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*The Effect of Feeding Different Amounts
of Calcium and Phosphorus upon
the Growth and Development
of Dairy Animals*

by H. O. HENDERSON and C. E. WEAKLEY, JR.

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*The Effect of Feeding Different Amounts of Calcium and Phosphorus upon the Growth and Development of Dairy Animals**

by H. O. HENDERSON and C. E. WEAKLEY, JR.†

IT HAS long been known that calcium and phosphorus are necessary in the rations of farm animals if they are to grow and have normal development. It was generally supposed, however, that any ration which supplied sufficient protein, carbohydrates, and fat to meet the requirements of the growing animal would also supply sufficient amounts of these two minerals. For this reason very little consideration has been given to the optimum amount of calcium and phosphorus required for normal growth and development of the dairy heifer.

In recent years much experimental work in mineral metabolism has emphasized the importance of calcium and phosphorus in the ration and has shown that these elements sometimes may not be present in sufficient amounts for normal growth and development. Within the last decade a dietary factor has been discovered which aids in the assimilation of calcium and phosphorus. The ultra-violet rays, when animals are exposed to them, also have the power to help with this assimilation. Most of such studies have been made with small laboratory animals or with such domestic animals as the chicken, the pig, or the dairy cow, but very little has been done with the growing dairy animal.

It has also been shown recently that in many sections of the country the soil is deficient in calcium or, more especially, in phosphorus, to the extent that the forage grown on it will not support normal growth and development in dairy heifers. This has led to the belief, fully substantiated, that some of the roughage grown in various parts of the country may be extremely low in one or both of these elements.

There has been a tendency also in late years toward the feeding of more and more by-products of milling and other industries. Such feeds are also quite low in one or both of these elements, with a correspondingly greater chance that a ration may be deficient in these minerals.

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With these points in mind it seemed important that a study of the requirements of the growing dairy heifer for calcium and phosphorus be made. The investigations herein reported were made to determine the effects upon the growing dairy heifer of feeding rations containing various amounts of calcium and phosphorus. The study was not intended to determine the exact requirements for calcium and phosphorus because of the many other factors involved, but was made as a contribution toward that end. When we consider that more than 4,500,000 dairy calves must be raised each year in order to maintain the present supply of dairy cows, the importance of additional information in this direction is readily apparent.

REVIEW OF LITERATURE

The deposition of calcium and phosphorus in the animal body has been a field of study for many years. Among the earlier investigators who studied the effects of rations low in minerals upon animals were Chossat (1842), who worked with pigeons; Weiske (1872), and Weiske and Wildt (1873), who used goats and lambs; Forster (1873) who employed pigeons and later dogs; Voit (1880) who also used pigeons and dogs; and Aron and Sebauer (1908) who worked upon rabbits and dogs. These investigators demonstrated the necessity of minerals in the diet.

Later studies by Osborne and Mendel (1918) have shown that the rat will not develop unless given sufficient minerals in its diet. Sherman and Pappenheimer (1921) and McCollum and co-workers (1921) have shown that rickets in rats can be produced by feeding rations low in one or both of these minerals.

A trouble known as leg weakness in chickens also has been attributed to the lack of sufficient minerals in the ration and has been described by Hart, Halpin, and Steenbock (1920) as a form of rickets which occurs when baby chicks are kept in confinement and when the chicks are from 4 to 8 weeks old.

Maynard, Goldberg, and Miller (1925), Bohstedt (1926) and co-workers (1927), and others have shown that when pigs are fed rations low in calcium, posterior paralysis results. This condition is due to a broken vertebra pressing upon the spinal cord. Somewhat similar results were obtained by Hart, McCollum, and Fuller (1909) when pigs were fed rations low in phosphorus.

The effects of feeding rations low in calcium and phosphorus to cattle also have been noted within recent years. The occurrence of abnormal conditions in cattle due to a mineral deficiency has been reported in various parts of the United States. In such sections the soil is so deficient in calcium, and more often in phosphorus, that the forage grown upon it is low in its content of these two minerals. In such sections the cattle very often are stunted, showing extreme emaciation, stiffness in the hind quarters and also at times in the front quarters, swollen joints, harshness of coats, dull eyes, unthrifty condition, and perverted appetite. Such areas are known as mineral deficiency areas. Studies of this subject have been made by Welch

(1924) in Montana, by Schmidt (1924) in Texas, by Eckles, Becker, and Palmer (1926) in Minnesota, and by Hart and co-workers (1927) in Wisconsin. The trouble has also been reported in West Virginia and Alabama by Halverson and Nixon (1924), in Florida by Bitting (1894), in Pennsylvania by Pearson (1898), and in various other states.

Similar areas have been reported in other countries. Von Gohren (1861) and Nessler (1873) reported such trouble in Germany, Aston (1905) in New Zealand, Murphy (1917) in Victoria, Theiler (1920) and associates (1924) in South Africa, Tuff (1924) in Norway, and Marcq (1925) in the Belgian Congo. This condition is no doubt much more widespread than these scattered reports indicate.

Very little experimental work has been reported concerning the effects of rations low in calcium and phosphorus upon growing dairy animals. Eckles and Swett (1918) found that a dairy heifer when fed a ration low in calcium made normal growth up to eighteen months of age, but that she then became stiff in her joints and could get up only with the greatest difficulty. Rose (1912) found that dairy cows receiving a low phosphorus ration for four weeks became stiff in the joints and decreased in milk flow. Salmon and Eaton (1925) were unable to increase the growth of heifers by the addition of bonemeal to the ration.

Forbes and co-workers (1922) after extensive mineral balance experiments with dairy cows in milk flow (1916, 1917, 1918, 1922a) concluded that the calcium metabolism of the milk cow while on winter feeds is characterized by rapid loss from the body during the early part of lactation, changing to retention late in lactation, and by continued retention during the dry period, with most rapid storage at the end of the period of gestation. Hart and associates (1921, 1922, 1923, 1923a) have found that green plant tissue contains the factor which assists in assimilation of minerals by the dairy cow. The same authors (1924) also found that the ultra-violet rays have a beneficial influence upon the assimilation of calcium and phosphorus by a milking goat.

METHODS EMPLOYED IN THE EXPERIMENT

The object of the experiment was to determine the effect upon growing dairy heifers of feeding rations which contained different amounts of calcium and phosphorus.

In order to make such a study 32 grade Holstein heifer calves were divided into 4 groups of 8 calves each. Each group was fed a ration as similar to the other rations in its digestible crude protein and total nutrient content as it was practical to obtain. All were fed according to the Morrison Feeding Standard for dairy heifers. The chief difference between the rations fed was in the calcium and phosphorus content.

Group I, the check group, during the trial was fed, as far as could be ascertained before the beginning of the experiment, a normal ration. This ration was selected as typical of those fed under practi-

cal farm conditions. Since this ration contained a legume hay as one-half of the roughage and a typical grain mixture which has been fed widely without apparent bad results, it was assumed that its calcium and phosphorus content was sufficient.

Group II was fed a ration very much lower in its calcium content but otherwise similar to that of Group I in its nutritive value. Group II was designated as the low-calcium group.

Group III, designated as the low-phosphorus group, was fed a ration similar in its nutritive value to that fed to Group I with the exception that it was much lower in its phosphorus content.

Group IV, designated as the low-calcium-low-phosphorus group, was fed a ration low in both calcium and phosphorus as compared to the ration fed to Group I, but otherwise similar in its nutritive value.

