the digestion of fiber and in building up proteins and the whole complex of water-soluble vitamins, including vitamin $B_{12}$.

It has been indicated that there is an interrelationship between manganese and vitamin $B_1$. Perla and Sandberg (23) found that the toxic manifestations of an excess of vitamin $B_1$ were dependent on the ratio of manganese to vitamin $B_1$ in the diet. The addition of manganese to the diet in amounts of 2 mg. per rat per day completely neutralized the unfavorable effects of the excess of vitamin $B_1$ (400 gamma daily). Perla et al. (24) observed that rats are enabled to store a greater part of an unusually high manganese intake if large amounts of vitamin $B_1$ are offered at the same time.

When fed at high levels of intake, manganese is toxic. Grummer et al. (14) found that growing pigs would not tolerate a high level of manganese (500 ppm. of ration). At this level the pigs exhibited a slowed rate of gain and poorer appetite. Toward the end of the experiment, the pigs exhibited a stiffness of limbs and stilted gait. Findlay (8) reported that large doses of manganese chloride administered subcutaneously are extremely toxic. It was possible to produce liver cirrhosis in rabbits, rats, and guinea pigs by repeated injections of manganese chloride. However, Becker and McCollum (2) were unable to verify these drastic results with rats, using similar procedures, although they did note a decreased rate of gain at the high levels of intake (99.8 mg. of manganese per rat per day).

Carratala and Carbonischi (5) determined the minimum lethal dose of various manganese compounds when injected intravenously into rabbits. Their results were as follows: 30 mg. manganese dioxide, killing in an average of 11 minutes; manganese carbonate, 50 mg. killing in 15 minutes; manganese chloride, 80 mg. killing in 2 minutes; and manganese sulfate, 120 mg. killing in 4 minutes. They also noted that chronic poisoning developed in rabbits after feeding 0.7 to 0.9 mg. manganese sulfate daily

for 20 to 25 days or after feeding 0.10 to 0.94 mg. of manganese chloride for a similar period of time. Greatest damage was done to the liver. The bone marrow was affected the least.

The soluble manganese salt, manganese chloride, in the absence of supplemental vitamin D, has been shown to produce a rachitic effect in rats, but the more difficultly soluble manganese dioxide did not produce this effect (3). This indicated, as would be expected, that the toxic effects produced by the supplemental salts are directly related to the solubility of that salt. 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 ration low in both calcium and phosphorus. In general, the absorption of manganese from the intestinal tract is very slow and incomplete under normal conditions (33). This fact could account for the relatively high value of 0.1 gram of manganese per pound of body weight, proposed by Monier-Williams (22), as the critical level of intake.

The principal place of manganese storage in animals is the liver and the kidneys. Richards (28) found a greater storage in the liver and pancreas of sheep than in the kidneys. The duodenum of sheep also contains considerable amounts of manganese. When manganese is fed, at least in the case of pigs, these organs show greater variations in manganese content than do the same organs in animals on normal rations (28).

Greenberg, Copp, and Cuthbertson (12) have shown that the bile plays an important role in the excretion of manganese. In their radioactive studies with rats given intraperitoneal injections of manganese, they found that 50 to 75 per cent of the manganese that makes its way from the body into the intestinal canal was carried by the bile. The amount of manganese excreted by way of the urine was only a small fraction of the

total. Only about 3 or 4 per cent of the manganese orally ingested was absorbed. A wide range of manganese intakes (0.1 to 20.0 mg./mother rat/day) was permissible during the lactation period without affecting the mother or her young (15). This observation is probably another reflection of the inefficient absorption of manganese from the intestinal tract of the mother, and inefficient passage of dietary manganese into the milk.

In a study with lactating cows, Reid et al. (25) showed that an unusually large number of negative calcium balances were obtained when a basal ration of natural feeds was supplemented with manganese sulfate. This marked depression of calcium metabolism appeared to be effected by the manganese sulfate supplementation. It is noteworthy that this depression of calcium metabolism could be largely prevented by the ingestion of other trace minerals (iodine, magnesium, copper, cobalt, zinc, and iron). The metabolism of phosphorus did not seem to be affected by the manganese supplement. Reid and Ward (26) later reported that, regardless of the quantity of manganese ingested (622.4 to 1325.6 mg. per day), lactating cows retained only 154.4 $\pm$ 9.8 mg. of manganese per day. The amount of manganese excreted in the feces was found to be directly proportional to the amount ingested. They also reported that the manganese sulfate was utilized equally as well as that provided by the feed material. As yet, however, these results have not been verified.

## EXPERIMENTAL PROCEDURE

Two experiments were conducted to determine the effect of high intakes of manganese on the retention of calcium and phosphorus by lambs.

In Experiment I, balance trials were conducted with lambs weighing from 54 to 77 pounds. The 12 animals were divided, according to weights, into three comparable groups of four lambs each. They were placed in metabolism stalls with provisions made for the quantitative separation and collection of urine and feces. For 10 days preceding and during the 10-day collection period, all lambs received a basal ration which consisted of prairie hay, 48.6 per cent; soybean meal, 20.4 per cent; corn, 28.9 per cent; salt (NaCl), 1.7 per cent; 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 supplemental manganese at levels of 0, 500, and 1000 ppm. of ration. Water was offered ad lib. but no record was kept of the amount consumed. The lambs were rotated in the second and third trials such that at the end of the experiment each lamb had received all three levels of manganese intake. Between trials, the animals were placed in a small pen for a few days in order that they might receive some exercise before the next trial was started. Individual feeding stanchions were provided in the pen so that the animals could be maintained on the experimental rations.

In Experiment II, essentially the same routine was followed. However, several changes were made in order to improve the second experiment. A record was kept of the consumption of water. This was deemed necessary in order to obtain an accurate measurement of calcium intake,

since it was found that the water supply contained an average of 0.03 gram of calcium per liter. Upon analysis of the feces and urine samples collected in Experiment I, it was obvious that considerable amounts of manganese were being carried over by the lambs from one trial to the next. This carry over was especially noticeable when the lambs receiving the high level of manganese (1000 ppm. of ration) in one trial were switched to the zero level in the following trial. In order to prevent, or at least diminish, this carry over of manganese, the preliminary feeding period was lengthened to 20 days.

Manganese carbonate was used as the source of supplemental manganese in this experiment. Thomas et al. (19) reported that lambs which were deficient in sulfur exhibited gradual failure of appetite, loss of body weight, emaciation, and death. Thus, it was felt that any benefical effect which the sulfur might have produced in Experiment I, when manganese sulfate was used as the supplement, could have overshadowed any detrimental effects caused by the high manganese intakes. Also, if the same results were obtained in both experiments, they could be attributed to the manganese intake and not to the anion which was present. A random plan for the rotation of rations was adopted. This plan was used so that any results which were obtained would have a greater statistical meaning.

Six lambs weighing from 60 to 64 pounds were used in this experiment. The basal ration consisted of prairie hay, 47.7 per cent; soybean meal, 20.8 per cent; and ground corn, 31.5 per cent. Salt and calcium carbonate were mixed with the corn. This ration supplied 15.44 per cent protein, 0.46 per cent calcium, and 0.27 per cent phosphorus (dry matter basis). The same levels of manganese supplementation were used in the second experiment as in the first one. In all trials of both experiments, adequate vitamin D was supplied by adding 1 ml. of high potency cod liver oil to the ration daily.

Feed samples which were collected during the 10-day collection period were, if necessary, ground in a Wiley mill, mixed well, and an appropriate size sample (8 ounce jar) taken for the various chemical analyses. Aliquots of the daily urine collections were made acid with hydrochloric acid and stored in a refrigerator. Aliquots of the daily fecal material were mixed thoroughly and 200 grams were dried to a constant weight to determine the moisture content of the fresh material. These dried samples were then ground in the Wiley mill, remixed and retained for analysis.

## Chemical Analyses

Routine analyses--protein, moisture, and ash--were run on all samples. Protein was determined by the Kjeldahl procedure (20). Moisture was determined by drying the samples in an electric oven at 100 to 105°C. for a period of four hours. Ash was determined by igniting the sample in a muffle furnace at 600 to 650°C. for two hours.

