A CHEMICAL INVESTIGATION OF PHOSPHORUS DEFICIENCY IN RANGE BEEF CATTLE

A CHEMICAL INVESTIGATION OF PHOSPHORUS DEFICIENCY IN RANGE BEEF CATTLE

By

MYRON E. GIBSON, JR.

Bachelor of Science

Ouachita College

Arkadelphia, Arkansas

1948

Submitted to the Department of Chemistry

From the Laboratory of the

Department of Agricultural Chemistry Research

Oklahoma Agricultural and Mechanical College

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1951

A CHEMICAL INVESTIGATION OF PHOSPHORUS DEFICIENCY IN RANGE BEEF CATTLE

MYRON E. GIBSON, JR.

MASTER OF SCIENCE

1950

THESIS AND ABSTRACT APPROVED:

Dennis D Sacamp
Chairman, Thesis Committee

Robert Mandigan
Member of the Thesis Committee

Head of the Department

D. P. McTurbah
Dean of the Graduate School

ACKNOWLEDGMENT

The author expresses his gratitude to Dr. Willis D. Gallup for his encouragement and constructive criticism throughout this investigation.

TABLE OF CONTENTS

INTRODUCTION

Beef cattle produced in certain areas of Southeastern Oklahoma are frequently described as unthrifty, lacking in finish and vigor, and even "creepy". Inability of the cattle to make rapid gains in weight and their failure to produce profitable calf crops constitute major economic problems in beef cattle production in these areas. In some areas, feed shortage and poor feeding practices may account for part of the unthriftiness, but in other areas, the cattle fail to make the desired gains in weight although forage is available in ample quantities.

In one such area near Wilburton, Oklahoma, the color of the soil indicates a very low organic matter content. Soil samples taken from this area by the Department of Agronomy show deficiencies of calcium, total and available phosphorus and potassium. A deficiency of phosphorus in forage produced in this area might be suspected since it is known that the content of certain minerals in plants is closely related to the amount of these minerals available in the soil.

Since the soil analysis and the appearance of the cattle in the Wilburton area indicated a phosphorus deficiency, a study was undertaken to determine the value of a mineral phosphorus supplement for beef cattle under practical range feeding and management conditions.

Two locations were selected for this study, one in the central portion of the state near Stillwater, the experimental range bordering Lake C. P. Blackwell where the soil is on the borderline of being phosphorus deficient, and one in the southeastern part of the state near Wilburton where the soil is definitely phosphorus deficient.

At the end of the second year of this study evidence was obtained that the Wilburton cattle not receiving a phosphorus supplement were suffering from a phosphorus deficiency and perhaps other unknown disorders of a nutritional nature. Phosphorus supplementation was only partially effective in preventing the disorders. The forages from the two areas contained approximately the same amount of total phosphorus yet the cattle at the Wilburton area did not do so well as comparable lots of cattle receiving similiar rations at the Lake C. P. Blackwell area. It became the aim of this investigation therefore to explore other possible causes for this apparent nutritional deficiency by making a more complete analysis of the forage from the two areas and by determining a number of constituents in the blood of cattle consuming the forage.

REVIEW OF LITERATURE

Mineral deficient areas are known to be present in many parts of the world. Although characteristic disorders among animals in some of these areas have been observed for over 150 years, the exact cause was not found until 1924 when Theiler and his coworkers (1) in South Africa undertook the task of solving the cause of a disease known as Lamsiekte in cattle grazed on the South African veld. These investigators found that animals contracted the disease from eating putrified bones. This depraved appetite for bones and also for wood, rocks, and other foreign material was found to be due to a lack of sufficient phosphorus in the diet of the cattle. The feeding of phosphorus in the form of steamed bone meal allieviated the depraved appetite and generally improved the health and well being of the animals.

A short time later in this country, Eckles, Becker, and Palmer (2) reported a nutritional disorder among dairy cattle in certain sections of Minnesota. Although the disorder was not so severe as that reported by Theiler, it was serious enough to endanger the dairy and cattle industries in some 30 counties in Minnesota. The chief cause of the disorder was traced to a deficiency of phosphorus in the roughages fed the animals.

Aphosphorosis, the term generally applied to phosphorus deficiency in animals, has been definitely recognized in recent years in Montana, Minnesota, Wisconsin, Michigan, Kansas, Utah, California, Texas, and Florida. Other areas where less severe deficiency conditions exist have been found in New York, Pennsylvania, West Virginia, South Carolina, Alabama, and Mississippi. The primary cause of the trouble which has been so disasterous to the cattle industry in such widespread areas can be traced to a deficiency of available phosphorus in the soil. These deficient soils give rise to forage crops containing inadequate amounts of phosphorus to meet the needs of farm animals (1).

Theiler (1) reported that lack of sufficient phosphorus in the diet of cattle produced an abnormal craving for bones, rocks, and wood which normal cattle will ordinarily shun. Becker and his associates in Florida (3) reported that cattle ate oyster shells, old leather shoes, stones, and many other curious objects. Eckles et al., (4) noted that this depraved appetite was most apparent during the early stages of phosphorus deficiency but that it was not so pronounced after the condition became chronic.

The condition of cattle grazing on phosphorus deficient forage has been similarly described by a number of investigators (5, 6, 7). Stunted growth of the cattle, low milk yield, and reduced calf crops are the main economic factors involved. Failure of the cattle to maintain a good finish and body weight has been noted in all affected areas. Eckles and associates (4) learned from questioning farmers that cows produced an average of only one calf in two years. Further studies by these men gave conclusive evidence that oestrum was inhibited by the deficiency. Both Theiler (1) and Eckles (4) found that the addition of a phosphorus supplement to the ration reduced mortality among young calves, increased the fertility of the cows, and resulted in the superior development of the calves.

It was shown by both the South African and the Minnesota workers that the content of inorganic phosphorus in the blood plasma of cows dropped to very low levels before any outward symptoms of a phosphorus deficiency were manifested. The calcium content of the blood did not change. The feeding of bone meal to affected cows increased inorganic blood phosphorus from 1.3 mg. percent to about 5 mg. percent. The normal amount of inorganic phosphorus in the blood plasma of cattle receiving sufficient phosphorus is from 4 to 9 mg. percent

depending on the age of the animal. Blood phosphorus is usually higher in young animals than in older ones (8).

A study by Black and his associates (7) was undertaken on the King Ranch at Kingsville, Texas, to determine the mineral requirements of cattle grazed on a pasture known to be deficient in phosphorus. Three different mineral supplements were fed to separate lots of cattle, one lot which received no mineral supplement serving as a control lot. Bone meal, disodium phosphate, and bone meal plus trace minerals were fed to the three supplemented lots. No significant difference in performance was found between the three supplemented lots but the difference between these lots and the control lot was very significant. The supplemented lots produced more calves, weaned a higher percentage of the calves dropped, and the calves in these lots were sold at higher prices than those of the control lot.

Although there is limited information on the phosphorus requirements of range beef cattle, the minimum phosphorus requirements of dairy cattle have been studied at several experiment stations. Beeson (9) has shown that for fattening steers a minimum intake of 2 grams of phosphorus per 100 pounds of body weight per day are required to produce good gains in weight. This requirement was not met by feeding a ration containing 0.18 percent phosphorus. Mitchell (10) states that roughages containing about 0.12 percent or less of phosphorus do not furnish an adequate amount of this element to meet the requirements of cattle and sheep. Watkins and Knox (11), working at the New Mexico Agricultural Experiment Station, are of the opinion, however, that the amount of phosphorus needed by cattle on the range is less than that usually recommended. Minimum requirements calculated from their data are 0.095 percent of phosphorus in the ration for dry cows and 0.113 percent of phosphorus in the ration for lactating cows. Thus, a 1000 pound cow consuming 20 pounds

of dry matter per day would be receiving only from 0.85 gram to 1.25 grams of phosphorus per 100 pounds liveweight. Huffman et al., (5) found that rations containing less than 0.2 percent of phosphorus were inadequate for dairy cattle. After reviewing available data, the National Research Council has recommended the use of a phosphorus-rich mineral supplement for range beef cattle whenever the phosphorus content of forage falls below 0.15 percent (12).

Although the form in which phosphorus occurs in plants is believed to be a factor affecting its availability for some animals, ruminants appear to be able to utilize organic as well as inorganic forms of phosphorus. In a series of metabolism trials, Reid, Franklin, and Hallsworth (13) found that phytate phosphorus, which made up over 50 percent of the total phosphorus in a number of natural feeds, was completely hydrolyzed in the alimentary tract of sheep. An "in vitro" experiment also conducted by these investigators demonstrated the complete hydrolysis of phytate phosphorus by ruminal contents within eight hours. Their results confirm those of earlier, though less critical, experiments carried out with cows by Jordan, Hart, and Patten (14).

Despite the importance of vitamin D in phosphorus metabolism, apparently no studies have been made of the comparative value of different sources or forms of vitamin D for most efficient utilization of phosphorus by ruminants. Animals on the range are believed to be adequately supplied with vitamin D (15).