The animals were handled and cared for in the same manner throughout the experiment, in order that, as far as could be ascertained, the rations consumed would constitute the only difference between the 4 groups. There was, of course, some difference in the individuality of the calves, but this probably was not pronounced, since the groups were quite uniform at the beginning of the experiment.

In order to study the difference between the rations, the heifers were weighed and the height at their withers was measured at weekly intervals so that the growth of the animals could be ascertained. The calves also were watched daily for all abnormalities, and notes were taken as to their general appearance, health, period of oestrus, unnatural cravings, or any other tendencies.

Analyses were made also of the blood for calcium and inorganic phosphorus at three-month intervals in order to ascertain the effects of the ration upon the calcium and inorganic phosphorus content of the blood.

To ascertain the effects upon the internal organs and upon the bones of the animals, two animals from each group were slaughtered at the end of 8 months, two at the end of 13 months, two at the end of 19 months, and two at the end of 25 months. At the time of slaughter the internal appearance of the animals was noted, and weights were taken of the different internal organs. Samples of the long bones also were obtained for analysis and for determination of breaking strength.

Throughout the experiment Group I was considered as normal, and the other groups were compared to it.

DESCRIPTION OF ANIMALS USED

Thirty-two grade Holstein calves from 2 to 4 weeks in age were obtained from northern Pennsylvania, arriving in the coldest part of the winter. Most of them took sick and several died. Replacements in most cases from other shipments from the same source were made as soon as possible. Many of the calves, however, were slow to recover, and it was necessary to feed them milk much longer than had been planned. No knowledge of the ancestry of the animals was at hand. They were well marked, only two of them showing any Jersey

characteristics. Since all came from the same locality it may be assumed that they probably had somewhat the same general ancestry. They were under the normal size as given by Eckles (1920) in weight but not in height at withers.

The calves were divided into 4 groups as nearly as possible the same as to age, weight, and height at withers. In certain groups there were more replacements than in others, and hence the difference in size became somewhat greater; but in spite of such replacements the 4 groups were fairly uniform at the beginning of the experiment. Table 1 gives the age, the time started on experiment, and the initial weight and height at withers for the various calves.

Housing and Care of the Animals

The animals were housed in a well-lighted and ventilated experimental barn. Each animal was kept in an individual pen so that it

TABLE 1.—*Date of Birth, Date of Starting on Experiment, Weight, and Height of Animals Used in the Experiment*

Number of Calf	Date Born	Date Started on Experiment*	Weight	Height at Withers
GROUP I. NORMAL GROUP				
			(pounds)	(cms.)
E-1	1-24-26	2-22-26	100	75.5
E-2	1-28-26	2-22-26	98	73.3
E-3	2-3-26	2-22-26	87	73.5
E-4	2-1-26	2-22-26	85	74.0
E-5	1-28-26	2-22-26	79	73.4
E-6	2-2-26	2-22-26	75	71.6
E-7	2-3-26	2-22-26	69	68.5
E-8	2-2-26	2-22-26	64	69.0
Average			82.1	72.3
GROUP II. LOW-CALCIUM GROUP				
E-9	1-25-26	2-22-26	100	78.7
E-10	4-10-26	4-20-26	80	71.0
E-11	2-5-26	2-22-26	87	72.5
E-12	2-5-26	2-22-26	85	72.8
E-13	2-2-26	2-22-26	76	70.3
E-14	2-4-26	2-22-26	75	71.6
E-15	3-15-26	4-1-26	79	68.5
E-16	2-8-26	2-22-26	67	74.4
Average			81.1	72.3
GROUP III. LOW-PHOSPHORUS GROUP				
E-17	1-23-26	2-22-26	109	75.5
E-18	2-6-26	2-22-26	96	75.0
E-19	1-27-26	2-22-26	90	72.9
E-20	1-31-26	2-22-26	82	74.2
E-21	1-28-26	2-22-26	81	75.0
E-22	2-4-26	2-22-26	74	70.3
E-23	2-1-26	2-22-26	70	72.3
E-24	3-15-26	4-1-26	82	72.0
Average			85.5	73.4
GROUP IV. LOW-CALCIUM-LOW-PHOSPHORUS GROUP				
E-25	1-23-26	2-22-26	109	80.5
E-26	2-24-26	3-1-26	110	74.0
E-27	2-5-26	2-22-26	90	72.9
E-28	1-30-26	2-22-26	84	71.1
E-29	1-31-26	2-22-26	80	71.3
E-30	4-10-26	4-15-26	90	73.0
E-31	2-1-26	2-22-26	69	71.5
E-32	3-15-26	4-1-26	89	75.0
Average			90.1	73.8

*The calves were put on the various rations outlined at about four months of age.

could be fed separately. The pens were about $3\frac{1}{2}$ by 4 feet in size. When the calves were 13 months old, after one-half their number had been slaughtered the pens were increased in size to about 5 by 6 feet. The animals remained in these pens all the time except once a week, when they were removed to be weighed and measured. This was done in the same barn. They were removed from the barn but once every six months, when they were being photographed.

Although the barn had an abundance of light, the ultra-violet rays were not a factor to be considered, since the windows were kept closed. Hess and associates (1922) have shown that common window glass will allow no wave lengths shorter than 334 millimicrons to pass through and so will filter out all of the light that is beneficial to calcium and phosphorus assimilation. Gullickson and Eckles (1927), however, have found that dairy heifers can be grown satisfactorily away from the light even on rations not very high in calcium.

Exercise. The animals were given no exercise except what they were able to obtain in their pens and when they were weighed each week. It was noted that many of them played a great deal in their pens, thus getting considerable exercise.

Bedding. The pens were bedded with shavings so that no nutrients were obtained from that source.

Weighing and Measuring. Weight of the animals and measurements at the height of the withers were taken at weekly intervals. These weights and measurements were averaged monthly.

Breeding. Since it was the object to study the effects of the rations on growing heifers, none of the animals was bred, in order that the factor of gestation might not enter into the problem. The times of oestrus, however, were observed in order to determine the effect of the rations upon the time of the first signs of oestrus and the regularity thereafter.

Salting. Once a week each heifer was given all the salt desired. The salt was carefully weighed and placed before each animal and left there for about one hour, when it was removed and again weighed. In this way the amount consumed was ascertained.

The amount consumed varied from month to month, but the heifers on the restricted ration did not appear to crave the salt and did not consume any more than did those fed the normal ration. It was planned to restrict all calves to the same amount of salt as was consumed by the ones on the normal ration, but since the other calves did not seem to crave the salt, restriction was not found necessary.

Water. Water was given twice a day in buckets. Three times per month—on the fifth, fifteenth, and twenty-fifth days of the month—weights were taken of the amount consumed and used as the average daily consumption of water. There was considerable variation in the consumption of water by individual animals. Some individuals would average as much as 90 pounds per day for certain months, while others would average as low as 20 pounds for the same month. Some were consistently heavy drinkers while others were just as consistently light drinkers. No apparent difference could be noted,

however, between heavy and light drinkers. Neither could it be noted that any one group had a larger number of heavy drinkers than any other group.

When the average daily consumption of water per animal in each group was considered, very little difference could be noted in the amount consumed. Group II, the low-calcium group, consumed on the average about 2½ pounds more per day than the normal-fed group, and Group IV, the low-calcium-low-phosphorus group, consumed a little over one-half pound more than did the calves receiving the normal ration. Group III, the low-phosphorus group, on the other hand, consumed almost a pound less per day than the normal-fed group.