In the preparation of samples for the mineral analyses, the dry ashing procedure was used exclusively. While it is generally recognized that wet ashing with combinations of nitric, sulfuric, and perchloric acids may, in some instances, be superior to dry ashing, lack of available laboratory equipment made it impossible to utilize this type of preparation. Dry ashing is most successfully accomplished in a muffle furnace which is equipped with controls to prevent overheating. This has the advantage of maintaining a constant temperature, while flame procedures do not. Sandell (30) shows that in samples ashed in a muffle furnace, the quantity of manganese forming insoluble silicates is considerably less than is formed by ashing over a flame. The quantity forming

insoluble silicates at a low muffle-furnace temperature of 500°C. is about 1.0 per cent of the total manganese present in the sample.

Samples on which calcium, phosphorus, and manganese were to be determined were prepared, therefore, in the following manner. An appropriate size sample was weighed into a 150 ml. pyrex beaker and ashed for 15 hours in a muffle furnace set at 500 to 550°C. The ash was taken up in 15 ml. of 1:1 hydrochloric acid (1 part of water to 1 part of concentrated hydrochloric acid) and digested for four hours on a steam plate. This solution was then evaporated to dryness on a steam plate and the silica dehydrated by further heating of the dry residue for a period of one hour. The residue was again taken up in 15 ml. of 1:1 hydrochloric acid and heated near the boiling temperature for two hours to insure the complete solution of all minerals present.

The entire contents of the beaker were then filtered into a 250 ml. volumetric flask. The filter paper was washed free of the filtrate by repeatedly washing with hot water, and the insoluble residue treated as indicated below for combined feces and urine samples. This solution was diluted to volume and suitable aliquots were taken for the determination of the various minerals.

In order to make an accurate analysis of the small amounts of minerals present in the urine, a proportionate amount of urine was added to the corresponding feces sample. This mixture was evaporated to dryness in an oven, and then ashed and digested according to the above outlined procedure. However, it was found that considerable amounts of manganese were retained in the insoluble residue when the ash of these combined samples of urine and feces were treated with hydrochloric acid. To correct this error in the manganese determination, it was necessary to volatilize the silica. The insoluble residue and filter paper, therefore,

were placed in platinum dishes and dried in an oven. The filter paper was then burned off in a muffle furnace. The ash was moistened with distilled water and six drops of concentrated sulfuric acid were added. After the addition of about 2 ml. of hydrofluoric acid to each sample, they were then placed on a steam plate which was located in the hood. They were allowed to remain on the steam plate for two hours to insure complete evaporation of all excess hydrofluoric acid. If large amounts of silica were present, the addition of hydrofluoric acid was repeated. The soluble residue was then dissolved in 15 ml. of 1:1 hydrochloric acid and added to the original hydrochloric acid extract. By using this procedure, the recovery of manganese was quite satisfactory.

The method used in determining manganese was a modification of that used by Willard and Greathouse (34). From the solution prepared as above, an aliquot containing from 0.01 to 0.06 mg. of manganese was taken. To this solution was added 5 ml. of 1:1 nitric acid and the solution was evaporated to dryness. Evaporation with nitric acid is necessary to remove chlorides and other oxidizable materials (27) which would require the use of excess periodate, or cause a fading of the permanganate color. This step was repeated three times to insure complete removal of the chlorides.

The chloride-free residue was taken up in 10 ml. of one normal nitric acid and 1 ml. of 85 per cent ortho-phosphoric acid. The latter was added to deionize and decolorize the ferric iron, and thus prevent its interference (1) (34). After heating this solution to near boiling, about 50 mg. of potassium periodate were added in small amounts to oxidize the manganous ion to permanganate ion. The reaction is as follows:

$$2Mn^{++} + 5IO_4^- + 3H_2O \longrightarrow 2MnO_4^- + 5IO_3^- + 6H^+$$

The beaker was covered with a watch glass and heated for one hour to insure complete oxidation. The solution was made to a volume of 25 ml. and then transferred to a colorimeter tube. Light transmittance was measured in an Evelyn photoelectric colorimeter, a 515 mu. filter being used. Manganese concentration was then calculated from a standard curve.

The concentration of acid in the solution during oxidation is of some importance. Unless the concentration of manganese is very small, the acid concentration may be increased considerably over the quantity required to prevent precipitation of periodates. The speed of oxidation is increased if the acid concentration is above 3.5 N. (27). In this regard, we found that the acid strength could be more easily controlled if the color were developed in calibrated test tubes instead of beakers, since the amount of solution lost by evaporation was considerably less. Because it was more difficult to stir the solutions thoroughly, the time allowed for color development was extended to two hours.

Calcium was determined by the oxalate method as proposed by the Association of Official Agricultural Chemists (20). In this procedure the aliquot was made to a volume of 100 ml. The solution was heated nearly to boiling and 15 ml. of saturated ammonium oxalate were added. Three drops of methyl red indicator were placed in the solution. It was then neutralized to a faint pink with ammonium hydroxide. The solution was kept hot for a period of two hours to insure complete precipitation and then filtered through a Gooch crucible. The crucible and precipitate were washed free of chlorides, the calcium oxalate dissolved in normal sulfuric acid, and the solution titrated with 0.05 N. potassium permanganate.

Phosphorus was determined by the method of Fiske and Subbarow (9). An aliquot of the original solution containing 0.003 to 0.02 mg.

phosphorus was placed in a colorimeter tube and to it was added 1 ml. of molybdate solution I. The solution was diluted to 9.6 ml., and then 0.4 ml. of the 1-amino-2-naphthol-4-sulfonic acid color reagent was added. The solution was mixed thoroughly, and allowed to stand 30 minutes for complete color development. Phosphorus was determined by measurement of light transmittance at 660 mu. in the Evelyn colorimeter.

## RESULTS AND DISCUSSION

In Table 1 is shown the effect of different levels of manganese intake on the average daily retention of calcium and phosphorus by lambs in Experiments I and II.

In the first trial of Experiment I, lamb number 5 was removed from the experiment because of feed refusals. In Experiment II, lambs number 5 and 6 refused small amounts of feed during trial 4. In trial 5, lamb number 5 again refused feed and in order to maintain comparable feed intakes within the triad the intakes of animals 4 and 6 were also decreased. In trial 6, lamb 5 was removed from the experiment. In all other trials of the two experiments the lambs maintained a constant daily feed intake.

From the data obtained in Experiment I, in which manganese sulfate was used as the manganese supplement, it was not possible to correlate the retention of calcium and phosphorus with the manganese intake. There was no indication that the high levels of manganese intake changed the path of excretion of either calcium or phosphorus since the fecal excretion of both minerals was essentially the same at all levels.

In Experiment II, when manganese carbonate was used as the supplemental salt, there was an indication that high levels of manganese, or other factors, caused an increase in the fecal excretion of both calcium and phosphorus. In all three trials of Experiment II, the fecal excretion of calcium and phosphorus was increased when manganese was supplemented at a level of 500 ppm. of ration. The excretion of calcium and phosphorus, in two of the three trials, was less at the 1000 ppm. level of manganese than at the 500 ppm. level; the excretion of these minerals

TABLE 1

Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. on Average Daily Retention of Calcium and Phosphorus by Sheep

| Trial | Ration ppm. Mn | Number of animals[1] | CALCIUM | | | | PHOSPHORUS | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Intake | Feces | Urine | Retention | Intake | Feces | Urine | Retention |
| Experiment I. Manganese supplied as manganese sulfate | | | | | | | | | | |
| | | | 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 | 0.00 | 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 |
| Experiment II. Manganese supplied as manganese carbonate | | | | | | | | | | |
| 4 | 0 | 2 | 2.68 | 2.55 | 0.19 | -0.06 | 1.60 | 1.59 | 0.02 | -0.01 |
| 4 | 500 | 2 | 2.60 | 2.87 | 0.25 | -0.52 | 1.54 | 1.84 | 0.01 | -0.31 |
| 4 | 1000 | 2 | 2.67 | 2.56 | 0.22 | -0.11 | 1.52 | 1.54 | 0.02 | -0.04 |
| 5 | 0 | 2 | 2.30 | 1.92 | 0.05 | +0.33 | 1.38 | 1.26 | 0.07 | +0.05 |
| 5 | 500 | 2 | 2.42 | 2.31 | 0.06 | +0.05 | 1.48 | 1.49 | 0.03 | -0.04 |
| 5 | 1000 | 2 | 2.44 | 2.52 | 0.16 | -0.24 | 1.47 | 1.63 | 0.01 | -0.17 |
| 6 | 0 | 2 | 2.89 | 1.86 | 0.11 | +0.92 | 1.61 | 1.24 | 0.03 | +0.34 |
| 6 | 500 | 2 | 2.87 | 2.28 | 0.05 | +0.54 | 1.62 | 1.58 | 0.00 | +0.04 |
| 6 | 1000 | 1 | 2.84 | 1.93 | 0.00 | +0.91 | 1.62 | 1.29 | 0.07 | +0.26 |

[1] One animal in trial 1 and one in trial 6 were eliminated from the experiment because of feed refusals.

was always as great or greater at the 1000 ppm. level than at the zero level. There is no ready explanation for the smaller excretion of calcium and phosphorus at the 1000 ppm. level of manganese intake than at the 500 ppm. level.