Wide calcium:phosphorus ratios which adversely effect the utilization of phosphorus by most animals have not been found to influence phosphorus utilization by ruminants (4, 13, 15). In fact, Axelsson and Eriksson (16) have recently shown that at least 6 grams of calcium and from 1 to 1.5 grams of phosphorus are required in a ration for a positive balance of these elements in the body of adult wethers. Such ratios of 6 parts of calcium to 1 of

phosphorus are not uncommon in native forage. Forage deficient in phosphorus has been found, almost without exception, to supply adequate amounts of calcium for grazing livestock (2, 17, 18).

The effect of mineral elements other than calcium on metabolism of phosphorus has received little attention in beef cattle nutrition. Certain ration minerals in large amounts have been found to impair phosphorus utilization by forming insoluble compounds of phosphorus in the alimentary tract. Cox and associates (19) reported that ferric and aluminum salts when fed to guinea pigs and rabbits in amounts in excess of the total phosphorus in the diet caused a lowering of the inorganic phosphorus content of the blood to a level 15 percent of normal. Addition of monosodium phosphate equivalent to the iron or aluminum resulted in normal phosphorus utilization. Theobald and Elvehjem (20) have reported similiar results with chicks.

Jones (21) found that the addition of aluminum or beryllium salts to a high-calcium, low-phosphorus rachitogenic diet hastened the development of rickets in rats. Rehm and Winters (22) have shown that the feeding of ferric chloride in such amounts as to combine with one-half of the phosphorus in the diet resulted in considerable reduction in total amounts of ash, calcium, and phosphorus in the bodies of rats at the end of 30 days.

Williams et al., (23) found that the phosphorus of low-phosphorus clover hay was less available than that of high-phosphorus hay when the two hays were compared as sources of phosphorus for rats. Analysis of the two hays showed that the greater amount of aluminum and iron in the low-phosphorus hay was sufficient to be a possible disturbing factor in the utilization of the phosphorus. Similiar comparisons made with the ash of the two hays showed that the phosphorus from the low-phosphorus hay was the least available.

Considerable research has been published on the effect of a deficiency of manganese in the diets of chicks, rats, mice and rabbits, but very little has been reported concerning the possible effect of a manganese excess on calcium and phosphorus metabolism (24). Apparently no studies have been made of manganese in beef cattle nutrition. Chornock and others (25) reported the growth of rats to be retarded by a high intake of manganese. Manganese sulfate, when added to rachitogenic diets of high-calcium and low-phosphorus content in amounts to provide from 0.3 to 1.73 percent of manganese, decreased the growth rate of rats in proportion to the amounts added. At the 0.3 percent level, the rats were consuming an average of 15 mg. of manganese per day over a 30-day period.

Smith and Ellis (26), in studying the manganese requirements of rabbits, found that intakes of manganese up to 4 mg. per day increased rate of growth whereas 8 mg. per day had the opposite effect. These investigators expressed the opinion that 8 mg. of manganese per day may be near the toxic level for rabbits.

Archibald and Lindquist (27) reported that dairy cattle, over a period of time, showed an increasing reluctance to eat a ration of mixed grain when 10 grams of manganese fed as manganous sulphate was mixed with the ration. The grain mixture was refused entirely after the cattle were turned out to pasture.

Elvehjem (28) has shown that both iron and copper are dietary essentials for the prevention and cure of nutritional milk anemia. A naturally occurring nutritional anemia exists among cattle in certain regions in Florida (29). Investigation has shown that forage produced in these regions contains less iron and copper than that from other more fertile soils. When affected cattle were given iron and traces of copper the condition was overcome in all but the most advanced cases.

Forage which contains a high percentage of lignin is usually low in nutritive value (30). Crampton and Jackson (31) found that the percentage of lignin in pasture herbage increased as the grasses matured and that these changes were associated with a decrease in the digestibility of the herbage. Phillips and Loughlin (32) have also shown the digestible energy of hays to be closely related to their lignin content. Forbes and Garrigus (33) found that the dry matter digestibility and total digestible nutrients of various forages varied inversely with lignin content. The possible effect of lignin on the availability of phosphorus in feeds has not been thoroughly investigated.

EXPERIMENTAL PROCEDURE

Locations selected for study. Two sites were selected for the study of the value of a mineral phosphorus supplement for range beef cattle, one site near Wilburton in the southeastern part of the state where the soils are definitely phosphorus deficient and one near Stillwater at Lake C. P. Blackwell where the soils are bordering on phosphorus deficiency.

Cattle[1]. At the beginning of the study in January 1947, thirty bred three-year-old cows and thirty weanling heifer calves were placed at each location. The cows and heifer calves were divided into three lots of ten cattle each and were allowed about ten acres of pasture per head at each location during the grazing season. During the winter, the cattle were placed in traps and fed prairie hay, produced on the area, and corn gluten meal. The corn gluten meal which was fed as a protein supplement supplied only a small amount of phosphorus. Two lots of cattle in each age group at each location were given dicalcium phosphate prepared from bone meal as a phosphorus supplement. The amount supplied was calculated to provide the animals in one lot with 1.5 grams of phosphorus per 100 pounds liveweight and those in the other lot with 2.5 grams of phosphorus per 100 pounds liveweight. One lot in each age group received no phosphorus supplement. In order to provide the medium-phosphorus lots with an estimated 1.5 grams of phosphorus per 100 pounds liveweight when on pasture, those at Lake C. P. Blackwell were not fed a mineral supplement other than salt while those at Wilburton were allowed free access to a mineral mixture of 9

---

[1] The cattle were purchased by the Department of Animal Husbandry, Oklahoma Agricultural Experiment Station. The many details of feeding and management of the cattle were attended to by members of the cooperating department of Animal Husbandry under the direct supervision of Professor C. B. Ross.

parts salt and 1 part dicalcium phosphate. The high phosphorus lots at both locations were allowed free access to a mineral mixture of 2 parts salt and 1 part dicalcium phosphate when on pasture.

Rations of the animals. Feeds supplied the animals in winter traps and during the summer grazing period were as follows:

Winter	Summer
Lot 1	
Prairie hay (free choice) Corn gluten meal, 1.25 lbs. per head per day. Ground rock salt (free choice)	Pasture Ground rock salt (free choice)
Lot 2	
Same as lot 1 plus enough dicalcium phosphate to provide a total intake of 1.5 grams of phosphorus per 100 lbs. body weight per day.	Same as lot 1 plus enough dicalcium phosphate to provide an estimated phosphorus intake of 1.5 grams per 100 lbs. body weight per day.
Lot 3	
Same as lot 1 plus enough dicalcium phosphate to provide a total intake of 2.5 grams of phosphorus per 100 lbs. body weight per day.	Same as lot 1 plus enough dicalcium phosphate to provide an estimated phosphorus intake of 2.5 grams per 100 lbs. body weight per day.

General sampling. Samples of all analytical material were obtained through the cooperation of the Department of Animal Husbandry, Oklahoma Agricultural Experiment Station. Samples of hay to be used for winter feeding were taken soon after baling. Additional samples were taken during the winter months for determinations of carotene. At any one sampling, a large composite sample was formed by combining small samples taken from ten or more representative bales. A portion of the composite sample was ground in a Wiley mill and used for the determination of usual feed constituents including calcium and

phosphorus. Carotene was determined immediately after grinding the sample. Another portion of the composite sample was clipped into lengths of about one inch with shears and used for mineral analysis. The same general procedure was followed whenever composite samples of different grass species which made up the hay were to be analyzed.

Representative samples of corn gluten meal and commercial dicalcium phosphate were secured by combining small samples taken from a number of sacks in each shipment.

During the grazing season samples of the predominate species of grasses in the pasture were taken at about 56-day intervals. These were transported to the laboratory in a small ice box and immediately cut into short lengths for the determination of carotene and moisture. Portions of these samples were air dried and handled similar to the hay samples for further chemical analysis.

Precautions were taken to protect all samples from dust and other sources of mineral contamination during collection, drying, and sampling. The samples were stored in sealed glass jars in the laboratory.

Blood samples were taken from each animal at about 56-day intervals. The blood was drawn from the juglar vein into tubes containing ¼ mg. of lithium citrate per ml. of blood and chilled in ice water. These samples were kept refrigerated previous to and between analytical determinations which were started immediately after the samples were received in the laboratory.

In addition to carotene and inorganic phosphorus which were determined at 56-day intervals on all animals in the experiment (60 animals initially at each location) determinations were made of calcium, magnesium, copper, plasma protein, hemoglobin, and hematocrit in blood of a representative number of cows in lots 1 and 3 at each location. These determinations were made in 1950

during February and April when the animals were on winter feed and in June when the animals had been on pasture for two months.

Blood analysis. Unless stated otherwise, all colorimetric determinations were made with the Evelyn photoelectric colorimeter equipped with the proper light filter.