TABLE 2.—Average Composition of Feeds Used

Feed	Water	Ash	Crude Protein	Carbohydrates		Ether Extract
	(percent)	(percent)	(percent)	Cr. Fiber	N.F.E.	
	(percent)	(percent)	(percent)	(percent)	(percent)	(percent)
Cornmeal	9.96	1.54	9.44	2.78	72.03	4.25
Oats (ground)	7.76	3.76	16.87	12.55	61.52	3.54
Linseed oilmeal	8.07	5.15	35.92	7.17	37.64	6.05
Soy beans	6.70	4.82	39.70	4.26	24.84	19.58
Beet pulp	7.66	3.21	10.25	19.07	59.12	0.69
Polished rice	10.91	0.52	5.94	0.47	80.77	0.39
Wheat gluten	7.76	0.97	80.22	0.66	9.94	0.45
Washed bran	7.40	1.50	14.70	13.12	61.15	2.13
Alfalfa hay	5.14	5.61	11.54	32.28	43.98	1.42
Timothy hay	5.33	4.70	5.85	30.96	51.36	1.80

The water was analyzed for calcium and phosphorus and found to contain 2.96 parts of calcium per million parts of water and only a slight trace of phosphorus. The amount of these elements obtained from the water therefore was very small. The amount of calcium received per month through the water would be about 12 grams, which would amount to but 0.4 of a gram per day.

Methods of Feeding

Early Feeding. Since the calves were received in a weakened condition, many had to be fed whole milk and all had to be fed skim milk for a much longer period than had been planned. It was impossible to make them consume the experimental ration for some time and so they were started on whole oats and later fed for a short period on a ration of equal parts of oats, corn, and linseed oilmeal. They were put on the experimental ration at 4 months of age, when they were growing normally. They were not weaned from skim milk until they were five months of age; some individuals were fed skim milk until they were six months old.

Analysis of Rations. Each animal was fed individually throughout the experiment. Necessary changes in the feeding were made at two-week intervals, and all refuse was carefully recorded daily. In this way a complete record was kept of all the feed consumed by each individual. A year's supply of feed was purchased at a time so that variations in the feeds would be kept at a minimum. These feeds were carefully sampled and analyzed each year. The results as given in Table 2 are the average analyses of the feeds for the two years of the experiment.

Rations. The rations used throughout the experiment are given in Table 3.

The rations numbered from I to IV were fed during the first year of the experiment and were fed to Groups I to IV, respectively. They were calculated for a normal 6-months old heifer and were fed so that each was receiving its requirements according to the Morrison standard.

When the heifers were a year old the rations were changed. This was made necessary because the requirements change slightly as the animals grow older, and, in the case of Rations III and IV, because analysis of the phosphorus content of the washed bran showed that only about one-third of the phosphorus was being removed by the method used in washing. Hart, McCollum, and Fuller (1909) had been able to wash practically all of the phosphorus from bran by allowing whole bran to soak over night in warm water, when an acid fermentation resulted. The bran subsequently was thoroughly washed with water and dried. The bran washed in their work contained only 0.11 percent of phosphorus as compared to 1.51 percent for whole bran. The results in the present experiment showed that the bran washed in the manner described above contained on the average 0.94 percent of phosphorus, and as it was being fed for its supposedly low phosphorus content it was at once omitted from the ration, and beet pulp was substituted.

TABLE 3.—Average Daily Ration Fed during Experiment

Feed	Ration Number							
	I	II	III	IV	Ia	IIa	IIIa	IVa
Alfalfa hay (pounds)....	3	...	3	...	4	...	4	...
Timothy hay "	3	6	3	6	4	8	4	8
Cornmeal "	1¼	1	...	...	3	2	...	...
Ground oats "	1¼	1	...	...	3	2	...	...
Linseed oilmeal "	¾	...	...	...	...	...	...	...
Ground soybeans "	...	1½	...	...	...	1¾	...	...
Polished rice "	...	...	2	1½	...	...	3	3
Washed bran "	...	...	1½	2	...	...	...	...
Wheat gluten "	...	...	¾	½	...	...	½	1
Beet pulp "	...	...	...	...	...	...	2	1½

Rations Ia to IVa were fed during the second year of the experiment and were fed to Groups I to IV respectively. They were calculated for normal 12-months old heifers and differed only from Rations I to IV in amounts, with the exception that linseed oilmeal was dropped out of Ration I to form Ration Ia, and beet pulp was substituted for washed bran in Rations III and IV to form Rations IIIa and IVa.

The average analyses as shown in Table 2 were converted to the digestible basis by the digestibility of American feed stuffs as given by Henry and Morrison (1923) whenever possible; otherwise the digestibility of some closely related feed was used. In this way the average amount of digestible protein and digestible nutrients consumed by each group was ascertained as is shown in Table 4. This table and many of those following are divided into periods, from the beginning of the experiment to 8 months, from 8 months to 13

months, from 13 months to 19 months, and from 19 months to 25 months to correspond to the time of slaughtering the animals, as studies and comparisons can more easily be made in that manner.

Table 4 shows that, whereas there is considerable difference in the total amount of digestible crude protein and total digestible nutrients consumed per day, on the live weight basis there is very little difference. The normal group consumed slightly less digestible protein than did the other groups. There was very little difference between the amount of digestible nutrients consumed by the different groups per 100 pounds of live weight. This would indicate that, as far as nutritive value was concerned, the animals should make similar growth.

Calcium and Phosphorus Content of the Ration. The different feeds were analyzed for their ash and calcium and phosphorus content, with the results as shown in Table 5.

The feeds, as a general rule, analyzed just a little higher in calcium than has been found at other stations. This was especially true of the calcium content of the alfalfa and timothy hay. Both contained more than twice as much calcium as was found by Forbes and co-workers (1913). Both also were lower in phosphorus than was found by Forbes. This was particularly true of the alfalfa hay, of second-

TABLE 4.—Average Daily Amount of Digestible Crude Protein and Total Digestible Nutrients Consumed by the Animals in Each Group, and Average Daily Amount Consumed Per 100 Pounds of Live Weight of the Animals, by Periods

Period End- ing when Animals Were at Age of	Average Weight	Pounds Protein Consumed per Day	Pounds Protein Consumed Daily per 100 Pounds Live Weight	Pounds Total Digestible Nutrients Consumed per Day	Pounds Total Digestible Nutrients Consumed Daily per 100 Pounds Live Weight
GROUP I. NORMAL GROUP					
8 mos.	196	0.67	0.34	3.86	1.97
13 mos.	408	0.91	0.22	7.09	1.74
19 mos.	646	0.91	0.14	9.74	1.51
25 mos.	880	1.07	0.12	10.82	1.23
GROUP II. LOW-CALCIUM GROUP					
8 mos.	184	0.66	0.36	3.84	2.09
13 mos.	381	1.11	0.29	7.61	2.00
19 mos.	585	1.12	0.19	8.67	1.48
25 mos.	750	1.26	0.17	10.09	1.34
GROUP III. LOW-PHOSPHORUS GROUP					
8 mos.	192	0.63	0.33	3.79	1.94
13 mos.	395	0.89	0.23	6.90	1.75
19 mos.	617	1.09	0.18	9.40	1.54
25 mos.	833	1.27	0.14	10.43	1.25
Group IV. LOW-CALCIUM-LOW-PHOSPHORUS GROUP					
8 mos.	180	0.62	0.34	3.55	1.97
13 mos.	366	0.93	0.25	6.80	1.86
19 mos.	570	1.10	0.19	8.36	1.47
25 mos.	731	1.28	0.17	9.37	1.28