In Table 2 the fecal, urinary, and total excretions of calcium and phosphorus are expressed as a per cent of the intake. It should be pointed out that in Experiment I the fecal excretion of calcium, with the exception of trial 3, represents more than 80 per cent of the intake. The same is true in Experiment II except that the calcium excretion in trial 6 was considerably less than in the other two trials. It should also be noted that the calcium excreted in the urine was less in Experiment II than in Experiment I. This decrease in urinary calcium was probably caused by the basic salt, manganese carbonate, used in Experiment II as compared to the acidic salt, manganese sulfate, used in the first experiment. Practically all of the phosphorus, in both experiments, was excreted by way of the feces. The urinary phosphorus never amounted to more than 4.7 per cent of the intake in either experiment. These data are shown graphically in Figures 1 and 2.

Table 3 shows a summary of the calcium and phosphorus excretions for both experiments. The average excretion of calcium in Experiment I, when expressed as a per cent of the intake, is 97.1, 96.4, and 96.4 per cent for the 0, 500, and 1000 ppm. levels, respectively. Corresponding values for phosphorus are 95.0, 94.4, and 93.7 per cent, respectively. In Experiment II, the calcium excretion at the 0, 500, and 1000 ppm. levels is 84.7, 99.2, and 94.3 per cent, respectively; for phosphorus the values are 92.2, 106.3, and 100.0 per cent, respectively. Thus, it may be seen that there is a greater difference in calcium and phosphorus excretion between the zero and 500 ppm. levels of manganese

TABLE 2

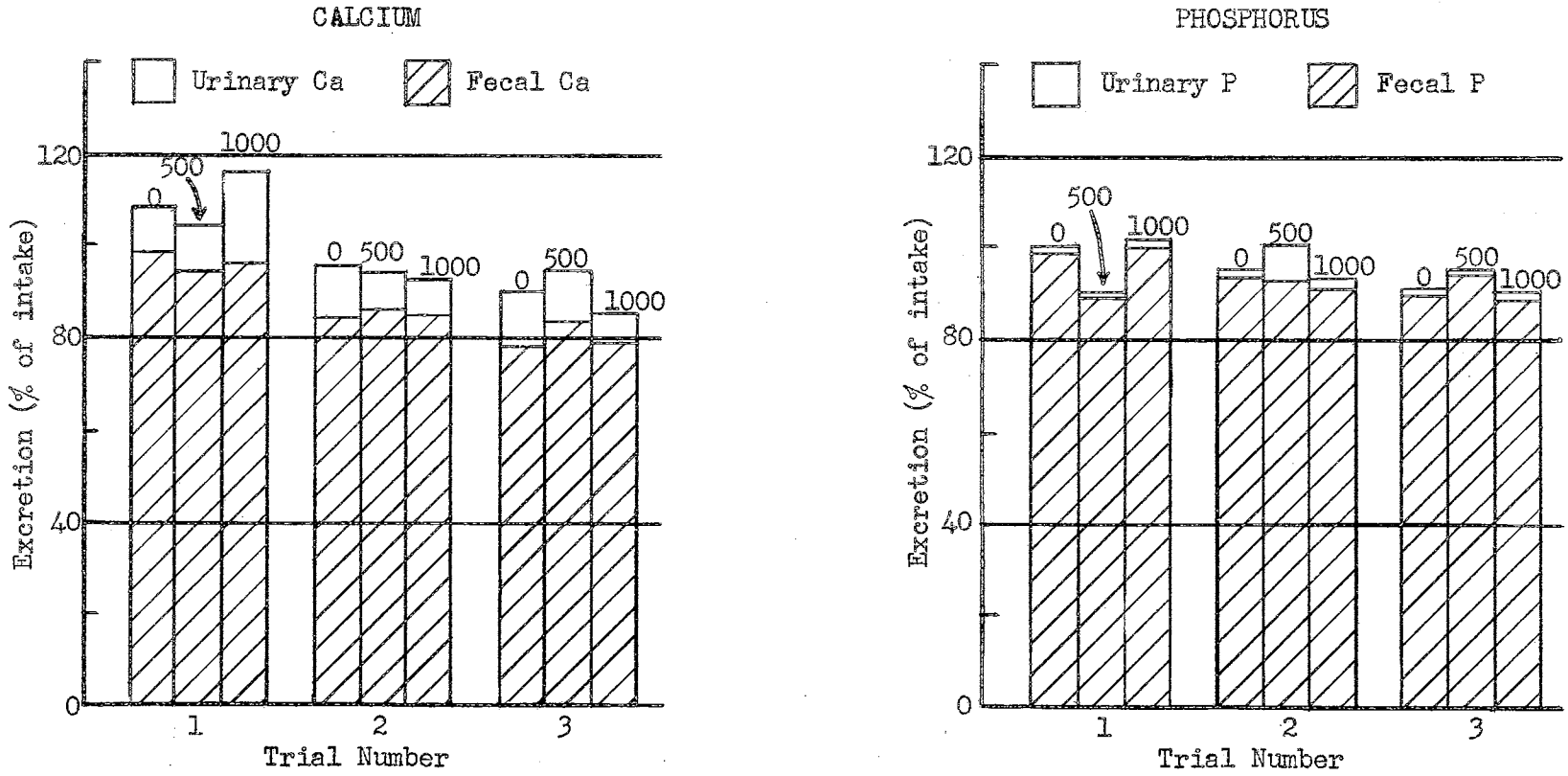
Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration on Excretion of Calcium and Phosphorus by Sheep[1]

| Trial | Ration ppm. Mn | Number of animals | CALCIUM | | | PHOSPHORUS | | |
|---|---|---|---|---|---|---|---|---|
| | | | Fecal excretion | Urinary excretion | Total excretion | Fecal excretion | Urinary excretion | Total excretion |
| Experiment I. Manganese supplied as manganese sulfate | | | | | | | | |
| | | | % | % | % | % | % | % |
| 1 | 0 | 4 | 96.2 | 11.3 | 107.5 | 97.8 | 1.2 | 99.0 |
| 1 | 500 | 3 | 92.9 | 10.9 | 103.8 | 89.4 | 0.6 | 90.0 |
| 1 | 1000 | 4 | 92.8 | 20.0 | 112.8 | 100.4 | 0.6 | 101.0 |
| 2 | 0 | 4 | 84.6 | 10.5 | 95.1 | 95.4 | 0.6 | 96.0 |
| 2 | 500 | 4 | 86.4 | 8.0 | 94.4 | 94.6 | 4.4 | 99.0 |
| 2 | 1000 | 4 | 83.9 | 8.4 | 92.3 | 89.5 | 2.5 | 92.0 |
| 3 | 0 | 4 | 78.9 | 10.0 | 88.9 | 88.1 | 0.9 | 89.0 |
| 3 | 500 | 4 | 81.2 | 10.3 | 91.5 | 92.4 | 0.6 | 93.0 |
| 3 | 1000 | 4 | 78.6 | 5.5 | 84.1 | 87.4 | 1.6 | 89.0 |
| Experiment II. Manganese supplied as manganese carbonate | | | | | | | | |
| 4 | 0 | 2 | 94.9 | 7.1 | 102.0 | 99.4 | 1.6 | 101.0 |
| 4 | 500 | 2 | 110.4 | 9.6 | 120.0 | 119.4 | 0.6 | 120.0 |
| 4 | 1000 | 2 | 95.8 | 8.2 | 104.0 | 101.7 | 1.3 | 103.0 |
| 5 | 0 | 2 | 84.0 | 2.0 | 86.0 | 91.3 | 4.7 | 96.0 |
| 5 | 500 | 2 | 95.3 | 2.7 | 98.0 | 101.0 | 2.0 | 103.0 |
| 5 | 1000 | 2 | 103.4 | 6.6 | 110.0 | 111.3 | 0.7 | 112.0 |
| 6 | 0 | 2 | 64.0 | 4.0 | 68.0 | 77.1 | 1.9 | 79.0 |
| 6 | 500 | 2 | 79.4 | 1.6 | 81.0 | 98.0 | 0.0 | 98.0 |
| 6 | 1000 | 1 | 68.0 | 0.0 | 68.0 | 79.7 | 4.3 | 84.0 |

[1]Excretions expressed as a per cent of the intake.