Inorganic phosphorus was determined in blood plasma by the procedure of Fiske and Subbarow as modified for use with the Evelyn photoelectric colorimeter (34). After precipitation of the plasma proteins with 10 percent trichloroacetic acid and centrifuging, an aliquot of the supernatent liquid was treated with molybdic acid and the phosphomolybdic acid so formed was reduced with p-aminonapthol sulfonic acid. The intensity of the resulting blue color was determined in the colorimeter.

Plasma calcium was precipitated with ammonium oxalate in accordance with the procedure of Clark and Collip (35). After repeated centrifuging and washing, the calcium oxalate was dissolved in sulfuric acid and the oxalic acid titrated with potassium permanganate. Centrifuge tubes drawn out to a small tip were used in this determination and in the determination of magnesium.

An aliquot of the supernatent liquid from the calcium precipitation was used for the determination of magnesium according to the method described by Denis (36). Magnesium was precipitated with ammonium phosphate, washed with dilute ammonium hydroxide as in the calcium determination, and dissolved in sulfuric acid. The phosphorus in the magnesium phosphate was determined by the Fiske and Subbarow colorimetric method (34).

Copper was determined by the procedure of Cartwright, et al., (37) in which the plasma proteins were precipitated and extracted with trichloroacetic acid and the extract treated with sodium diethyldithiocarbamate. The color intensity of the copper compound was determined with the colorimeter.

Carotene was determined by Kimble's method (38) in which the plasma proteins were precipitated with ethyl alcohol and the carotene extracted by shaking with Skellysolve F. Carotene was determined colorimetrically in an aliquot of the Skellysolve F solution.

For the determination of vitamin A (38), an aliquot of the above Skellysolve solution was evaporated to dryness at 45° C. under reduced pressure and the residue taken up in chloroform. Antimony trichloride in chloroform was added to the solution and the intensity of blue color produced was measured colorimetrically. A correction was made for the reaction of carotene with antimony trichloride in calculating vitamin A.

For the determination of hemoglobin, a color method was used in which 0.02 ml. of blood was measured from a serological pipette into 10 ml. of physiological saline solution, two drops of HCl were added and the solution allowed to stand for 15 minutes. The intensity of the resulting color was measured in a colorimeter. The value obtained in this manner was checked by a method described by Koch and Hanke (39) in which the specific gravity of the blood and of the plasma were determined by the copper sulfate method and the hemoglobin calculated from these specific gravity values.

Plasma protein was calculated using the specific gravity of the plasma which was determined by the copper sulfate method described by Koch and Hanke (39). Plasma protein was calculated using the following formula:
$Ps = 343(Gs - 1.0070)$, where Ps is the plasma protein concentration in grams per 100 ml. of plasma and Gs is the specific gravity of the plasma.

Analyses of feeds. Feed nutrients in hay, grass and supplemental feed samples were determined by the conventional scheme of feed analysis as described in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, sixth edition, 1945. The constituents were moisture,

ash, protein, ether extract, crude fiber, and nitrogen-free extract. Minerals in the hay and different grass species were also determined by methods prescribed by the Association (40). Many of these methods are so well known that they need only brief description.

Moisture was determined by drying at 105° C. in a convection draft electric oven for four hours.

Samples were ashed in an electric muffle furnace maintained at 600° C. for two hours.

Protein was determined by the Kjeldahl-Gunning procedure with copper as a catalyst. A factor of 6.25 was used to convert all nitrogen values to protein.

Ether extract was determined by extraction of the dry sample with anhydrous diethyl ether for 16 hours.

Crude fiber, the combustible, insoluble residue left after digesting the sample with dilute boiling sulfuric acid and sodium hydroxide, was filtered from the extracts with the aid of suction.

Nitrogen-free extract (soluble carbohydrates) was determined by difference.

For calcium and phosphorus determinations, samples were ashed overnight in a muffle furnace at 400° C. The ash was dissolved in dilute hydrochloric acid and the solution made to a known volume. An aliquot of the solution was used for the precipitation of calcium as calcium oxalate. The calcium oxalate was filtered by suction, dissolved in dilute sulphuric acid and the oxalic acid titrated with 0.05 N potassium permanganate. Phosphorus was precipitated from another aliquot of the solution as ammonium phosphomolybdate. After filtering by suction, the precipitate was dissolved in excess 0.2 N sodium hydroxide and the unreacted sodium hydroxide titrated with 0.2 N hydrochloric acid.

The procedure of Peterson (41) was followed for the determination of carotene in air-dry forages. The samples were saponified with alcoholic KOH and the resulting mixture extracted with Skellysolve B, followed by dilution with water to bring about a separation of two layers. The lower layer, which contained the chlorophylls and other pigments, was drawn off and discarded. The upper layer, which contained a mixture of carotene and xanthrophyll was washed with 90 percent methyl alcohol to remove the xanthrophyll and a large proportion of the yellow pigments other than carotene. After further washing and drying, the total yellow color of the Skellysolve solution was determined with the Evelyn photoelectric colorimeter and reported as crude carotene. Solutions of pure beta-carotene in Skellysolve B were used to calibrate the colorimeter.

The crude carotene was then further purified as described by Wall and Kelly (42) by pouring the Skellysolve extract through an adsorption column of magnesium oxide and Hyflo Supercell. Carotene was eluted from the column with a 4 percent solution of acetone in Skellysolve B. Many of the colored impurities in the crude carotene extract not removed by previous extraction with methyl alcohol were retained in the column. The color of the acetone-Skellysolve B solution was taken as a measure of the true carotene of the sample.

Beginning in the winter of 1948, additional analytical determinations were made to include the percentage of silica, iron, aluminum, manganese, magnesium, sodium, and potassium in predominate grass species which made up the hay used in winter feeding. In addition, determinations were made of cellulose and lignin in these samples.

For mineral analysis, 10-gram samples of the clipped hay were weighed into flat-bottom platinum dishes and ignited in a muffle furnace at 450° C. overnight. The ash was treated with dilute HCl and allowed to simmer on the

hotplate for several hours and then evaporated to dryness to render the silica insoluble. The residue was then taken up with dilute HCl and allowed to simmer on a hotplate for several hours in order to dissolve all soluble elements. The solution was filtered and the insoluble portion weighed as SiO_2. The combined filtrate and washings were made to volume in a volumetric flask. This solution will be referred to as solution A.

For the determination of iron and aluminum (40) $(NH_4)_2HPO_4$ was added to an aliquot of solution A and the acidity of the solution adjusted with thymol blue being used as the indicator. Iron and aluminum were precipitated as the phosphate upon the addition of 25 ml. of ammonium acetate. After stirring and allowing the solution to stand overnight, the precipitate was filtered off, washed with 5 percent ammonium nitrate, ignited and weighed as iron and aluminum phosphate.

Iron was determined in an aliquot of solution A, following its oxidation with bromine, by addition of thiocyanate to produce the red color of iron thiocyanate. The intensity of the color produced was compared with that of a known solution of iron treated in a similar manner. Color comparisons were made in a Klett visual colorimeter.

Aluminum was calculated from the results of the iron and aluminum phosphate determination and the colorimetric iron determination.

For the determination of manganese (43), an aliquot of solution A was made free of chlorides by boiling with nitric acid. The manganese was then oxidized by additions of potassium periodate in small amounts and boiling until the resulting solution showed no further increase in color. After adjustment of volume of the solution, the color produced was compared (in the visual colorimeter) with standard solutions containing known amounts of potassium permanganate.

Magnesium (40) was determined in the combined filtrate and washings from the calcium determination. After addition of nitric acid the solution was evaporated to dryness to decompose the ammonium salts and the residue taken up in dilute HCl. Sodium citrate was added to hold iron in solution and magnesium precipitated with $(NH_4)_2HPO_4$ in an alkaline solution. The solution was stirred until the precipitate was granular, allowed to stand at room temperature overnight, filtered, and washed free from chlorides with dilute ammonium hydroxide. The precipitate was then ignited and weighed as $Mg_2P_2O_7$.

An aliquot of solution A was neutralized with ammonium hydroxide for the determination of sodium and potassium. The amounts of these elements in the solution were determined with the aid of a Perkin and Elmer flame photometer.

Lignin was determined by the 72 percent sulfuric acid method of Ellis, Matrone, and Maynard (44). The sample was extracted with a benzene-ethanol mixture to remove fat-soluble material and digested with pepsin to break down protein. It was then digested with 5 percent sulfuric acid, dried, and redigested with 72 percent sulfuric acid to remove cellulose. After digesting in 3 percent sulfuric acid, the residue was filtered with suction and lignin determined by the loss of weight on ignition.

The Norman-Jenkins method was used for cellulose determination as modified by Matron, Ellis, and Maynard (45). The sample was extracted with a benzene-ethanol mixture and treated with sodium sulphite and sodium hypochlorite until free from lignin. The residue was then filtered with suction and cellulose determined by the loss of weight on ignition.