TABLE 5.—Average Amount of Ash, Calcium, and Phosphorus Found in 100 Pounds of Feed Used in Experiment

Feed	Pounds Ash	Pounds CaO	Pounds Calcium	Pounds P ₂ O ₅	Pounds Phosphorus
Cornmeal	1.34	0.038	0.027	0.69	0.301
Oats (ground) ...	3.37	0.130	0.093	0.88	0.383
Linseed oilmeal .	5.72	0.530	0.379	1.90	0.828
Ground soybeans	4.51	0.290	0.208	1.36	0.593
Beet pulp	3.56	1.100	0.788	0.20	0.087
Polished rice	0.55	0.015	0.011	0.23	0.100
Wheat gluten ...	1.01	0.100	0.072	0.73	0.315
Washed bran	5.45	0.218	0.156	2.16	0.942
Alfalfa hay	8.53	3.300	2.369	0.35	0.159
Timothy hay	4.63	0.530	0.379	0.30	0.131

cut alfalfa obtained from Michigan. Reed and Huffman (1928) have found that the alfalfa hay from parts of Michigan is quite high in calcium and low in phosphorus—a possible explanation of results. The fact that the analyses varied from the average has caused the rations to be higher in calcium and the normal phosphorus rations to be lower in phosphorus than was desired. The fact also that the phosphorus was not washed from the bran caused Rations III and IV to be much higher in phosphorus than was desired.

Table 6 shows the total amount of calcium and phosphorus in each of the rations used. It also shows the amount of each mineral per hundredweight of the animals and per 100 grams of the ration, and also the ratio between the phosphorus and calcium in each ration. The rations vary from 2.78 to 11.56 grams of calcium per 100 pounds of live weight and from 0.249 to 0.936 grams of calcium per 100 grams of the ration.

In phosphorus the rations vary from 1.43 to 3.86 grams per 100 pounds of live weight and from 0.131 to 0.298 grams per 100 grams of the ration. The ratio between the phosphorus and calcium varies from 1:0.9 to 1:7.2.

The average amount of calcium and phosphorus consumed by the calves in each group per day and the amount consumed per 100 pounds of the animal are shown in Table 7.

Table 7 shows that during the first period all of the animals were fed rations containing considerably more of both calcium and phosphorus in relation to their weight than they received during later periods. This was due largely to the milk, which was fed in large amounts during the first period. Toward the latter part of the period, of course, the ration contained much less of these elements. In the ration of Groups I and III the calcium was supplied in fairly large amounts at all times, due largely to the alfalfa hay fed. The calcium in the rations fed to Groups II and IV, however, was much restricted in relation to the weight of the animals.

During the first two periods the phosphorus was supplied fairly well in the rations of all 4 groups. It, however, was not extremely high in any of the rations. During the last two periods the rations of Groups III and IV were quite low in phosphorus. The ration of

Group I was quite low in its content of phosphorus during the last period, illustrating the fact that rations which are thought normal may sometimes be very close to the lower limit of the requirements of the animals.

Discussion of the Rations Fed

Amount of Phosphorus and Calcium Fed. While there is no definite knowledge as to the correct amount of calcium and phosphorus to include in a ration for a dairy animal, several investigators have stated what they believed to be the requirements for such animals, while others have calculated the amount retained by such animals.

Kellner (1907) computed the calcium and phosphorus retention of growing calves as 21 grams of CaO and 19 grams of P_2O_5 per day during the first year, which amounts to about 15 grams of calcium and 8.3 grams of phosphorus per day. He states that the feed should contain from 40 to 60 grams of each per day.

Armsby (1917) computed from Lawes and Gilbert's analyses of the ash of the entire body of farm animals that during the first year, cattle gain a total of 5,609 grams of calcium and 2,972 grams of phosphorus. This is an average daily retention of 15.37 grams of calcium and 8.14 grams of phosphorus per animal. On a 100-pound basis, cattle during the first year require 3.2 grams of calcium and 1.7 grams of phosphorus daily in their bodies.

McCollum and co-workers (1922) have found that for rats a content of 0.641 grams of calcium per 100 grams of the ration is very near to the optimum concentration of this element. They (1922a) also found that the optimum amount of phosphorus for growing rats is not less than 0.4146 percent. It may be considerably higher.

If Kellner's and Armsby's figures, as given above, which check very closely with each other and which apply to the conditions in the present experiment, are considered, it can be seen by Tables 6 and 7 that Group I was being fed ample calcium and sufficient phosphorus, if there is a fairly high percentage of retention, throughout the entire experiment, although the amount of phosphorus during the latter part of the experiment was dangerously near the lower limits of the requirements. Group II was fed just about the amount of calcium

TABLE 6.—*Total Amount of Calcium and of Phosphorus, Amount of Each per Hundredweight of the Animal and per 100 Grams of the Ration, and Ratio between Phosphorus and Calcium in the Various Rations Indicated in Table 3*

Ration Number	Grams Calcium in Daily Ration	Grams Ca per cwt. of Animal	Grams Ca per 100 Grams of Ration	Grams Phosphorus in Daily Ration	Grams P per cwt. of Animal	Grams P per 100 Grams of Ration	Ratio between P and Ca
I.....	39.32	11.23	0.936	10.53	3.01	0.251	1:3.7
II.....	12.34	3.53	0.286	10.72	3.06	0.248	1:1.2
III.....	40.47	11.56	0.914	11.54	3.30	0.261	1:3.5
IV.....	12.05	3.44	0.265	13.52	3.86	0.298	1:0.9
1a.....	51.42	9.18	0.809	13.94	2.49	0.220	1:3.7
IIa.....	15.59	2.78	0.249	15.67	2.79	0.251	1:1
IIIa.....	57.25	10.23	0.934	8.03	1.43	0.131	1:7.2
IVa.....	19.69	3.51	0.321	8.15	1.45	0.133	1:2.4

that should be retained during the first year of the experiment and considerably less than is required for retention during the latter part of the experiment. The amount of phosphorus probably was ample throughout the entire experiment. Group III was fed an abundance of calcium and probably enough phosphorus during the first year, but during the second year the amount of phosphorus was considerably less than is required for retention. Group IV also was fed just about the amount of calcium that should be retained, and during the first year probably was fed sufficient phosphorus. During the last year, however, the phosphorus was considerably below the amount which is required for retention.

TABLE 7.—Average Daily Amount of Calcium and of Phosphorus Consumed by the Animals in Each Group, and Average Daily Amount of Each Consumed per 100 Pounds of Live Weight of the Animals, by Periods

Period End- ing When Animals Were at Age of	Average Weight	Grams Calcium Consumed per Day	Grams Calcium Consumed per cwt. of Animal	Grams Phos- phorus Consumed per Day	Grams Phos- phorus Consumed per cwt. of Animal
GROUP I. NORMAL GROUP					
8 mos.	196	25.7	13.1	9.3	4.7
13 mos.	408	45.7	11.2	13.0	2.5
19 mos.	646	54.9	8.5	14.4	2.2
25 mos.	880	64.2	7.3	16.9	1.9
GROUP II. LOW-CALCIUM GROUP					
8 mos.	184	13.1	7.1	8.6	4.7
13 mos.	381	14.5	3.8	13.5	3.5
19 mos.	585	16.6	2.8	15.0	2.5
25 mos.	750	20.1	2.1	17.3	2.3
GROUP III. LOW-PHOSPHORUS GROUP					
8 mos.	192	24.7	12.9	9.2	4.8
13 mos.	395	44.8	11.3	13.5	3.4
19 mos.	617	60.8	9.8	8.5	1.2
25 mos.	833	71.3	8.5	10.0	1.2
GROUP IV. LOW-CALCIUM-LOW-PHOSPHORUS GROUP					
8 mos.	180	11.3	6.3	9.4	5.2
13 mos.	366	13.6	3.7	15.0	4.1
19 mos.	570	20.0	3.5	8.2	1.4
25 mos.	731	22.3	3.0	9.1	1.2

If the optimum requirements for calcium and phosphorus, as found by McCollum for rats as given above, is applied to the rations for dairy calves, it will be seen that Rations II, IV, IIa, and IVa are all below the optimum requirements for calcium, and that all of the rations are considerably below the optimum requirements for phosphorus.