FIGURE 1

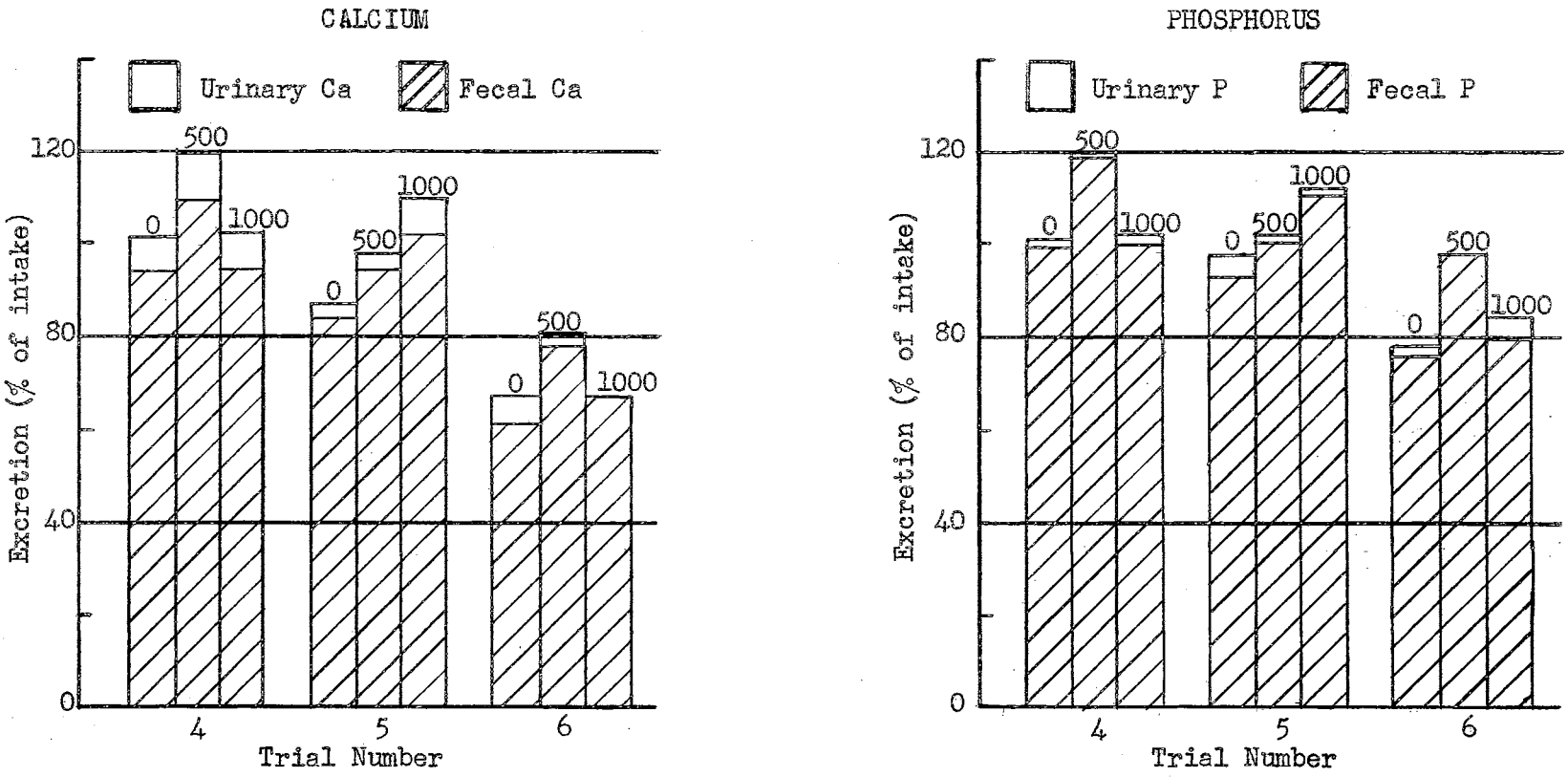
Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration
on Excretion of Calcium and Phosphorus by Sheep
(Manganese Supplied as Manganese Sulfate)




Values shown represent the average of 4 sheep fed each level of manganese.

FIGURE 2

Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration
on Excretion of Calcium and Phosphorus by Sheep
(Manganese Supplied as Manganese Carbonate)




Values shown represent the average of 2 sheep fed each level of manganese.

intake than between the 500 and 1000 ppm. levels. Although the differences in calcium and phosphorus excretion between treatments are small, it should be pointed out that whatever effect manganese has on calcium excretion, it appears to have a similar effect on phosphorus. This could mean that the effect of large amounts of manganese is on the calcification mechanism in bone as proposed by Chornock et al. (6).

TABLE 3

Summary of Calcium and Phosphorus Excretions for Experiments I and II

| Expt. | Ration ppm. Mn | CALCIUM Intake gm. | CALCIUM Excretion gm. | CALCIUM Excretion %[1] | PHOSPHORUS Intake gm. | PHOSPHORUS Excretion gm. | PHOSPHORUS Excretion %[1] |
|---|---|---|---|---|---|---|---|
| 1 | 0 | 2.74 | 2.66 | 97.1 | 1.61 | 1.53 | 95.0 |
| 1 | 500 | 2.74 | 2.64 | 96.4 | 1.61 | 1.52 | 94.4 |
| 1 | 1000 | 2.74 | 2.64 | 96.4 | 1.61 | 1.51 | 93.7 |
| 2 | 0 | 2.62 | 2.22 | 84.7 | 1.53 | 1.41 | 92.2 |
| 2 | 500 | 2.63 | 2.61 | 99.2 | 1.55 | 1.65 | 106.3 |
| 2 | 1000 | 2.61 | 2.46 | 94.3 | 1.52 | 1.52 | 100.0 |

[1]Values expressed as a per cent of the intake.

Differences in calcium excretion at different levels of manganese intake in Experiment II may not have been due entirely to manganese alone. Manganese carbonate is a basic salt and thus would cause the ration to be somewhat more basic than the ration used in Experiment I. It is known that an excess of basic anions in the intestinal tract is detrimental to the absorption of calcium (31).

Chornock et al. (6) showed that rats fed the Steenbock diet developed a rachitic condition that was intensified by the presence of large amounts of manganese. Upon addition of phosphate to the diet the effect of the manganese was nullified. They were also able to show that the administration of vitamin D to rats receiving high manganese diets

improved the condition of the animals regardless of the level of manganese fed. Therefore, with animals fed a ration containing adequate calcium, phosphorus, and vitamin D, perhaps only small differences in calcium and phosphorus retention are to be expected.

Calcium and inorganic phosphorus were determined in the blood plasma of the lambs during trials 2 and 3. In trial 2 the calcium content of the plasma ranged from 9.6 to 11.4 mg. per cent and phosphorus ranged from 3.76 to 5.60 mg. per cent. In trial 3 the calcium was somewhat lower, 8.2 to 10.5 mg. per cent. Plasma phosphorus in trial 3 was essentially the same as in trial 2 and ranged from 4.72 to 6.04 mg. per cent. These data are shown in Table 1 of the Appendix. Neither the calcium nor the phosphorus content of the plasma was correlated with the manganese intake. Blood was not analyzed during Experiment II.