RESULTS

Calving data. At Wilburton in 1948, the cows in low-phosphorus lot 1 weaned a 50 percent calf crop; the average weight of the calves at birth was 67 pounds. The medium-phosphorus lot 2 cows weaned a 20 percent calf crop with an average birth weight of 68 pounds. Lot 3, which was the high-phosphorus lot, weaned a 30 percent calf crop with an average birth weight of 75 pounds. The average weaning weights of the calves in lots 1, 2, and 3 were 351, 390, and 369 pounds, respectively. The weaning weights of the calves in all lots were considered to be lower than desirable.

In 1948, the cows at Lake C. P. Blackwell in the low-phosphorus lot 1 weaned a 70 percent calf crop which averaged 67 pounds at birth. The medium-phosphorus lot 2 cows weaned an 80 percent calf crop which averaged 67 pounds at birth, and lot 3, the high-phosphorus lot, weaned a 70 percent calf crop with an average birth weight of 63 pounds. The weaning weights of these calves were lot 1, 444 pounds; lot 2, 395 pounds; and lot 3, 435 pounds.

In 1949, the Wilburton cows, which were started on the experiment as weanling heifers, dropped their first calves. These heifers and the cattle placed on experiment as bred heifers made a total of 19 animals in lot 1. A 68 percent calf crop was weaned from this lot of cows with an average birth weight of 68 pounds. From a total of 13 cows in lot 2, a 69 percent calf crop was weaned with an average birth weight of 69 pounds. The twenty cows in lot 3 weaned a 65 percent calf crop averaging 68 pounds at birth. The weaning weights of calves at Wilburton from lots 1, 2, and 3 were 302, 383, and 388 pounds respectively.

From the 20 cows in each lot at Lake C. P. Blackwell in 1949, a calf crop of 85 percent was weaned in lot 1, 85 percent in lot 2, and 80 percent in lot 3,

the average birth weight for calves in the three lots being 75, 71, and 73 pounds respectively. The average weaning weights of the calves were lot 1, 465 pounds; lot 2, 417 pounds; and lot 3, 457 pounds. More complete details on the effect of a phosphorus supplement on reproduction are given in Oklahoma Experiment Station Miscellaneous Publication 17, May 1950.

Blood values. The average plasma inorganic phosphorus and carotene content of the blood for the various age groups in the three lots of cattle at both locations are given in tables 1, 2, and 3. These values have been presented together since there appears to be a negative correlation between plasma phosphorus and carotene as is discussed later.

Plasma carotene values were within the average range found in beef cattle at different seasons of the year. There were only small differences between locations or age groups.

Plasma inorganic phosphorus values, which were directly related to phosphorus intakes, showed a seasonal variation. These values for cows in lots 1, 2, and 3 are presented in table 1. At Wilburton, these values for older cows in lot 1 dropped to between 2 to 3 mg. percent during the winter and reached a general level of only about 3 to 4 mg. percent during the summer. Animals of the same age at Lake C. P. Blackwell showed the same trend but the values were generally about 1 mg. percent higher on corresponding bleeding dates.

Phosphorus values for Wilburton cows in lot 2 receiving medium amounts of phosphorus were only slightly lower than corresponding values for cows at Lake C. P. Blackwell. These values ranged from 3.0 to 7.0 mg. percent for the Wilburton cows and from 3.9 to 7.4 mg. percent for the Lake C. P. Blackwell cows.

Cows in lot 3 receiving the high-phosphorus supplement at both Lake C. P. Blackwell and Wilburton had plasma inorganic phosphorus values similar to those of cows in lot 2. It may be noted, however, that phosphorus values in both

TABLE 1

Seasonal variation in carotene and phosphorus content of blood plasma of original cows in lots 1, 2, and 3 at Wilburton and Lake C. P. Blackwell areas

Date	Lot 1				Lot 2				Lot 3			
	Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.	
	B[1]	W[2]	B	W	B	W	B	W	B	W	B	W
1948												
February	74	186	2.8	1.8	85	200	5.8	5.1	77	182	5.9	6.1
April	278	427	3.4	1.8	784	335	6.3	5.4	339	351	5.4	6.5
June	746	679	4.3	3.0	781	475	4.7	4.7	866	442	4.6	4.1
August	841	810	5.1	3.9	702	787	4.9	4.7	738	707	5.0	4.3
November	357	283	4.4	3.1	380	232	5.6	4.3	348	219	5.4	4.5
1949												
January	142	133	5.6	2.3	126	138	7.4	6.4	96	92	7.5	6.5
February	152	170	3.4	2.3	110	198	5.4	7.0	112	142	6.6	5.7
April	248	218	2.4	2.7	153	251	5.3	5.0	138	184	6.3	7.2
June	996	836	4.7	3.2	832	503	4.4	4.4	899	703	5.2	3.3
September	662	490	4.4	3.1	583	753	4.0	3.0	510	479	4.7	3.6
November	678	734	3.4	2.7	549	791	3.9	3.1	554	631	3.7	2.5
1950												
January	137	230	4.4	3.4	156	273	6.1	6.0	167	174	5.3	7.0
March	176	173	3.4	2.6	158	219	4.9	5.2	180	154	4.6	6.1
April	352	222	4.3	3.0	357	362	5.8	5.1	480	173	6.0	5.9
June	848	833	3.5	5.0	831	875	4.4	4.3	870	692	3.3	4.2

[1] B signifies Lake C. P. Blackwell area.
[2] W signifies Wilburton area.

lots 2 and 3 at both Lake C. P. Blackwell and Wilburton dropped whenever the animals were turned out to pasture.

Carotene and inorganic phosphorus content of the blood plasma of the original heifers are shown in table 2. Plasma inorganic phosphorus values and differences in these values between lots followed the same general pattern for the heifers as for the cows discussed above.

Plasma carotene and inorganic phosphorus values for the calves of the original cows and heifers are shown in table 3. Inorganic phosphorus values for calves in lot 1 dropped to between 3 and 4 mg. percent during the winter months. As a rule, the calves in this lot at Wilburton had lower inorganic phosphorus values than those in the same lot at Lake C. P. Blackwell. Inorganic phosphorus values for calves in lots 2 and 3 were similar at the two locations and were above 4 mg. percent at all seasons.

As previously mentioned, it was first noted during the winter of 1948-49 that a possible negative correlation existed between the average carotene and inorganic phosphorus content of the blood plasma. Table 4 shows the average plasma carotene values of each age group of animals in lot 1, which received no phosphorus supplement, expressed as percentages of those in lot 3, which received a high-phosphorus supplement. This apparent negative correlation was most pronounced in the older animals at Wilburton where, without exception, the cows receiving no phosphorus supplement had higher average plasma carotene levels than those in the high-phosphorus supplement lot. This relationship among animals of the same age group was evident at Lake C. P. Blackwell only about 50 percent of the time. In the next oldest age group, listed in table 4 as heifers, the Wilburton cattle followed this trend on about 50 percent of the bleeding dates whereas those at Lake C. P. Blackwell followed it on about 80

TABLE 2

Seasonal variation in carotene and phosphorus content of blood plasma of original heifers in lots 1, 2, and 3 at Wilburton and Lake C. P. Blackwell areas

Date	Lot 1				Lot 2				Lot 3			
	Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.	
	B[1]	W[2]	B	W	B	W	B	W	B	W	B	W
1948												
February	54	133	3.9	2.7	51	154	7.4	5.4	51	168	7.4	7.2
April	277	400	3.9	3.2	293	267	7.0	5.9	293	344	7.0	6.6
June	668	562	6.0	5.4	693	339	5.4	5.7	693	308	5.4	4.7
August	850	736	5.3	5.6	719	837	5.2	5.4	719	754	5.2	4.9
November	364	262	5.0	3.7	260	258	5.1	4.6	260	232	5.1	4.8
1949												
January	140	151	6.2	2.8	91	124	7.7	6.6	91	116	7.7	8.4
February	159	143	3.9	2.6	96	172	6.7	5.9	96	157	6.7	6.9
April	227	231	3.8	2.8	150	245	6.5	5.1	150	204	6.5	8.1
June	954	716	5.2	2.7	817	815	4.6	5.3	817	725	4.6	3.9
September	661	496	4.7	2.9	462	566	4.3	3.4	462	512	4.3	3.4
November	672	681	3.8	3.2	502	700	3.4	3.0	502	613	3.4	3.3
1950												
January	130	202	4.7	3.5	153	231	4.9	5.7	153	196	4.9	7.1
March	199	146	3.6	2.8	111	187	5.4	5.4	111	165	5.4	6.4
April	346	305	4.7	3.2	339	175	6.7	5.8	339	191	6.7	6.3
June	921	822	3.9	4.6	829	963	5.0	3.5	743	886	3.8	3.3

[1] B signifies Lake C. P. Blackwell area.
[2] W signifies Wilburton area.