The amount of phosphorus and calcium required in a ration depends largely upon the retention of these elements by the animal. It is of no use to feed a large amount of these elements if they are not retained. No definite results have been obtained as to the percentage of these elements which are retained in the body. It is now known that this depends upon several factors.

Ratio of Calcium and Phosphorus. That a proper ratio between the amount of calcium and phosphorus in a ration is important has been shown by several workers.

McCollum and co-workers (1921a) found that in the rat the profound disturbances in the deposition of lime salts in cartilage and bone and the changes in the cells of those tissues which give rise to rickets, may be produced by a disturbance in the diet of the optimal ratio between calcium and phosphorus. They stated that it would seem that in so far as calcium and phosphorus are concerned the proper ratio between the two is of vastly more importance in insuring normal calcification than the absolute amount of the elements themselves.

Haag and Palmer (1928) with rats obtained results which indicated that a more or less balanced condition of calcium, magnesium, and phosphorus salts in the ration is essential to normal growth and functioning.

Gullickson and Eckles (1927), however, with growing dairy heifers did not notice any ill effects of feeding rations in which the phosphorus content almost invariably exceeded the calcium content—most of the time by nearly two to one.

The rations under study varied greatly in the ratio between calcium and phosphorus. Ration IIIa had a ratio of phosphorus to calcium of 1 to 7.2, while Ration IV had a ratio of 1 to 0.9. The others varied between these two extremes.

Factor Which Assists in the Assimilation of Calcium and Phosphorus. That there is a specific factor connected with the assimilation of calcium and phosphorus was first shown by Mellanby (1919), but he associated it with vitamin A. McCollum, Simmonds, and Becker (1922) clearly demonstrated the existence of a vitamin distinct from vitamin A which regulates the metabolism of the bones. That this vitamin is carried in green plant tissues has been shown by Hart and associates (1923a). They have shown that green plant tissue contains more of this vitamin than plant tissue exposed to long curing processes, and that alfalfa hay when fed as a supplement to grains and grain by-products may be so cured as to retain qualities of this vitamin sufficient to maintain liberal milking cows in positive calcium and phosphorus balance. Under practical conditions of curing hay in the windrow with exposure to air and light, the hay is not capable of maintaining calcium and phosphorus equilibrium in milking cows. Hart and associates (1924) also have shown that the ultra-violet rays have a beneficial influence upon the assimilation of calcium and phosphorus by milking goats.

In this experiment no attempt was made to control the effects of this factor. Alfalfa hay was fed to Groups I and III, but it was of such a nature that it is doubtful that very much of this factor was available. Sunlight likewise was not a factor, since the heifers were kept indoors continuously, and any sunlight reaching them first passed through the window glass.

FIGURE 1.—Gain in Weight of the Four Groups of Animals as Compared to Each Other and to Eckles' Normal

RESULTS OF THE EXPERIMENT

Effect of Rations on Growth and Development of the Animals

Growth. The gain in weight and in height at withers is shown by Figures 1 and 2. The average monthly gains are shown by periods in Tables 8 and 9, where they are also converted to a percentage basis, using the normal-fed group as the basis of comparison.

It will be noted that during the first year none of the groups averaged as large as the figures given by Eckles (1920) for the normal purebred Holstein. The animals may not have been of as large a strain as those used by Eckles, but more probably the difference was due to the start which they received. As stated before, the calves when quite young were shipped a distance of several hundred miles

TABLE 8.—*Average Monthly Gain in Weight of Animals in the Various Groups, by Periods; Expressed also as Percentage of the Normal Group*

Period End- ing When Animals Were at Age of	Average Monthly Gain in Weight of Group I (Normal)	Average Monthly Gain in Weight of Group II	Percent of Normal Group	Average Monthly Gain in Weight of Group III	Percent of Normal Group	Average Monthly Gain in Weight of Group IV	Percent of Normal Group
	(pounds)	(pounds)		(pounds)		(pounds)	
8 mos.	27.75	25.55	92.1	27.47	99.0	24.81	89.4
13 mos.	28.93	27.77	96.0	27.36	96.0	24.94	89.7
19 mos.	35.76	31.35	87.6	32.60	91.4	32.73	91.5
25 mos.	31.36	23.92	76.3	33.93	108.2	17.17	54.8

in the coldest part of the winter and many of them had a long sick period before they were able to start normal growth. Beginning when they were about 13 months of age the animals in all groups made very good gains. Those in Groups I and III passed the Eckles normal growth curve both in weight and in height at withers and at the end of the experiment averaged more than 50 pounds in weight over those used to establish the Eckles normal, and were also considerably above them in height at withers.

The animals in Groups II and IV, however, failed by more than 50 pounds to reach the size of the Eckles normal in weight and were also below them in height at withers. The animals in all of the groups were still growing at the end of the experiment. The rate of gain, however, was considerably slower than at first.

It will be seen that the animals in Group I in most cases grew faster than the animals in the other groups, although the animals in Group III gained almost as well as did the normal-fed group, especially in weight. They did not seem to gain as well, however, in height at withers, especially during the last periods.

The animals in Groups II and IV grew almost as well as the animals in the normal-fed group during the first two periods, but during the last two periods they failed to gain as rapidly either in weight or in height at withers. At the end of the experiment they averaged

FIGURE 2.—Gain in Height at Withers of the Four Groups of Animals as Compared to Each Other and to Eckles' Normal

more than 100 pounds less in weight and more than 5 centimeters (2 inches) less in height at withers than the animals fed the normal ration.

Discussion of Results. It would seem that the slower growth made by the animals in Groups II and IV as compared to those in Groups I and III must be due to the lower calcium content of the ration. It will be noted that these two groups (II and IV) were both low in calcium throughout the experiment. Group IV was low also in phosphorus during the last two periods. However, the animals in Group III also were low in phosphorus during the same two

TABLE 9.—Average Monthly Gains in Height at Withers of Animals in the Various Groups; Expressed also as Percentage of the Normal Group

Period End- ing When Animals Were at Age of	Average Monthly Gain in Height Group I (Normal)	Average Monthly Gain in Height of Group II	Percent of Normal Group	Average Monthly Gain in Height of Group III	Percent of Normal Group	Average Monthly Gain in Height of Group IV	Percent of Normal Group
	(cms.)	(cms.)		(cms.)		(cms.)	
8 mos.	3.31	2.83	85.5	3.23	97.6	2.93	88.5
13 mos.	2.45	2.30	93.8	2.70	110.2	2.27	92.6
19 mos.	1.94	1.84	94.8	1.74	89.5	1.75	90.2
25 mos.	1.00	0.74	74.0	0.85	85.0	0.83	83.0

periods but still made gains almost equal to normal for some time. Table 3 shows that the animals in both Groups II and IV consumed slightly more digestible crude protein per hundredweight of the animals and per pound of gain than did those in Groups I and III. The difference in growth therefore must have been due to some factor other than protein and total nutrients. It is possible that the greater growth may have been due to some factor contained in the alfalfa hay which was included in the rations of Groups I and III and not in those of Groups II and IV. However, the only great difference as far as could be noted by chemical analyses was in the calcium content.