The amount of manganese absorbed by the animals in Experiments I and II is shown in the Appendix in Tables 2 and 4, respectively. In Experiment I it was found that the amount of manganese absorbed increased as the manganese intake was increased. Although the manganese absorbed by the animals in Experiment II follows this same trend, the amount absorbed at the high levels of intake was considerably less than in Experiment I. Therefore, differences between Experiment I and II in average excretion of calcium and phosphorus (Table 3) were not related to the amount of manganese absorbed.

The animals receiving the zero level of supplemental manganese were frequently in negative manganese balance. This was attributed to the carry over of manganese from the preceding trial. The extremely high fecal excretion of manganese for animal 7 in trial 1 and for animal 4 in trial 4 could not be explained. However, it was suspected that those

animals received one of the higher levels of manganese supplementation, one or more times, during the collection period.

As a check on the accuracy and repeatibility of the manganese determination, replicate samples of feeds and feces were ashed and oxidized on different days. These results are shown in Tables 4 and 5. Table 4 shows the repeatibility of the manganese determination on hay samples. It should be noted that samples 58a, 84a, and 112a are all considerably higher than repeat determinations for these samples. Since all three samples showed a higher content of manganese it would appear that they had in some manner become contaminated. If the "a" samples are disregarded, the maximum variation between determinations is 0.48 mg. manganese per 100 grams. When aliquots of the same solution were oxidized on different days, the following results were obtained: 8.84, 8.84, 8.74, and 8.84 mg. manganese per 100 grams of sample. The determination of replicate feces samples is shown in Table 5. It may be seen that with proper precautions the determination is quite accurate even at relatively high concentrations of manganese. The maximum variation in sample number 64 was 18.9 mg. or about 4.4 per cent of the total manganese present. It would appear, therefore, that repeat determinations on this type of material should check within at least 5 per cent.

In Tables 3 and 5 of the Appendix is shown the effect of manganese intake on the digestibility of organic matter and on the retention of nitrogen for Experiments I and II. It is apparent that the manganese intake had no effect on either the organic matter digestibility or the nitrogen retention in either experiment.

TABLE 4

Manganese Content of Replicate Hay Samples Determined on Different Days

| Sample number | Galvanometer reading | Mg. Mn/ 100 gm. |
|---|---|---|
| 58a | 77.50 | 9.3 |
| 58b | 79.00 | 8.6 |
| 58c | 79.00 | 8.6 |
| Average | | 8.8 |

| Sample number | Galvanometer reading | Mg. Mn/ 100 gm. |
|---|---|---|
| 84a | 76.25 | 9.9 |
| 84b | 78.50 | 8.8 |
| 84c | 77.75 | 9.2 |
| 84d | 78.75 | 8.7 |
| 84e | 78.50 | 8.8 |
| Average | | 9.1 |

| Sample number | Galvanometer reading | Mg. Mn/ 100 gm. |
|---|---|---|
| 112a | 74.00 | 11.0 |
| 112b | 77.75 | 9.2 |
| 112c | 77.50 | 9.3 |
| 112d | 77.00 | 9.5 |
| 112e | 77.50 | 9.3 |
| Average | | 9.7 |

TABLE 5

Manganese Content of Replicate Feces Samples Determined on Different Days

| Sample number | Mg. Mn/ 100 gm. |
|---|---|
| 62a | 20.1 |
| 62b | 22.2 |
| 62c | 22.2 |
| 62d | 19.3 |
| Average | 21.0 |

| Sample number | Mg. Mn/ 100 gm. |
|---|---|
| 63a | 238.5 |
| 63b | 238.4 |
| 63c | 245.0 |
| 63d | 231.9 |
| Average | 238.5 |

| Sample number | Mg. Mn/ 100 gm. |
|---|---|
| 64a | 429.3 |
| 64b | 410.6 |
| 64c | 423.6 |
| 64d | 415.5 |
| Average | 419.8 |

## SUMMARY

The effect of high levels of manganese intake on calcium and phosphorus metabolism was investigated in two balance experiments with sheep fed rations containing 0, 500, and 1000 ppm. of added manganese. The manganese was added as manganese sulfate in Experiment I and as manganese carbonate in Experiment II. In both experiments the rations contained about 0.47 per cent calcium, 0.28 per cent phosphorus, and supplemental vitamin D.

In Experiment I, the level of manganese intake had no measurable effect on the retention of either calcium or phosphorus. The calcium and inorganic phosphorus content of the blood plasma determined during two trials of Experiment I was found to be within the normal range at all levels of manganese intake.

In Experiment II, with manganese carbonate being used as the supplemental salt, there appeared to be a small increase in the excretion of both calcium and phosphorus. This increase was more evident at the level of 500 ppm. of manganese intake than at the level of 1000 ppm. Since the pattern of excretion was similar for both calcium and phosphorus, this could mean that manganese had some effect on normal calcification processes in the bone.

The absorption of manganese increased in both experiments as the level of manganese intake was increased. However, the amount absorbed at the same levels of intake in Experiment II were considerably less than in Experiment I. Differences between Experiments I and II in calcium and phosphorus absorption, therefore, were not directly related to differences in manganese absorption.

The high levels of manganese intake had no effect on the digestibility of organic matter or on the retention of nitrogen by the lambs.

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# APPENDIX

## TABLE 1

Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration on the Calcium and Inorganic Phosphorus Content of the Blood Plasma of Sheep

| Lamb no. | TRIAL 2 | | | TRIAL 3 | | |
|---|---|---|---|---|---|---|
| | Ration | Phosphorus | Calcium | Ration | Phosphorus | Calcium |
| | ppm. Mn | mg. % | mg. % | ppm. Mn | mg. % | mg. % |
| 1 | 500 | 4.36 | 10.8 | 1000 | 5.20 | 10.0 |
| 2 | 1000 | 5.24 | 10.8 | 0 | 5.64 | 8.4 |
| 3 | 0 | 4.76 | 10.8 | 500 | 5.16 | 8.4 |
| 4 | 500 | 4.24 | 9.6 | 1000 | 4.72 | 8.2 |
| 5 | 1000 | 5.36 | 11.4 | 0 | 5.96 | 9.8 |
| 6 | 0 | 5.60 | 10.8 | 500 | 4.60 | 8.2 |
| 7 | 500 | 3.76 | 10.4 | 1000 | 5.76 | 8.2 |
| 8 | 1000 | 4.88 | 10.4 | 0 | 5.88 | 9.4 |
| 9 | 0 | 4.88 | 10.8 | 500 | 5.08 | 10.0 |
| 10 | 500 | 4.12 | 10.4 | 1000 | 5.20 | 10.0 |
| 11 | 1000 | 5.08 | 10.1 | 0 | 6.04 | 9.0 |
| 12 | 0 | 5.20 | 10.4 | 500 | 5.80 | 9.2 |