TABLE 3

Seasonal variation in carotene and phosphorus content of blood plasma of calves[1] of original cows and heifers in lots 1, 2, and 3 at Wilburton and Lake C. P. Blackwell areas

Date	Lot 1				Lot 2				Lot 3			
	Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Carotene mcg./100 ml.		Phosphorus mg./100 ml.	
	B[2]	W[3]	B	W	B	W	B	W	B	W	B	W
1948												
February	57	78	3.2	2.8	51	106	5.1	4.2	64	82	7.8	5.9
April	275	158	4.1	3.8	434	145	5.3	5.8	401	128	6.5	8.3
June	352	453	7.6	5.3	541	607	7.9	5.4	454	328	6.8	5.5
August	513	421	8.0	6.0	511	584	5.9	5.0	401	530	6.1	5.9
November	257	300	5.9	3.8	391	221	6.3	4.6	356	188	6.0	6.9
1949												
January	101	126		3.2	112	108	6.7	4.0	75	94	6.3	7.1
February	433	134	4.7	3.6	91	161	6.2	7.1	76	103	7.8	7.5
April	171	235	4.5	3.9	134	235	6.7	6.1	102	170	8.2	7.3
June	730	573	6.2	4.2	642	627	5.4	5.0	526	492	6.1	5.4
September	428	471	5.0	6.0	441	495	4.1	4.1	376	400	5.0	5.0
November	420	638	3.7	3.4	478	671	4.4	4.4	460	456	4.5	4.6
1950												
January	156	204	5.6	7.0	180	216	6.0	7.4		188		6.7
March	171	135	5.2	6.0	203	134	5.2	7.3	180	117	6.2	6.9
April	371	192	6.0	6.5	397	210	7.3	6.1	629	226	9.7	5.5
June	692	1007	4.2	3.6	591	1076	5.2	3.5	750	906	4.5	3.2

[1] Animals were born in Spring of 1947.
[2] B signifies Lake C. P. Blackwell area.
[3] W signifies Wilburton area.

percent of the bleeding dates. The youngest age group, or the calves, also followed this trend on about 80 percent of the bleeding dates at Wilburton and 50 percent at Lake C. P. Blackwell.

TABLE 4

Average plasma carotene value of animals in lot 1 expressed as a percentage of the average plasma carotene value of animals in lot 3[1]

Date	Cows		Heifers		Calves	
	B[2]	W[3]	B	W	B	W
1948						
February	96.1	102.2	105.9	79.2	89.1	95.1
April	82.0	121.7	94.5	116.3	68.6	123.4
June	86.1	153.6	96.4	182.5	77.5	138.1
August	114.0	114.6	118.2	97.6	127.9	79.4
November	102.6	129.2	140.0	112.9	72.2	159.6
1949						
January	147.9	144.6	153.8	130.2	134.7	134.0
February	135.7	119.7	165.6	91.1	569.7	130.1
April	179.7	118.5	151.3	113.2	167.6	138.2
June	110.8	118.9	116.8	98.8	138.8	116.5
September	129.8	102.3	143.1	96.9	113.8	117.8
November	122.4	116.3	133.9	111.1	91.3	131.3
1950						
January	82.0	132.2	85.0	103.1		108.5
March	97.8	112.3	179.3	88.5	95.0	115.4
April	73.3	128.3	102.1	159.7	59.0	85.0
June	97.5	120.4	124.0	92.8	92.3	111.1

[1] $\frac{\text{Average plasma carotene value for lot 1}}{\text{Average plasma carotene value for lot 3}} \times 100$

[2] Lake C. P. Blackwell area.
[3] Wilburton area.

The seasonal variation in average vitamin A content of the blood plasma of cows in lots 1, 2, and 3 are given in table 5. Data for Wilburton cows in lots

TABLE 5

Vitamin A content of blood plasma of representative cows[1] from lots 1, 2, and 3 at Wilburton and Lake C. P. Blackwell areas (Values in mcg. per 100 ml. of plasma)

Date	Lot 1		Lot 2		Lot 3	
	B[2]	W[3]	B	W	B	W
1948						
February	5.0		10.8		4.7	
April	11.4	23.1	4.8	19.1	5.5	22.1
June						
August	33.7	21.9	29.7		24.5	
November	27.0		30.1		34.3	
1949						
January	17.4	19.0	12.0		20.4	
February	16.4	9.2	16.4		14.0	
April	13.1	14.8	10.0		15.2	
June	15.4	27.1	16.1		17.3	
September	30.1	23.3	28.8	31.2	26.2	20.3
November	20.6	16.6	27.4	17.6	22.9	13.7
1950						
January	23.9	17.9	20.0	20.9	21.0	20.3
March	5.5	10.4	9.2	12.6	13.2	9.8
April	10.8	11.3	12.0	13.9	18.2	9.0
June	24.0	43.7	17.6	39.0	23.7	42.8

[1] Average values for 5 animals.
[2] B signifies Lake C. P. Blackwell area.
[3] W signifies Wilburton area.

2 and 3 for late 1948 and early 1949 are incomplete due to an outbreak of bangs disease among the cows in lot 2. During the same period, through misunderstanding, vitamin A was determined on the plasma of the younger cows and heifers in lot 3. The results, therefore, do not permit comparison with those obtained

with the older cows and have been omitted. The vitamin A values ranged from about 5 to 40 mcg. per 100 ml. of plasma in all lots at both locations. Values of 25 mcg. per 100 ml. and above were usually obtained during the latter part of the grazing season, just before the cows were confined to winter traps. During winter feeding, vitamin A values were frequently below 20 mcg. per 100 ml. and were unrelated to phosphorus intake or location.

Table 6 gives the results of the more complete analysis of blood from cows in lots 1 and 3 at each location.

As might be expected, the carotene values for both lots at each location were highest during the summer grazing season. They increased from about 170 mcg. per 100 ml. in February and April to over 800 mcg. per 100 ml. in June.

Plasma inorganic phosphorus values showed the same characteristic differences between lots as were shown for the entire group of cows in table 1. In lot 1, inorganic phosphorus values were about 4 mg. percent for cows at Lake C. P. Blackwell and about 2.8 for cows at Wilburton. In lot 3, inorganic phosphorus values were about 6 mg. percent for cows at both locations while on winter feed and dropped to values below 4 mg. percent during the first two months on pasture.

Normal values of 9 to 12 mg. percent of plasma calcium were obtained for cows in both lots at each location. These results are in agreement with earlier observations.

Although plasma magnesium values ranged from 1.27 to 2.28 mg. percent, they appeared to be unrelated to location or phosphorus intake of the cows.

Plasma copper values were almost identical for cows at Lake C. P. Blackwell and Wilburton in lot 1, ranging from 63 to 81 mcg. per 100 ml. Similar values were obtained for the cows in lot 3, the highest values in both lots being observed in June.

TABLE 6

Composition of blood of cows in lots 1 and 3 at the Lake C. P. Blackwell and Wilburton areas

Date	Carotene mcg./100 ml.		Phosphorus mg./100 ml.		Calcium mg./100 ml.		Magnesium mg./100 ml.		Copper mcg./100 ml.		Plasma Protein gm./100 ml.		Hemoglobin gm./100 ml.		Hematocrit vol. percent	
							Lot 1									
	B[1]	W[2]	B	W	B	W	B	W	B	W	B	W	B	W	B	W
Feb.	179	177	3.8	2.5	9.7	11.0	1.28	1.72	72	73	7.2	6.5	9.9	9.3	34	32
Apr.	323	253	4.3	2.8	10.1	10.6	1.87	1.72	63	64	7.1	6.9	10.0	8.7	33	30
June	661	889	3.6	2.9	10.2	12.0	1.27	1.97	81	81	7.0	7.5	10.6	9.7	37	34
							Lot 3									
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W
Feb.	166	170	5.0	6.5	9.7	9.3	1.54	1.69	76	66	7.0	6.0	10.1	9.3	33	33
Apr.	462	192	6.6	6.0	9.4	9.6	2.28	1.73	61	53	7.4	6.1	10.1	8.6	33	30
June	739	904	3.9	3.1	9.3	10.9	2.05	2.24	78	84	7.3	7.2	10.7	10.5	37	35

[1] B signifies Lake C. P. Blackwell area.
[2] W signifies Wilburton area.

The cows at Lake C. P. Blackwell in lots 1 and 3 had plasma protein values of 7 gm. per 100 ml. and above while on winter rations and after being turned to pasture. These values were generally higher than those found at Wilburton which were as low as 6 gm. per 100 ml. in February and April, but above 7 gm. per 100 ml. in June.

Hemoglobin values for both lots of cows at Lake C. P. Blackwell were consistently higher than those of the corresponding lots at Wilburton. Values of 10 gm. per 100 ml. of blood were obtained for cows in lots 1 and 3 at Lake C. P. Blackwell while cows in lots 1 and 3 at Wilburton ranged from 8.6 to 10.5 gm. per 100 ml.

The same trend was observed in red cell volume or hematocrit values as was shown by hemoglobin values, the cows of both lots at Lake C. P. Blackwell having a higher percentage of red blood cells than the cows at Wilburton. The red blood cell volume ranged from 33 to 37 percent in lots 1 and 3 at Lake C. P. Blackwell and from 30 to 35 percent in these lots at Wilburton.