It has been found by several investigators that animals will still continue to gain in weight and especially in height at withers under adverse conditions as to nutrition. Eckles (1915) found that deficient rations fed to growing heifers restricted gain in weight more than in height at withers. This same result was found by Reed, Fitch, and Cave (1924) and by Davis and co-workers (1922). It has been shown by Trowbridge and co-workers (1915, 1918) and by Moulton and co-workers (1918) that young steers maintained at constant weight even on a sub-normal plane of nutrition continued to grow in height and in width of skeleton. They found that although the scantily fed steers grew less rapidly in all respects, they attained the same height at maturity as a group of full-fed steers. They tended to recover it by making more rapid gains when put on full feed and by continuing to grow for a longer period of time.

Investigators have not found, however, that rations low in calcium or low in phosphorus retard growth. Eckles and Swett (1918) found that the heifer fed a low-calcium ration continued to grow just as well as one which was being fed normally. The heifer at last became stiff and had difficulty in getting up and walking but gained normally up until this time. Similar results were obtained by Aron and Sebauer (1908) with dogs; by Maynard and co-workers (1925), Bohstedt (1926), and other investigators with pigs which were fed rations low in calcium; and by Hart and co-workers (1909) with pigs which were fed rations low in phosphorus. The animals in these cases continued to grow normally for some time, often up until the time of complete collapse.

On the other hand the cattle grown in the deficiency areas were almost invariably stunted. Eckles, Becker, and Palmer (1926) found that cattle grown in such areas in western Minnesota were decidedly stunted in size. Animals 4 years of age on some farms weigh less than 600 pounds as compared to a normal of 1,000 to 1,100 pounds for animals of similar size and breeding. Cattle were also reported stunted by Theiler and co-workers (1924), Tuff (1924), and by Hart and co-workers (1927). These report that after bonemeal is fed, the cattle make rapid gains. The trouble in these areas is caused by the

FIGURE 3.—
Number E 4,
Showing General
Appearance at Various
Stages of De-
velopment. Fed
Normal Ration

Ages
A—5 mos.
B—13 mos.
C—2 yrs.

low phosphorus content of the feed produced. The stunted condition may be caused by the fact that the animal fed such rations does not consume enough for normal growth, but just as soon as the deficiency is replaced it begins to grow and to take on weight in the normal manner. This, however, was not true with the animals in the present experiment. All were fed according to their requirements as to digestible crude protein and digestible nutrients. These, however, did not appear to utilize their feed as well as did the normal-fed animals, since they consumed more digestible crude protein and digestible nutrients per pound of gain and per 100 pounds of live weight than did the normal-fed group.

The animals in Groups II, III, and IV continued to gain in weight practically as well as in height at withers in comparison with the animals in the normal-fed group.

In summary, dairy animals fed rations low in calcium or low in both calcium and phosphorus may continue to grow in weight and in height at withers for some time almost as well as dairy animals fed a normal ration, but over a period of two years they will not make the gains either in weight or height at withers as will animals fed a normal ration. The animals fed rations low in phosphorus, however, continued to grow almost as well as did the normal-fed group.

Physical Abnormalities. Figures 3 to 10 inclusive show pictures of representatives of each group at various stages of development.

All animals in Group I developed normally as far as general appearances were concerned. There were, of course, considerable differences in appearance, but no more than could be attributed to individuality.

All the animals in Group II developed normally as far as general appearances were concerned. They were not as large as the animals in Group I but otherwise appeared normal.

In Group III, No. E 20, the largest and fastest growing heifer in the group, became stiff in her joints in September, 1927, at 19 months of age, or 5 months after being put on Ration VIIa, which was very low in phosphorus. At the time the stiffness appeared she refused her feed for several days and seemed to be in considerable pain. She gradually recovered her appetite and continued to grow normally, but her legs never fully recovered from their stiffness and were slightly bent, as shown in Figure 11, the picture being taken in April, 1928. No. E 21, the mate of No. E 20, was a short-legged, sturdy type of heifer, and as far as could be noted her legs remained normal. Her back, however, did take on a slight sway at about the same age, whereas it had been straight when she was younger.

In Group IV, No. E 31 when a calf had a very severe case of scours and it was uncertain for some time whether she would recover. She gradually did recover but was never a thrifty, normal-looking or acting calf. In January and February, 1927, when she was about a year old, she became very nervous. Whenever anyone would enter her stall she would tremble violently. On January 25, 1927, and again on February 23, 1928, 4 months after she had received no milk and

was on the low-calcium-low-phosphorus ration, she suffered a fit-like spasm. She fell over in her stall, frothed at the mouth, became stiff, and lay prostrate. These symptoms were very similar to those noted when calves were fed milk or milk and grain as the sole diet, as reported by McCandlish (1923) and Reed and Huffman (1925). The joints in her legs became swollen and her legs slightly bent. For several weeks she failed to gain in weight or height. However, she seemed to gain a little shortly before she was slaughtered, April 1, 1927. Probably the weakened condition of the calf, combined with the abnormal ration, brought on such trouble in this calf when more hardy calves, fed similar rations, seemed normal. If such was the case the amount of calcium in such a ration certainly must have been below the optimal requirements in calcium for such animals.

The other calves on this ration seemed to grow normally, although at a slower rate than the normal-fed group, until about September, 1927, when No. E 28 developed a sway in the back. This calf, as shown in Figure 9, had a straight back when she was a calf but gradually developed a sway until at the time of her death, April, 1928, she was extremely sway-backed. This is shown in Figure 12. Her

FIGURE 4.—
Number E 5,
Showing General Appearance at Various Stages of Development. Fed Normal Ration

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

legs also were not very straight at this time. It is very doubtful if this heifer could have withstood the strain incident to pregnancy and lactation. She appeared to be getting worse as she grew older.

No. E 29, the mate of No. E 28, however, did not develop as abnormally in this respect. She was an extremely healthy heifer from the start and as a calf had a very straight, strong back. It could be noted, however, that her back had begun to sway toward the end of the experiment, but this was not nearly as evident as it was with No. E 28.

These observations suggest the possibility that swaybacks in mature animals are partially due to the ration which the heifers receive when growing, but that the individuality of an animal may partially overcome defects in the ration. A good strong individual may be able to withstand poorer rations than animals that are not as strong. This may be due to the greater storage of minerals that the stronger individual may have stored up when younger.

Abnormal Cravings. Most of the animals, even those fed the normal rations, chewed the wood in their stalls. This began at an early age, even before they were weaned from milk, and continued throughout the experiment. The animals in the normal group seemed just as abnormal in this regard as the others. Some of the animals ate a few shavings with which they were bedded, but this habit, too, seemed just as strong with the normally fed group as with the others.

Drinking Habits. The animals in Groups III and IV developed the habit toward the last of the experiment of grinding their teeth. They would stand for 15 to 20 minutes at a time grinding their teeth with a peculiar noise.