TABLE 2

Daily Intake and Excretion of Manganese, Calcium, and Phosphorus by Lambs in Experiment I (Manganese Supplied as Manganese Sulfate)

| Ration | Trial no. | Lamb no. | MANGANESE | | | CALCIUM | | |
|---|---|---|---|---|---|---|---|---|
| | | | Intake | Feces | Absorbed | Intake | Feces | Urine |
| ppm. Mn | | | mg. | mg. | mg. | gm. | gm. | gm. |
| 0 | 1 | 1 | 31.7 | 29.8 | +1.9 | 2.65 | 2.63 | 0.30 |
| 0 | 1 | 4 | 31.7 | 25.6 | +6.1 | 2.65 | 2.63 | 0.37 |
| 0 | 1 | 7 | 31.7 | 54.6 | -22.7 | 2.65 | 2.53 | 0.23 |
| 0 | 1 | 10 | 31.7 | 25.5 | +6.2 | 2.65 | 2.41 | 0.31 |
| 0 | 2 | 3 | 34.5 | 43.2 | -8.7 | 2.86 | 2.44 | 0.29 |
| 0 | 2 | 6 | 34.5 | 60.8 | -26.3 | 2.86 | 2.44 | 0.28 |
| 0 | 2 | 9 | 34.5 | 68.6 | -34.1 | 2.86 | 2.35 | 0.30 |
| 0 | 2 | 12 | 34.5 | 30.5 | +4.0 | 2.86 | 2.43 | 0.34 |
| 0 | 3 | 2 | 34.7 | 34.3 | +0.4 | 2.71 | 2.25 | 0.23 |
| 0 | 3 | 5 | 34.7 | 32.1 | +2.6 | 2.71 | 1.96 | 0.48 |
| 0 | 3 | 8 | 34.7 | 34.1 | +0.6 | 2.71 | 2.26 | 0.18 |
| 0 | 3 | 11 | 34.7 | 36.9 | -2.2 | 2.71 | 2.08 | 0.19 |
| Average | | | 33.6 | 39.7 | -3.8 | 2.74 | 2.37 | 0.29 |
| 500 | 1 | 2 | 398.4 | 327.3 | +71.1 | 2.65 | 2.56 | 0.25 |
| 500 | 1 | 8 | 398.4 | 316.5 | +81.9 | 2.65 | 2.68 | 0.39 |
| 500 | 1 | 11 | 398.4 | 298.4 | +100.0 | 2.65 | 2.15 | 0.22 |
| 500 | 2 | 1 | 401.3 | 343.7 | +57.6 | 2.86 | 2.50 | 0.25 |
| 500 | 2 | 4 | 401.3 | 333.0 | +68.3 | 2.86 | 2.52 | 0.23 |
| 500 | 2 | 7 | 401.3 | 354.9 | +46.4 | 2.86 | 2.70 | 0.18 |
| 500 | 2 | 10 | 401.3 | 329.1 | +72.2 | 2.86 | 2.17 | 0.25 |
| 500 | 3 | 3 | 401.6 | 343.3 | +58.3 | 2.71 | 2.22 | 0.28 |
| 500 | 3 | 6 | 401.6 | 325.8 | +75.8 | 2.71 | 2.16 | 0.33 |
| 500 | 3 | 9 | 401.6 | 347.2 | +54.4 | 2.71 | 2.22 | 0.34 |
| 500 | 3 | 12 | 401.6 | 389.1 | +12.5 | 2.71 | 2.22 | 0.16 |
| Average | | | 400.4 | 337.1 | +63.5 | 2.74 | 2.37 | 0.26 |
| 1000 | 1 | 3 | 765.1 | 603.0 | +162.1 | 2.65 | 2.55 | 0.39 |
| 1000 | 1 | 6 | 765.1 | 620.5 | +144.6 | 2.65 | 2.52 | 0.44 |
| 1000 | 1 | 9 | 765.1 | 642.0 | +123.1 | 2.65 | 2.71 | 0.75 |
| 1000 | 1 | 12 | 765.1 | 601.9 | +163.2 | 2.65 | 2.60 | -- [1] |
| 1000 | 2 | 2 | 768.0 | 632.4 | +135.6 | 2.86 | 2.50 | 0.26 |
| 1000 | 2 | 5 | 768.0 | 565.4 | +202.6 | 2.86 | 2.38 | 0.27 |
| 1000 | 2 | 8 | 768.0 | 589.1 | +178.9 | 2.86 | 2.62 | 0.19 |
| 1000 | 2 | 11 | 768.0 | 610.4 | +157.6 | 2.86 | 2.11 | 0.23 |
| 1000 | 3 | 1 | 768.4 | 582.1 | +186.3 | 2.71 | 2.00 | 0.22 |
| 1000 | 3 | 4 | 768.4 | 587.7 | +180.7 | 2.71 | 2.22 | 0.10 |
| 1000 | 3 | 7 | 768.4 | 623.5 | +144.9 | 2.71 | 2.28 | 0.13 |
| 1000 | 3 | 10 | 768.4 | 551.0 | +217.9 | 2.71 | 2.04 | 0.13 |
| Average | | | 767.2 | 600.8 | +166.5 | 2.74 | 2.38 | 0.28 |

[1] Sample accidently destroyed while in storage.

TABLE 2 (continued)

| CALCIUM | | PHOSPHORUS | | | | |
|---|---|---|---|---|---|---|
| Absorbed | Retained | Intake | Feces | Urine | Absorbed | Retained |
| gm. | gm. | gm. | gm. | gm. | gm. | gm. |
| +0.02 | -0.28 | 1.64 | 1.66 | 0.00 | -0.02 | -0.02 |
| +0.02 | -0.35 | 1.64 | 1.56 | 0.04 | +0.08 | +0.04 |
| +0.12 | -0.11 | 1.64 | 1.58 | 0.03 | +0.06 | +0.03 |
| +0.24 | -0.07 | 1.64 | 1.59 | 0.00 | +0.05 | +0.05 |
| +0.42 | +0.13 | 1.59 | 1.61 | 0.00 | -0.02 | -0.02 |
| +0.42 | +0.14 | 1.59 | 1.50 | 0.00 | +0.09 | +0.09 |
| +0.51 | +0.21 | 1.59 | 1.47 | 0.04 | +0.12 | +0.08 |
| +0.43 | +0.09 | 1.59 | 1.49 | 0.01 | +0.10 | +0.09 |
| +0.46 | +0.23 | 1.60 | 1.52 | 0.03 | +0.08 | +0.05 |
| +0.75 | +0.27 | 1.60 | 1.36 | 0.03 | +0.24 | +0.21 |
| +0.45 | +0.27 | 1.60 | 1.44 | 0.00 | +0.16 | +0.16 |
| +0.63 | +0.44 | 1.60 | 1.33 | 0.00 | +0.27 | +0.27 |
| +0.37 | +0.08 | 1.61 | 1.51 | 0.02 | +0.10 | +0.09 |
| +0.09 | -0.16 | 1.64 | 1.56 | 0.02 | +0.08 | +0.06 |
| -0.03 | -0.42 | 1.64 | 1.63 | 0.00 | +0.01 | +0.01 |
| +0.50 | +0.28 | 1.64 | 1.20 | 0.02 | +0.44 | +0.42 |
| +0.36 | +0.11 | 1.59 | 1.60 | 0.07 | -0.01 | -0.08 |
| +0.34 | +0.11 | 1.59 | 1.61 | 0.00 | -0.02 | -0.02 |
| +0.16 | -0.02 | 1.59 | 1.62 | 0.03 | -0.03 | -0.06 |
| +0.69 | +0.44 | 1.59 | 1.22 | 0.17 | +0.37 | +0.20 |
| +0.49 | +0.21 | 1.60 | 1.49 | 0.04 | +0.11 | +0.07 |
| +0.55 | +0.22 | 1.60 | 1.46 | 0.00 | +0.14 | +0.14 |
| +0.49 | +0.15 | 1.60 | 1.51 | 0.00 | +0.09 | +0.09 |
| +0.49 | +0.33 | 1.60 | 1.47 | 0.00 | +0.13 | +0.13 |
| +0.38 | +0.11 | 1.61 | 1.49 | 0.03 | +0.12 | +0.09 |
| +0.10 | -0.20 | 1.64 | 1.53 | 0.04 | +0.11 | +0.07 |
| +0.13 | -0.31 | 1.64 | 1.63 | 0.00 | +0.01 | +0.01 |
| -0.06 | -0.81 | 1.64 | 1.77 | 0.00 | -0.13 | -0.13 |
| +0.05 | -- [1] | 1.64 | 1.64 | -- [1] | 0.00 | -- [1] |
| +0.36 | +0.10 | 1.59 | 1.53 | 0.01 | +0.06 | +0.05 |
| +0.48 | +0.21 | 1.59 | 1.38 | 0.05 | +0.21 | +0.16 |
| +0.24 | +0.05 | 1.59 | 1.52 | 0.10 | +0.07 | -0.03 |
| +0.75 | +0.52 | 1.59 | 1.29 | 0.00 | +0.30 | +0.30 |
| +0.71 | +0.49 | 1.60 | 1.32 | 0.04 | +0.28 | +0.24 |
| +0.49 | +0.39 | 1.60 | 1.43 | 0.03 | +0.17 | +0.14 |
| +0.43 | +0.30 | 1.60 | 1.47 | 0.00 | +0.13 | +0.13 |
| +0.67 | +0.54 | 1.60 | 1.35 | 0.03 | +0.25 | +0.22 |
| +0.36 | +0.12 | 1.61 | 1.49 | 0.03 | +0.12 | +0.10 |