Seasonal changes in the composition of predominate species of pasture grasses collected during 1948 from the Lake C. P. Blackwell and Wilburton areas are presented in table 7. It was hoped to relate these changes to changes in blood composition of the animals.

At the Blackwell area, the protein content of the grasses decreased as the grazing season progressed from a value of about 10 percent in May to the low value of about 3 percent in October. In general, as the protein content decreased the crude fiber increased from a value of about 31 percent to over 40 percent. Calcium values, which were between 0.3 to 0.4 percent during May, June, and July, decreased to less than 0.2 percent in October. Likewise, phosphorus values decreased from about 0.12 percent in the early part of the grazing

TABLE 7

Composition of predominate species of native grasses

Date	Description	Dry Matter Composition							
		Ash	Protein	Ether Extract	Crude Fiber	N-free Extract	Ca	P	Carotene
	Lake C.P. Blackwell Area	%	%	%	%	%	%	%	ppm
1948									
May	Switch	5.98	9.98	1.98	30.72	51.34	0.286	0.146	337.4
	Little Bluestem	7.08	8.77	1.32	33.06	49.77	0.356	0.103	260.7
	Big Bluestem	7.10	10.26	2.10	31.32	49.32	0.327	0.148	316.9
	Indian	7.86	9.26	1.87	31.87	49.14	0.368	0.118	279.4
June	Little Bluestem	7.15	7.71	2.51	31.73	50.90	0.350	0.102	172.4
	Big Bluestem	6.49	7.87	4.07	31.24	50.33	0.300	0.119	163.6
	Indian	6.77	7.50	3.21	31.46	51.06	0.360	0.111	177.9
July	Switch	5.19	5.57	2.67	40.58	45.99	0.250	0.080	101.0
	Little Bluestem	6.81	6.94	2.75	35.06	48.44	0.388	0.080	186.6
	Big Bluestem	6.01	6.80	3.30	36.86	47.03	0.332	0.085	214.8
	Indian	7.04	6.76	3.20	37.20	45.80	0.302	0.076	237.2
August	Switch	5.60	5.22	2.36	46.54	40.28	0.214	0.085	127.6
	Little Bluestem	3.55	5.19	2.17	45.81	43.28	0.249	0.098	109.9
	Big Bluestem	5.90	5.96	3.30	41.18	43.66	0.282	0.092	209.0
	Indian	5.66	4.81	2.22	46.60	40.71	0.206	0.084	171.1
October	Switch	6.03	2.92	1.65	40.42	48.98	0.264	0.048	18.0
	Little Bluestem	3.85	3.72	1.30	35.62	55.51	0.160	0.055	14.0
	Big Bluestem	4.07	3.33	1.40	36.49	54.71	0.165	0.050	17.0
	Indian	4.98	2.02	1.34	41.32	50.34	0.194	0.049	7.5

TABLE 7 (continued)

	Wilburton Area								
1945									
April	Little Bluestem	8.58	14.47	4.14	24.45	48.36	0.390	0.170	
	Big Bluestem	8.44	12.35	2.47	26.87	49.87	0.300	0.230	
	Indian	8.46	14.97	3.67	23.78	49.12	0.350	0.210	
June	Little Bluestem	6.75	6.84	2.09	32.60	51.72	0.370	0.088	74.4
	Big Bluestem	5.38	7.28	2.07	32.75	52.52	0.320	0.081	106.0
	Indian	5.96	7.84	2.57	31.97	51.68	0.430	0.093	118.3
August	Little Bluestem	10.18	5.79	1.95	29.84	53.14	0.490	0.066	62.3
	Big Bluestem	7.32	6.89	2.39	28.33	55.07	0.450	0.085	90.1
	Indian	8.91	4.47	2.01	31.65	52.96	0.460	0.059	15.4
	Switch	5.88	5.18	1.69	34.00	53.25	0.320	0.084	52.7
November	Little Bluestem	10.55	2.90	1.63	34.36	50.56	0.460	0.030	
	Big Bluestem	10.65	2.46	1.62	35.26	50.01	0.430	0.032	
	Indian	10.03	2.25	1.48	34.72	51.52	0.410	0.025	

season to values of 0.05 percent in October. The carotene content of the grasses during 1948 was well over 100 p.p.m. during most of the grazing season but dropped to values of less than 20 p.p.m. in October.

Grasses at the Wilburton area showed changes in protein and crude fiber content similar to those at the Lake C. P. Blackwell area. The calcium values, however, remained high throughout the season and were about 0.43 percent in the dead grass collected in November. Although the phosphorus content of the grasses at Wilburton were high in April and similar to those of grasses at the Blackwell area during June and August, they dropped to about 0.03 percent in November.

The major feed nutrients, and the lignin, cellulose, and carotene content of the native hay from these areas and other feed supplies fed during the winter are given in table 8. The same supplies of corn gluten meal and bone meal were fed at both locations. The composition of the corn gluten meal used in 1948-49 was very similar to that used in 1949-50. A single supply of bone meal was used during both years.

Prairie hay produced and fed at the two locations during the two winter periods contained between 4 and 5 percent of protein, about 33 percent of crude fiber, and slightly over 50 percent of nitrogen-free extract. Lignin values ranged from 8.8 to 9.8 percent and cellulose from 33 to 45 percent. The average carotene content of the hay was about the same at both locations during the same year but was slightly lower in 1948-49 than in 1949-50.

Differences between the hays in the above several constituents did not appear to be related to differences in location. The hay produced at Lake C. P. Blackwell, however, contained a very high proportion of the climax grasses, principally Big Bluestem and Little Bluestem, whereas that produced during the same year at Wilburton contained only 60 percent of these species and 40 percent of the weedy and unclassified grasses.

TABLE 8

Proximate composition, lignin, cellulose, and carotene content of winter feeds

	Description	Dry Matter	Dry Matter Composition							
			Ash	Protein	Ether Extract	Crude Fiber	N-free Extract	Lignin	Cellulose	Carotene[1]
		%	%	%	%	%	%	%	%	ppm
1948-49	Lake C.P. Blackwell									
	Prairie hay	94.68	7.52	4.43	2.05	32.82	53.18	8.82	40.28	9.1
	Corn gluten meal	94.71	5.02	43.35	3.18	3.89	44.56			
	Bone meal	96.52	81.05	0.65						
1948-49	Wilburton									
	Prairie hay	93.54	7.16	4.28	2.10	35.01	51.45	8.83	37.82	12.2
	Corn gluten meal	94.71	5.02	43.35	3.18	3.89	44.56			
	Bone meal	96.52	81.05	0.65						
1949-50	Lake C.P. Blackwell									
	Prairie hay									
	Composite sample[2]	94.20	7.80	4.95	2.11	34.82	50.32	9.56	45.33	15.3
	Climax grasses	94.34	8.37	5.11	2.06	32.71	51.75			15.0
	Weedy grasses	94.58	10.11	6.25	3.59	29.69	50.36			
	Corn gluten meal	92.96	5.94	45.92	2.78	4.63	40.73			
	Bone meal	96.52	81.05	0.65						

[1] Average values for the winter feeding periods.

[2] The composite sample contained 96% climax grasses composed of Big and Little Bluestem and 4% of weeds and unclassified grasses designated as weedy grasses.

TABLE 8 (continued)

1949-50 Wilburton									
Prairie hay									
Composite sample[3]	94.22	7.55	4.15	3.29	31.24	53.77	9.66	39.16	14.4
Climax grasses	93.95	7.43	3.86	2.61	31.28	54.82	9.80	42.15	14.5
Weedy grasses	94.65	8.81	5.58	4.38	30.89	50.84	9.92	38.14	27.4
Foreign matter	93.64	6.31	4.30	3.95	32.61	52.83	11.84	33.65	12.8
Prairie hay[4]	93.39	8.21	4.82	2.14	30.93	53.90	9.42	41.22	9.2
Corn gluten meal	92.96	5.94	45.92	2.78	4.63	40.73			
Bone meal	96.52	81.05	0.65						

[3] The composite sample contained 60% climax grasses composed of Big and Little Bluestem, 15% was weedy grasses and foreign material, and 25% was unclassified grasses.

[4] Purchased February 20, 1950, to complete winter feeding.

Differences between the two hays in their content of certain minerals are shown in the results presented in table 9. Hay produced at the Blackwell area had a slightly lower calcium and higher phosphorus content than that produced at the Wilburton area during similar years. Calcium values ranged from 0.42 to 0.75 percent. The hay produced at the Blackwell area contained 0.061 percent phosphorus the first year and 0.064 percent the second year. That at Wilburton contained 0.053 percent phosphorus the first year and 0.047 percent the second year.

There was little important difference between the hays in their content of iron, aluminum, potassium, and sodium. The Wilburton hay, however, contained over 0.025 percent manganese and over 0.40 percent magnesium while hay produced in the Blackwell area contained only from 0.003 to 0.007 percent manganese and from 0.16 to 0.19 percent magnesium. Although these differences in themselves may not be important, they reflect the relationship between mineral content of the plant and factors associated with locality and the soil on which the plant is grown.