The animals in these two groups and also those in Group II developed the habit of lapping the water with their tongues in cold weather. Some of them would froth considerably at time of drinking. No. E 12 in Group II, Nos. E 20 and E 21 in Group III, and Nos. E 28, E 29, and E 30 in Group IV all showed this characteristic before they were slaughtered. No. E 28 in Group IV and No. E 20 in Group III were probably the worst in this habit.

When the teeth were examined at time of slaughter it was found that they had become so badly worn that some of them were down to the gums. The teeth of the animals in Groups II, III, and IV were all badly worn at the end of the experiment, although the teeth even of Group I were worn to some extent. They, however, were not worn nearly so badly as were those of the other groups. The same condition was noted by Reed and Huffman (1928) when they fed a lot of heifers raw rock phosphate in their ration. The teeth of these animals became sensitive to cold water when they were about $2\frac{1}{2}$ years of age. An examination of their mouths revealed that the raw rock phosphate had brought about a softening of the teeth and in some instances the teeth were worn down to the gums.

TABLE 10.—*Age at First Signs of Oestrus, Total Number of Times Noted, and Regularity of Oestrus*

Number of Calf	Age at First Signs of Oestrus			Number of Times Noted	Regularity of Oestrus
	Years	Months	Days		
GROUP I. NORMAL GROUP					
E-3	1	1	11	10	regular
E-4	1	0	4	14	regular
E-5	1	1	22	13	regular
E-6	1	3	20	6	fairly regular
E-7	0	11	20	1	regular
Average	1	1	9	10.8*	
GROUP II. LOW-CALCIUM GROUP					
E-11	1	0	25	11	regular
E-12	1	9	23	5	irregular
E-13	1	0	11	5	very irregular
E-14	1	3	25	6	fairly regular
E-15	0	10	8	2	regular
Average	1	2	18	6.8*	
GROUP III. LOW-PHOSPHORUS GROUP					
E-19	1	2	29	12	regular
E-20	1	3	13	9	irregular
E-21	0	11	28	21	regular
E-22	1	1	13	10	regular
Average	1	1	28	13.0	
GROUP IV. LOW-CALCIUM-LOW-PHOSPHORUS GROUP					
E-27	1	3	16	7	fairly regular
E-28	1	4	20	10	irregular
E-29	1	4	5	10	irregular
E-30	1	1	7	3	irregular
Average	1	3	12	7.5	

*Average of first four animals only.

Effect of Rations upon the Oestrus of the Animals

Records were kept of the time the heifers in the various groups showed signs of oestrus. Of course some may have been missed but the attendant was on the watch at all times and recorded all that were noted. Table 10 gives the age of the heifers at the time of the first signs of oestrus, together with the total number of times of oestrus noted and the regularity of oestrus of the different heifers in the experiment.

Nos. E 1, E 2, E 8, E 9, E 10, E 16, E 17, E 18, E 23, E 24, E 25, E 26, E 31, and E 32 were slaughtered before they showed signs of oestrus.

It will be noted that the normal group averaged 1 year, 1 month, and 19 days of age at the time of the first sign of oestrus. They varied from 11 months and 20 days to 1 year, 3 months, and 20 days. All were fairly regular in the period between signs of oestrus, and were observed showing signs of oestrus a total of 44 times.

The animals in Group II averaged 1 year, 2 months, and 18 days of age when they first showed signs of oestrus. They were 1 month and 18 days older on the average than the normal group. No. E 12 did not show signs of oestrus until an advanced age, which was largely responsible for this difference. The animals were noted show-

ing signs of oestrus a total of 27 times, or 17 times less than Group I. As a group, the period between signs of oestrus was irregular.

The animals in Group III averaged 1 year, 1 month, and 28 days of age when they first showed signs of oestrus. This was only 19 days older than the average of the animals in the normal group. They were observed showing signs of oestrus a total of 52 times, or 8 times more than the animals in the normal group. They were fairly regular in period between times of oestrus. Since their ration was not greatly different from that of Group I in its calcium and phosphorus content up until the animals were a year old, very little difference in time of first signs of oestrus between the animals in this group and those in Group I would be expected.

The animals in Group IV averaged 1 year, 3 months, and 12 days when they first showed signs of oestrus. This was 2 months and 3 days older than the animals fed the normal ration. They were observed to show signs of oestrus a total of 30 times, or 14 times less than the group fed the normal ration. They were quite irregular in the period between times of oestrus.

Discussion of Results. The results indicate that the heifers in Groups I and III showed signs of oestrus when they were $1\frac{1}{2}$ to 2 months younger than the heifers in Groups II and IV.

FIGURE 5.—
Number E 12,
Showing General
Appearance at Various
Stages of De-
velopment. Fed
Low - Calcium
Ration

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

The heifers in Groups I and III also were much more regular in period between times of oestrus than were the heifers in the other two groups.

This is in agreement with results obtained by other investigators, as there is found evidence in literature which indicates that rations quite low in the minerals often cause serious disturbances in reproduction. Eckles, Becker, and Palmer (1926) found that in the deficient area in western Minnesota considerable trouble was experienced with breeding. On many farms the cows were not expected to produce a calf more than once in two years. Heifers often did not show signs of oestrus until two years of age. The average calf crop in the worst-affected districts, as observed after a dry season, was estimated at less than 50 percent of the normal. Tuff (1924) also found that in seasons of low rainfall the trouble incident to reproduction in the cattle in deficient areas of Norway was increased. The cattle seem to fail to ovulate. Meigs and Woodward (1921) also found difficulty in reproduction when cows were fed a ration low in calcium. Jordan, Hart, and Patten (1906), studying the physiological effect of certain phosphorus compounds, found that when cows were fed rations very low in phosphorus they showed entire cessation of oestrus. Hart and co-workers (1924) in working with the rations derived from the oat and wheat plants found that normal reproduction would not

FIGURE 6.—
Number E 13,
Showing General
Appearance at V-
arious Stages of
Development. Fed
Low-Calcium Ra-
tion

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

take place on account of lack of calcium. They state that 0.45 percent CaO in the ration is the least amount which can be fed in a ration to insure normal reproduction.

It can be seen by referring to Table 6 that the rations fed to the animals in Groups II and IV were slightly below the amount stated by Hart and co-workers to be the least amount of calcium that could be fed to insure normal reproduction. In their studies, however, the animals conceived but failed to drop normal offspring, or would abort. The animals in the present experiment were not bred.

Effect of the Rations upon the Calcium and Inorganic Phosphorus Content of the Blood

Since the blood carries the food nutrients to the parts of the body where they are needed, it would naturally be supposed that a lack of any required nutrient in the food would result in a lower concentration of this nutrient in the blood. This, however, is not always the case, since the blood is maintained fairly uniform in its constitution, even in time of low supply, by using some of the reserve supply which has been stored up in various parts of the body.

The calcium and inorganic phosphorus content of the blood, however, has been used for several years as a criterion in the demonstration of rickets in animals. Steenbock and co-workers (1923) used it as a criterion in the demonstration of the existence of a specific anti-rachitic vitamin. They were able to increase the calcium and inorganic phosphorus in the serum of dogs fed rachitic rations by the addition of cod-liver oil. They were also able to increase the concentration of calcium and inorganic phosphorus in the serum of chickens by the addition of cod-liver oil to a ration which otherwise caused leg weakness.

A study was therefore made of the blood of the animals in the present experiment to determine the effect of the rations upon its calcium and inorganic phosphorus content.

Analyses of Blood. Samples of blood were taken from each animal in the experiment at 9-month intervals and analyzed for calcium and inorganic phosphorus by the Briggs (1922, 1924) modification of the Bell and Doisy (1920) method.