TABLE 3

Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration on Digestibility of Organic Matter and Nitrogen Retention (Manganese Supplied as Manganese Sulfate)

| Ration | Trial no. | Lamb no. | Dry Matter intake | ORGANIC MATTER | | | NITROGEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Intake | Feces | Digested | Intake | Feces | Urine | Apparent digested | Retained |
| | | | gm. | gm. | gm. | % | gm. | gm. | gm. | gm. | gm. |
| 0 | 1 | 1 | 576.0 | 533.0 | 119.5 | 77.58 | 13.87 | 3.12 | 8.27 | 10.75 | 2.48 |
| 0 | 1 | 4 | 576.0 | 533.0 | 115.4 | 78.35 | 13.87 | 3.05 | 8.06 | 10.82 | 2.76 |
| 0 | 1 | 7 | 576.0 | 533.0 | 129.0 | 75.80 | 13.87 | 3.38 | 6.54 | 10.49 | 3.95 |
| 0 | 1 | 10 | 576.0 | 533.0 | 131.3 | 75.37 | 13.87 | 3.51 | 7.19 | 10.36 | 3.17 |
| 0 | 2 | 3 | 581.0 | 535.0 | 127.0 | 76.26 | 13.98 | 3.35 | 8.39 | 10.63 | 2.24 |
| 0 | 2 | 6 | 581.0 | 535.0 | 131.8 | 75.36 | 13.98 | 3.43 | 8.52 | 10.55 | 2.03 |
| 0 | 2 | 9 | 581.0 | 535.0 | 123.9 | 76.84 | 13.98 | 3.20 | 7.19 | 10.78 | 3.59 |
| 0 | 2 | 12 | 581.0 | 535.0 | 131.2 | 75.48 | 13.98 | 3.21 | 7.81 | 10.77 | 2.96 |
| 0 | 3 | 2 | 586.0 | 539.0 | 117.9 | 78.13 | 14.21 | 3.23 | 7.69 | 10.98 | 3.29 |
| 0 | 3 | 5 | 586.0 | 539.0 | 110.8 | 79.44 | 14.21 | 2.89 | 7.56 | 11.32 | 3.76 |
| 0 | 3 | 8 | 586.0 | 539.0 | 116.1 | 78.46 | 14.21 | 3.14 | 8.31 | 11.07 | 2.76 |
| 0 | 3 | 11 | 586.0 | 539.0 | 124.6 | 76.88 | 14.21 | 3.19 | 7.94 | 11.02 | 3.08 |
| Average | | | 581.0 | 535.7 | 123.2 | 77.01 | 14.02 | 3.23 | 7.79 | 10.80 | 3.01 |
| 500 | 1 | 2 | 576.0 | 533.0 | 115.5 | 78.33 | 13.87 | 3.25 | 8.27 | 10.62 | 2.35 |
| 500 | 1 | 8 | 576.0 | 533.0 | 121.6 | 77.19 | 13.87 | 3.28 | 8.15 | 10.59 | 2.44 |
| 500 | 1 | 11 | 576.0 | 533.0 | 114.7 | 78.45 | 13.87 | 3.16 | 6.92 | 10.71 | 3.79 |
| 500 | 2 | 1 | 581.0 | 535.0 | 124.6 | 76.71 | 13.98 | 3.01 | 9.68 | 10.97 | 1.29 |
| 500 | 2 | 4 | 581.0 | 535.0 | 127.0 | 76.26 | 13.98 | 3.17 | 8.29 | 10.81 | 2.52 |
| 500 | 2 | 7 | 581.0 | 535.0 | 138.9 | 74.04 | 13.98 | 3.68 | 7.04 | 10.30 | 3.26 |
| 500 | 2 | 10 | 581.0 | 535.0 | 135.3 | 74.71 | 13.98 | 3.44 | 7.67 | 10.54 | 2.87 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 500 | 3 | 3 | 586.0 | 539.0 | 118.6 | 78.00 | 14.21 | 3.21 | 8.43 | 11.00 | 2.57 |
| 500 | 3 | 6 | 586.0 | 539.0 | 122.1 | 77.35 | 14.21 | 3.29 | 7.97 | 10.92 | 2.95 |
| 500 | 3 | 9 | 586.0 | 539.0 | 122.4 | 77.29 | 14.21 | 3.20 | 8.01 | 11.01 | 3.00 |
| 500 | 3 | 12 | 586.0 | 539.0 | 123.8 | 77.03 | 14.21 | 3.14 | 7.07 | 11.07 | 4.00 |
| Average | | | 581.0 | 535.7 | 124.0 | 76.85 | 14.02 | 3.26 | 7.95 | 10.78 | 2.82 |
| 1000 | 1 | 3 | 576.0 | 533.0 | 120.8 | 77.34 | 13.87 | 3.35 | 8.17 | 10.52 | 2.35 |
| 1000 | 1 | 6 | 576.0 | 533.0 | 131.8 | 75.27 | 13.87 | 3.56 | 7.91 | 10.31 | 2.40 |
| 1000 | 1 | 9 | 576.0 | 533.0 | 131.0 | 75.42 | 13.87 | 3.50 | 7.93 | 10.37 | 2.44 |
| 1000 | 1 | 12 | 576.0 | 533.0 | 115.7 | 78.30 | 13.87 | 3.17 | 7.74 | 10.70 | 2.96 |
| 1000 | 2 | 2 | 581.0 | 535.0 | 129.9 | 75.72 | 13.98 | 3.50 | 7.53 | 10.48 | 2.95 |
| 1000 | 2 | 5 | 581.0 | 535.0 | 141.5 | 73.55 | 13.98 | 3.37 | 7.17 | 10.61 | 3.44 |
| 1000 | 2 | 8 | 581.0 | 535.0 | 130.4 | 75.63 | 13.98 | 3.29 | 8.52 | 10.69 | 2.17 |
| 1000 | 2 | 11 | 581.0 | 535.0 | 113.5 | 78.79 | 13.98 | 3.21 | 8.53 | 10.77 | 2.24 |
| 1000 | 3 | 1 | 586.0 | 539.0 | 115.8 | 78.52 | 14.21 | 2.73 | 9.17 | 11.48 | 2.31 |
| 1000 | 3 | 4 | 586.0 | 539.0 | 115.1 | 78.65 | 14.21 | 2.95 | 7.98 | 11.26 | 3.28 |
| 1000 | 3 | 7 | 586.0 | 539.0 | 125.8 | 76.66 | 14.21 | 3.30 | 7.49 | 10.91 | 3.42 |
| 1000 | 3 | 10 | 586.0 | 539.0 | 124.0 | 77.00 | 14.21 | 3.24 | 8.25 | 10.97 | 2.72 |
| Average | | | 581.0 | 535.7 | 124.6 | 76.74 | 14.02 | 3.26 | 8.03 | 10.76 | 2.72 |