TABLE 9

Mineral content of winter feeds[1]

	Description	Dry Matter Composition								
		Ca	P	SiO_2	Fe[4]	Mn[4]	Mg	K	Na	Al[4]
		%	%	%	%	%	%	%	%	%
1948-49	Lake C.P. Blackwell									
	Prairie hay	0.420	0.061	5.63	0.0091	0.0075	0.159	0.549	0.010	0.0148
	Corn gluten meal	0.245	0.689							
	Bone meal	25.1	18.74							
1948-49	Wilburton									
	Prairie hay	0.520	0.053	4.04	0.0095	0.0253	0.561	0.438	0.037	0.0152
	Corn gluten meal	0.245	0.689							
	Bone meal	25.1	18.74							
1949-50	Lake C.P. Blackwell									
	Prairie hay									
	Composite sample[2]	0.460	0.064	5.00	0.0091	0.0032	0.188	0.685	0.010	0.0131
	Climax grasses	0.400	0.067	5.52			0.162	0.672	0.022	
	Weedy grasses	0.370	0.069	6.64			0.234			
	Corn gluten meal	0.110	0.837							
	Bone meal	25.1	18.74							

[1] Minerals other than calcium and phosphorus were not determined on corn gluten meal or bone meal since these feeds were from the same supply at both locations and constituted only a small part of the total ration.

[2] See footnote 2, table 8.

TABLE 9 (continued)

1949-50	Wilburton									
	Prairie hay									
	Composite sample[3]	0.750	0.047	4.45	0.0082	0.0287	0.431	0.366	0.046	0.0136
	Climax grasses	0.570	0.045	5.30	0.0077	0.0309	0.351	0.399	0.037	0.0069
	Weedy grasses	0.410	0.056	6.27			0.318	0.507	0.011	
	Foreign material	1.220	0.052	2.27			0.544	0.628	0.021	
	Prairie hay[4]	0.530	0.086	6.11	0.0042	0.0182	0.358	0.385	0.017	0.0153
	Corn gluten meal	0.110	0.837							
	Bone meal	25.1	18.7[4]							

[3] See footnote 3, table 8.

[4] Results are omitted for grass samples which were ground in a Wiley mill.

DISCUSSION

The condition of the cattle in all lots at the Blackwell area was satisfactory as judged by standards of the Animal Husbandry Department for beef cattle managed as described in this experiment. The satisfactory performance of the cattle in lot 1 is evidence that adequate phosphorus was provided in the native pasture grasses grazed in the summer and the prairie hay produced in this area and fed with a low-phosphorus protein supplement in the winter. Further evidence of the adequacy of phosphorus intake is provided by the results of the blood phosphorus determinations. Although these values for the original cows in lot 1 dropped to between 3 and 4 mg. percent toward the end of the winter period, similar values were obtained during the early part of the grazing season for comparable animals in lots 2 and 3 receiving a mineral phosphorus supplement. From these observations and those reported by Watkins and Knox at New Mexico, it appears that blood phosphorus values of 3-4 mg. percent are not below the normal, or expected, range for beef cows.

The amount of feed phosphorus required to maintain blood phosphorus at these levels can be closely approximated from records of feed intake and feed composition. During the winter months the cows in lot 1 consumed an average of 17 pounds of hay and 1.25 pounds of corn gluten meal, per head, daily. The only other available source of phosphorus was from occasional weeds and winter grasses which, during mild weather, started growth within the winter trap. On the assumption that these materials in the amounts present were of negligible value, it is estimated that the cows consumed 9.70 grams of phosphorus from the daily feed containing 0.117 percent of phosphorus. Expressed in relation to average weight of the cows during the winter, the daily phosphorus intake would be 1.21 grams per 100 pounds of liveweight, a figure considerable lower than

that usually recommended. Phosphorus intake calculated in the same manner for calves in lot 1 would be about 1.25 grams per 100 pounds of liveweight.

There appeared to be no reliable method for calculating the exact phosphorus intake of the cattle while on pasture, consequently, intakes were estimated on the basis of the phosphorus content of the predominate species of grasses during different months of the grazing season. In addition to phosphorus these grasses supplied carotene and protein in considerably greater amounts than the hay fed during the winter, which accounts for the increased percentage of phosphorus and carotene in the blood of the cattle in lot 1 during the summer. The initial drop in phosphorus in the blood of animals in lots 2 and 3 is indicative of decreased intake associated perhaps with selective grazing or refusal of mineral supplement.

The apparent effect of low-phosphorus intake on blood carotene which was observed during periods of constant carotene intake in this study has become the subject of another investigation (46). It appears that a low-phosphorus intake interfers with carotene conversion to vitamin A. This effect, however, had no serious consequence in the present investigation since symptoms of vitamin A deficiencies were not observed and blood vitamin A values were at all times within the range reported for beef cattle (6, 47).

The condition of the cattle in all lots at Wilburton has been described on different occasions as unsatisfactory with respect to general appearance, growth, and calving records (47, 48). Phosphorus appeared to be the first limiting factor in the nutrition of these animals since those in lots 2 and 3 maintained higher blood-phosphorus levels and were in better condition at all times than comparable animals in lot 1. The pasture grasses and hay fed at Wilburton contained only slightly less phosphorus than those of the Blackwell area yet, extreme cases of phosphorus deficiency were common among the cows in lot 1.

During the winter feeding period, blood phosphorus values in this lot were dangerously low. Despite the improved condition of the animals in lots 2 and 3, attributed to phosphorus and the maintenance of normal blood phosphorus values, even the best lots of cows and calves at Wilburton were judged inferior to those in the poorest lot at the Blackwell area. An explanation was sought in a more complete analysis of the blood of the cattle and of the forage produced at the two areas.

The relatively high content of manganese, 253 p.p.m., found in the forage at Wilburton was found to be associated with a high percentage of this element in the soil.[1] During winter feeding when feed intake could be calculated with reasonable accuracy, the cows at Wilburton were consuming about 2.0 grams of manganese per day. Although manganese in these amounts may not be detrimental, the fact that it was present in amounts 8 times that found in the forage at Lake C. P. Blackwell leads to the suggestion that other trace elements of greater physiological importance may likewise be present in relatively large amounts. The possible presence of such elements is being investigated. Pilot experiments with rabbits have yielded evidence that rations supplying over 300 p.p.m. of manganese inhibit growth.

The low values obtained for hemoglobin and red cell volume are strong evidence of an anemic condition among the cattle at Wilburton, particularly during the period of confinement to winter traps. Both the hemoglobin and the hematocrit values were found to be higher after the cattle had been on pasture for about two months. All other blood constituents determined were found to be in the normal range for beef cattle.

---

[1] Soil analysis and description supplied by the Department of Agronomy.

SUMMARY

Satisfactory production of range beef cattle at Lake C. P. Blackwell was obtained by grazing the cattle on native pasture grass during the summer and feeding them native hay supplemented with corn gluten meal during the winter. Salt was the only mineral supplement. The winter ration supplied 0.105 percent of phosphorus. Additional phosphorus, fed as dicalcium phosphate, added to the protein supplement during the winter and mixed with salt for the cattle during the summer was of no apparent value.

Under similar conditions of management and feeding at Wilburton, in Southeastern Oklahoma, beef cattle developed a severe phosphorus deficiency. The summer pasture grasses provided only slightly less phosphorus than those at Lake C. P. Blackwell and the winter ration contained 0.101 percent of phosphorus. A mineral phosphorus supplement only partially alleviated the condition of the cattle.

Analysis of blood from the phosphorus deficient cattle revealed a low inorganic phosphorus content of the plasma and low values for plasma proteins, hemoglobin, and red cell volume. Values for calcium, magnesium, copper, carotene, and vitamin A were within the normal range. Blood from the phosphorus supplemented cattle contained normal amounts of inorganic phosphorus but was low in plasma protein, hemoglobin, and red cell volume.

The native grass hays at the two areas were similar in their content of total feed nutrients, cellulose, lignin, carotene, calcium, iron, aluminum, potassium, and sodium. The Wilburton hay was slightly lower in phosphorus, higher in magnesium, and contained from 4 to 8 times as much manganese as that grown at the Blackwell area.

Anemia, unavailability of phosphorus, and possible interference with normal phosphorus metabolism by manganese or some closely associated material in the Wilburton hay are offered as possible explanations for the poor condition of cattle in the Wilburton area. In addition, low fertility of the soil, the prevalence of weeds in the pastures, and low availability of nutrients in the forage of this area are suggested as contributing factors.

BIBLIOGRAPHY

1. Theiler, A. and H. H. Green.
   Aphosphorosis in ruminants.
   Nutrition Abs. and Rev. 1: 353-485 (1932).

2. Eckles, C. H., R. B. Becker, and L. S. Palmer.
   A mineral deficiency in the rations of cattle.
   Minnesota Agri. Exp. Sta. Bull. 229, 49 pps. (1926).