TABLE 4

Daily Intake and Excretion of Manganese, Calcium, and Phosphorus by Lambs in Experiment II (Manganese Supplied as Manganese Carbonate)

| Ration | Trial no. | Lamb no. | MANGANESE | | | CALCIUM | | |
|---|---|---|---|---|---|---|---|---|
| | | | Intake | Feces | Absorbed | Intake | Feces | Urine |
| ppm. Mn | | | mg. | mg. | mg. | gm. | gm. | gm. |
| 0 | 4 | 1 | 27.2 | 35.6 | -8.4 | 2.68 | 2.62 | 0.27 |
| 0 | 4 | 4 | 27.2 | 93.2[1] | -66.0[1] | 2.68 | 2.48 | 0.10 |
| 0 | 5 | 3 | 26.2 | 30.1 | -3.9 | 2.53 | 2.17 | 0.02 |
| 0 | 5 | 5 | 19.9 | 19.5 | +0.4 | 2.06 | 1.66 | 0.07 |
| 0 | 6 | 2 | 32.5 | 49.2 | -16.7 | 2.89 | 1.97 | 0.06 |
| 0 | 6 | 6 | 32.5 | 34.2 | -1.7 | 2.89 | 1.74 | 0.16 |
| Average | | | 27.6 | 33.7 | -6.1 | 2.62 | 2.11 | 0.11 |
| 500 | 4 | 2 | 317.9 | 315.5 | +2.4 | 2.77 | 2.91 | 0.26 |
| 500 | 4 | 5 | 285.8 | 306.4 | -20.6 | 2.42 | 2.84 | 0.23 |
| 500 | 5 | 1 | 316.5 | 297.2 | +19.3 | 2.50 | 2.56 | 0.07 |
| 500 | 5 | 6 | 284.6 | 264.5 | +20.1 | 2.33 | 2.06 | 0.06 |
| 500 | 6 | 3 | 329.3 | 282.6 | +46.7 | 2.88 | 2.01 | 0.09 |
| 500 | 6 | 4 | 329.3 | 269.9 | +59.4 | 2.85 | 2.55 | 0.00 |
| Average | | | 310.6 | 289.4 | +21.2 | 2.63 | 2.49 | 0.12 |
| 1000 | 4 | 3 | 623.1 | 584.6 | +38.5 | 2.78 | 2.37 | 0.18 |
| 1000 | 4 | 6 | 564.5 | 513.3 | +51.2 | 2.55 | 2.75 | 0.26 |
| 1000 | 5 | 2 | 593.5 | 539.4 | +54.1 | 2.52 | 2.85 | 0.22 |
| 1000 | 5 | 4 | 523.5 | 490.4 | +33.1 | 2.35 | 2.18 | 0.10 |
| 1000 | 6 | 1 | 601.5 | 469.6 | +131.9 | 2.84 | 1.93 | 0.00 |
| Average | | | 583.3 | 519.5 | +61.8 | 2.61 | 2.42 | 0.15 |

[1]Values omitted from the averages.

TABLE 4 (continued)

| CALCIUM | | PHOSPHORUS | | | | |
|---|---|---|---|---|---|---|
| Absorbed | Retained | Intake | Feces | Urine | Absorbed | Retained |
| gm. | gm. | gm. | gm. | gm. | gm. | gm. |
| +0.06 | -0.21 | 1.60 | 1.69 | 0.03 | -0.09 | -0.12 |
| +0.20 | +0.10 | 1.60 | 1.49 | 0.02 | +0.11 | +0.09 |
| +0.36 | +0.34 | 1.55 | 1.38 | 0.09 | +0.17 | +0.08 |
| +0.40 | +0.33 | 1.21 | 1.14 | 0.04 | +0.07 | +0.03 |
| +0.92 | +0.86 | 1.61 | 1.29 | 0.05 | +0.32 | +0.27 |
| +1.15 | +0.99 | 1.61 | 1.19 | 0.01 | +0.42 | +0.41 |
| +0.52 | +0.40 | 1.53 | 1.36 | 0.04 | +0.17 | +0.13 |
| -0.14 | -0.40 | 1.60 | 1.88 | 0.02 | -0.22 | -0.30 |
| -0.42 | -0.65 | 1.48 | 1.80 | 0.00 | -0.32 | -0.32 |
| -0.06 | -0.13 | 1.56 | 1.67 | 0.02 | -0.11 | -0.13 |
| +0.25 | +0.21 | 1.40 | 1.30 | 0.04 | +0.10 | +0.06 |
| +0.87 | +0.78 | 1.62 | 1.44 | 0.00 | +0.18 | +0.18 |
| +0.30 | +0.30 | 1.62 | 1.71 | 0.00 | -0.09 | -0.09 |
| +0.12 | +0.02 | 1.55 | 1.63 | 0.01 | -0.08 | -0.10 |
| +0.41 | +0.22 | 1.59 | 1.45 | 0.02 | +0.14 | +0.12 |
| -0.20 | -0.46 | 1.45 | 1.63 | 0.02 | -0.18 | -0.20 |
| -0.33 | -0.55 | 1.55 | 1.82 | 0.02 | -0.27 | -0.29 |
| +0.17 | +0.07 | 1.38 | 1.43 | 0.00 | -0.05 | -0.05 |
| +0.91 | +0.91 | 1.62 | 1.29 | 0.07 | +0.33 | +0.26 |
| +0.19 | +0.04 | 1.52 | 1.52 | 0.03 | +0.01 | +0.03 |

TABLE 5

Effect of Manganese Intake at Levels of 0, 500, and 1000 ppm. of Ration on Digestibility of Organic Matter and Nitrogen Retention (Manganese Supplied as Manganese Carbonate)

| Ration | Trial no. | Lamb no. | Dry Matter intake | ORGANIC MATTER | | | NITROGEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Intake | Feces | Digested | Intake | Feces | Urine | Apparent digested | Retained |
| | | | gm. | gm. | gm. | % | gm. | gm. | gm. | gm. | gm. |
| 0 | 4 | 1 | 582.0 | 546.0 | 153.5 | 71.90 | 14.51 | 4.42 | 7.48 | 10.09 | 2.61 |
| 0 | 4 | 4 | 582.0 | 546.0 | 145.2 | 73.42 | 14.51 | 3.73 | 6.96 | 10.78 | 3.82 |
| 0 | 5 | 3 | 587.0 | 545.0 | 167.9 | 69.19 | 14.36 | 3.96 | 7.91 | 10.40 | 2.49 |
| 0 | 5 | 5 | 467.0 | 428.0 | 115.4 | 73.02 | 10.91 | 2.68 | 6.34 | 8.23 | 1.89 |
| 0 | 6 | 2 | 591.0 | 546.0 | 153.8 | 71.84 | 14.66 | 3.77 | 7.27 | 10.89 | 3.62 |
| 0 | 6 | 6 | 591.0 | 546.0 | 155.4 | 71.54 | 14.66 | 3.67 | 7.40 | 10.99 | 3.59 |
| Average | | | 567.0 | 526.0 | 148.5 | 71.82 | 13.94 | 3.71 | 7.23 | 10.23 | 3.00 |
| 500 | 4 | 2 | 582.0 | 541.0 | 171.3 | 68.31 | 14.47 | 4.25 | 8.15 | 10.22 | 2.07 |
| 500 | 4 | 5 | 518.0 | 478.0 | 162.2 | 66.05 | 13.38 | 4.43 | 7.27 | 8.95 | 1.68 |
| 500 | 5 | 1 | 588.0 | 547.0 | 179.0 | 67.28 | 14.36 | 4.58 | 8.20 | 9.78 | 1.58 |
| 500 | 5 | 6 | 535.0 | 492.0 | 142.8 | 70.99 | 12.93 | 3.54 | 7.50 | 9.39 | 1.89 |
| 500 | 6 | 3 | 591.0 | 548.0 | 160.9 | 70.63 | 14.76 | 3.77 | 7.92 | 10.99 | 3.07 |
| 500 | 6 | 4 | 591.0 | 548.0 | 171.4 | 68.71 | 14.76 | 4.32 | 7.40 | 10.44 | 3.04 |
| Average | | | 568.0 | 526.0 | 164.6 | 68.66 | 14.11 | 4.15 | 7.74 | 9.96 | 2.22 |
| 1000 | 4 | 3 | 582.0 | 540.0 | 154.5 | 71.39 | 14.44 | 3.86 | 7.55 | 10.58 | 3.03 |
| 1000 | 4 | 6 | 549.0 | 509.0 | 151.6 | 70.23 | 12.92 | 4.24 | 7.42 | 8.68 | 1.26 |
| 1000 | 5 | 2 | 588.0 | 546.0 | 169.0 | 69.05 | 14.33 | 4.46 | 8.52 | 9.87 | 1.35 |
| 1000 | 5 | 4 | 534.0 | 491.0 | 139.2 | 71.67 | 12.90 | 3.25 | 7.32 | 9.65 | 2.33 |
| 1000 | 6 | 1 | 591.0 | 548.0 | 154.1 | 71.88 | 14.71 | 3.79 | 7.51 | 10.92 | 3.41 |
| Average | | | 569.0 | 527.0 | 153.7 | 70.84 | 13.86 | 3.92 | 7.66 | 9.94 | 2.28 |

# VITA

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