3. Becker, R. B., W. M. Neal, and A. L. Shealy.
   Stiffs or sweeny (phosphorus deficiency) in cattle.
   Florida Agri. Exp. Sta. Bull. 264, 25 pps. (1933).

4. Eckles, C. H., T. W. Gullickson, and L. S. Palmer.
   Phosphorus deficiency in the rations of cattle.
   Minnesota Agri. Exp. Sta. Tech. Bull. 91, 118 pps. (1932).

5. Huffman, C. F., C. W. Duncan, C. S. Robinson, and L. W. Lamb.
   Phosphorus requirement of dairy cattle when alfalfa furnished the principal source of protein.
   Michigan Agri. Exp. Sta. Tech. Bull. 134, 75 pps. (1933).

6. Knox, J. H., J. W. Benner, and W. E. Watkins.
   Seasonal calcium and phosphorus requirements of range cattle, as shown by blood analysis.
   New Mexico Agri. Exp. Sta. Bull. 282, 28 pps. (1941).

7. Black, W. H., L. H. Tash, J. M. Jones, and R. J. Kleberg, Jr.
   Effects of phosphorus supplements on cattle grazing on range deficient in this mineral.
   U. S. D. A. Tech. Bull. 856, 23 pps. (1943).

8. Maynard, L. A.
   Animal Nutrition, second edition, pp. 120-121. McGraw-Hill Book Company, New York, N. Y. 1947.

9. Beeson, W. M.
   Phosphorus needs for fattening beef steers.
   Idaho Agri. Exp. Sta. Circular 83, 6 pps. (1941).

10. Mitchell, H. H.
    The mineral requirements of farm animals.
    Jour. Ani. Sci. 6: 365-377 (1947).

11. Watkins, W. E. and J. H. Know.
    The monthly protein and phosphorus contents of two important range grasses of Southern New Mexico.
    Jour. Ani. Sci. 4: 297-305 (1945).

12. Guilbert, H. R., P. Gerlaugh, and L. L. Madsen.
Recommended nutrient allowances for beef cattle.
Nat'l. Res. Council, No. 4, 32 pps. (1945).

13. Reid, R. L., M. F. Franklin, and E. G. Hallsworth.
The utilization of phytate phosphorus by sheep.
Australian Vet. Jour. 23: 136-140 (1947).

14. Jordan, W. H., E. B. Hart, and A. J. Patton.
Am. Jour. Physiol. 16: 268 (1906).
(Quoted by Reid, Franklin, and Hallsworth) (13).

15. Mitchell, H. H. and J. F. McClure.
Mineral nutrition of farm animals.
Nat'l. Res. Council Bull. 99, 133 pps. (1937).

16. Axelsson, J. and S. Eriksson.
Factors influencing the calcium and phosphorus metabolism of adult sheep.
The Annals of the Royal Agri. College of Sweden 16: 711-721 (1949).

17. Watkins, W. E.
Composition of range grasses and brouse at varying stages of maturity.
New Mexico Agri. Exp. Sta. Bull. 311, 43 pps. (1943).

18. Fudge, J. F. and G. S. Fraps.
The chemical composition of forage grasses from the gulf coast prairie as related to soils and to requirements for range cattle.
Texas Agri. Exp. Sta. Bull. 664, 39 pps. (1944).

19. Cox, G. J., M. L. Dodds, H. B. Wigman, and J. F. Murphy.
The effect of high doses of aluminum and iron on phosphorus metabolism.
J. Biol. Chem. 92: XI (1931).

20. Deobald, H. J., and C. A. Elvehjem.
The effect of feeding high amounts of soluble iron and aluminum salts.
Am. J. Physiol. 111: 118-123 (1935).

21. Jones, J. H.
The metabolism of calcium and phosphorus as influenced by the addition to the diet of salts of metals which form insoluble phosphates.
Am. J. Physiol. 124: 230-237 (1938).

22. Rehm, P. and J. C. Winters.
The effect of ferric chloride on the utilization of calcium and phosphorus in the animal body.
J. Nutrition 19: 213-222 (1940).

23. Williams, D. E., F. L. MacLeod, and E. Morrell.
Availability to rats of phosphorus in red clover hays of widely varying phosphorus content.
J. Nutrition 23: 501-511 (1942).

24. Maynard, L. A.
Relation of soil and plant deficiencies and of toxic constituents in soils to animal nutrition.
Ann. Rev. Biochem. 10: 451 (1941).

25. Chornock, C. S., M. B. Guereant, and R. A. Dutcher.
Effect of manganese on calcification in the growing rat.
J. Nutrition 23: 445 (1942).

26. Smith, S. E. and G. H. Ellis.
Studies of the manganese requirements of rabbits.
J. Nutrition 34: 33-41 (1947).

27. Archibald, J. G. and H. G. Lindquist.
Manganese in cows milk.
J. Dairy Sci. 26: 325-330 (1943).

28. Elvehjem, C. A.
The biological significance of copper and its relation to iron metabolism.
Physiol. Rev. 15: 471-507 (1935).

29. Becker, R. B., W. M. Neal, and A. L. Shealy.
Salt sick: its cause and prevention.
Florida Agri. Exp. Sta. Bull. 231, 23 pps. (1931).

30. Crampton, E. W. and L. A. Maynard.
The relation of cellulose and lignin content to the nutritive value of animal feeds.
J. Ani. Sci. 3: 446 (1944).

31. Crampton, E. W. and I. R. C. Jackson.
Seasonal variation in chemical composition of pasture herbage and the relation to its digestibility by steers and sheep.
J. Ani. Sci. 3: 333-339 (1944).

32. Phillips, T. G. and M. E. Loughlin.
Composition and digestible energy of hays fed to cattle.
J. Agri. Res. 78: 389-395 (1949).

33. Forbes, R. M. and W. P. Garrigus.
Application of a lignin ratio technique to the determination of the nutrient intake of grazing animals.
J. Ani. Sci. 7: 373-382 (1948).

34. Fiske, C. H. and Y. Subbarow.
The colorimetric determination of phosphorus.
J. Biol. Chem. 66: 375 (1925).

35. Clark, E. P. and J. B. Collip.
A study of the Tisdall method for the determination of blood serum calcium with a suggested modification.
J. Biol. Chem. 63: 461 (1925).

36. Denis, W.
The determination of magnesium in blood, plasma, and serum.
J. Biol. Chem. 52: 411 (1922).

37. Cartwright, G. E., P. J. Jones, and M. M. Wintrobe.
A method for the determination of copper in blood serum.
J. Biol. Chem. 160: 593-600 (1945).

38. Kimble, M. S.
The photo-colorimetric determination of vitamin A and carotene in human plasma.
Jour. Lab. and Clin. Med. 24: 1055 (1939).

39. Koch, F. C. and M. E. Hanke.
Practical Methods in Biochemistry, fifth edition, 419 pps., illus., The Williams and Wilkins Company, Baltimore, Md. 1948.

40. Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, sixth edition, 932 pps., illus., Washington, D. C. 1945.

41. Peterson, W. J.
Recent developments in methods for determining carotene.
Ind. Eng. Chem. Anal. Ed. 13: 212-216 (1941).

42. Wall, M. E. and E. G. Kelley.
Determination of pure carotene in plant tissue. A rapid chromatographic method.
Ind. Eng. Chem. Anal. Ed. 15: 18-20 (1943).

43. Willard, H. H. and L. H. Greathouse.
Colorimetric determination of manganese by oxidation with periodate.
J. Am. Che. Soc. 39: 2366-2377 (1917).

44. Ellis, G. H., G. Matrone, and L. A. Maynard.
A 72 percent H_2SO_4 method for the determination of lignin and its use in animal nutrition studies.
J. Ani. Sci. 5: 285-297 (1946).

45. Matrone, G., G. H. Ellis, and L. A. Maynard.
A modified Norman-Jenkins method for the determination of cellulose and its use in the evaluation of feedstuffs.
J. Ani. Sci. 5: 306-311 (1946).

46. Ross, O. B. and W. D. Gallup.
The effect of phosphorus deficiency on blood plasma carotene.
J. Ani. Sci. 8: 628 (1949).

47. Ross, O. B., W. D. Gallup, D. F. Stephens, C. C. Chamberlain, and W. D. Campbell.
Phosphorus studies of range beef cattle.
Oklahoma Agri. Exp. Sta. Misc. Pub. 15, p. 10 (1949).

48. Ross, O. B., W. D. Gallup, J. A. Nance, W. D. Campbell, and A. E. Darlow. Effect of phosphorus upon the reproduction of beef cows. Oklahoma Agri. Exp. Sta. Misc. Pub. 17, pp. 28-35 (1950).

THESIS TITLE: A Chemical Investigation of Phosphorus Deficiency in Range Beef Cattle

NAME OF AUTHOR: Myron E. Gibson, Jr.

THESIS ADVISER: Willis D. Gallup

The content and form have been checked and approved by the author and thesis adviser. "Instructions for Typing and Arranging the Thesis" are available in the Graduate School office. 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.

NAME OF TYPIST: Leota L. McOsker