The samples were taken from the jugular vein of the animals at the same hour each day in order that the samples might be as uniform as possible. After the first two times samples were taken on 3 consecutive days, and a composite of each day's sampling was used for the analyses.

Calcium. The average results obtained with the calcium analyses for each period are given in Table 11.

Table 11 shows that the rations had very little effect upon the calcium content of the blood. The blood of the calves fed the rations low in calcium had just as much calcium in it as the blood of the animals fed rations rich in calcium. Possibly if the calcium content of the rations had been somewhat lower, the blood would have been affected.

It can be noted also that in general, as the animal became older, there was a slight diminution in the calcium concentration of the blood.

Phosphorus. The average results obtained with the phosphorus analyses for each period are given in Table 12.

Table 12 indicates that the rations fed to animals exert a very profound influence upon the inorganic phosphorus content of the blood. When the ration fed contained ample phosphorus, as they all did in the first two periods of the trial, the inorganic phosphorus content of the blood remained normal. However, just as soon as the phosphorus in the feed was lowered, the inorganic phosphorus content of the blood also went down.

There appears some evidence that dairy animals are unable to keep the inorganic phosphorus content of their blood normal if they do not obtain sufficient phosphorus in the feed, even though they have ample storage in their bones. This is indicated in the experiment by the following facts: when the animals in Groups III and IV were fed a ration containing as much as or slightly more phosphorus than those in Groups I and II, they maintained the inorganic phosphorus content of their blood. On March 24, 1927, their rations were chang-

FIGURE 7.—
Number E 20,
Showing Gen-
eral Appear-
ance at Various
Stages of De-
velopment. Fed
Low-Phosphorus
Ration

Ages
A—5 mos.
B—13 mos.
C—2 yrs.

ed from Rations III and IV, which contained 3.4 gms. and 4.1 gms. respectively of phosphorus per hundredweight of the animals, to Rations IIIa and IVa, which contained 1.2 gms. and 1.4 gms. respectively of phosphorus per hundredweight of the animals. Samples of blood were taken beginning March 28, 1927, 4 days after the ration had been changed. In that short period the inorganic phosphorus content of the blood had dropped from an average of 8.15 mg. per

TABLE 11.—Average Calcium Content of 100 cc. of Blood of the Animals in the Various Groups, by Periods

Period Ending When Animals Were at Age of	Average Calcium Content per 100 cc. of Blood			
	Group I	Group II	Group III	Group IV
	(mgs.)	(mgs.)	(mgs.)	(mgs.)
8 months	14.86	14.75	14.80	14.54
13 months	14.19	14.72	14.23	14.66
19 months	14.62	13.79	14.16	14.73
25 months	12.54	13.02	13.17	13.44

100 cc. of blood to 4.86 mg. in the case of Group III, and from 7.93 mg. per 100 cc. of blood to 4.35 mg. in the case of Group IV. The phosphorus content of the rations was kept at that level, and the inorganic phosphorus in the blood gradually diminished. This would indicate that the animals must depend upon their feed as the source of the inorganic phosphorus of their blood. These animals had had time to store ample phosphorus in their bones which they could have drawn upon in time of need. This, however, they did not seem able to do, depending entirely upon their food for their supply of phosphorus.

As a general rule the concentration of inorganic phosphorus in the blood of normal-fed animals seemed to increase slightly until the animal was about a year old and then to decrease slightly as the animal grew older.

Study of Individuals. No. E 31, which already has been noted as showing signs of convulsions and bent legs at an early age, showed on her third test the lowest calcium concentration in her blood of any of the animals. Just shortly after this test she developed nervousness, convulsions, and bent legs. On her fourth test the ration had been changed to one containing slightly more calcium and less phosphorus. The concentration of calcium in the blood had increased, but the inorganic phosphorus of the blood then was very low, although the ration had been changed to the low-phosphorus one less than a week previously. This indicates that the concentration of inorganic phosphorus in the blood changes almost as soon as the phosphorus content of the ration is changed.

No. E 28, which developed a sway in her back together with slightly bent legs, was the lowest of all the heifers during the last 3 tests in the inorganic phosphorus content of her blood. The concentration of inorganic phosphorus seemed to be growing less, and at the time of her death it was only 3.14 mg. per 100 cc. of blood.

No. E 29, which was fed the same ration as No. E 28, did not show as marked abnormal characteristics as did No. E 28, and her

blood was not as low in inorganic phosphorus. It, however, was much lower than the blood of the normal animals. Her failure to show marked abnormal characteristics perhaps was due to her individuality, for she was always a strong animal. But she probably would have succumbed later to the effects of the abnormal ration.

The blood of No. E 20, which developed the stiffness in her legs, was low in inorganic phosphorus, but not quite as low as was the blood of No. E 29. It was a little lower than that of her mate, E 21, which did not develop as abnormal characteristics.

This would indicate that the inorganic phosphorus concentration in the blood would be a fairly good index as to whether an animal is receiving sufficient of this element. This apparently is not as true with calcium, although in this particular experiment the amount of calcium in the ration was not reduced to the same extent as it was in the phosphorus rations.

It is difficult to assign a specific value for the normal content of calcium and inorganic phosphorus in the blood of normal animals. Different methods for such determinations have been proposed, many of which do not give concordant results.

Discussion of Results. Meigs, Blatherwick, and Cary (1919) found that with calves the plasma calcium tended to become lower

FIGURE 8.--
Number E 21,
Showing General
Appearance at Various
Stages of
Development. Fed
Low - Phosphorus
Ration

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

with advancing age up at least to 6 months. The inorganic phosphorus was fairly high at birth but tended to increase until the animal was 6 months of age. Then as a rule it fell off again before the age of 18 months. The calcium in the serum of normal calves they found to be about 10 mgs. per 100 cc., with variations from 9.1 mgs. to 13.7 mgs. The inorganic phosphorus in the serum was found to average 6.5 to 7 mgs. per 100 cc., with variations from 5.6 to 9.2 mgs.

Meigs was able to reduce the inorganic phosphorus in the serum by feeding rations low in phosphorus, but was unable to increase to any extent the calcium in the serum by feeding calcium in the ration. His results indicate that in cattle above two months of age the plasma calcium is little, if at all, altered by pregnancy, lactation, or by feeding rations rich in calcium. This was found also by Halverson and co-workers (1917) who found that the calcium content of the serum was little affected by the feeding of calcium in the feed and that it is kept nearly constant by the enormous calcium reserve stored in the bones.

On the other hand, Bethke and co-workers (1923) found that in the absence of normal growth in rats there was a general tendency for a reduced calcium content in the blood and ash content of the bones. The phosphorus content of the blood was found more constant, but apparently as the calcium increased there was a tendency for the phosphorus to be depressed.

TABLE 12.—Average Phosphorus Content of 100 cc. of Blood of Animals in the Various Groups, by Periods

Period Ending When Animals Were at Age of	Average Phosphorus Content per 100 cc. of Blood			
	Group I	Group II	Group III	Group IV
	(mgs.)	(mgs.)	(mgs.)	(mgs.)
8 months	6.63	7.44	6.79	7.37
13 months	7.08	7.49	8.15*	7.93*
19 months	6.50	7.09	4.93†	4.05†
25 months	5.98	6.31	4.69	3.86

*Only one composite was used, since ration had been changed before the second one was run.

†Three composites were used, since ration had been changed so that they represented the same feeding method.

With the experimental animals it was found that the calcium content of the blood varied from 10.29 to 19.95 mgs. per 100 cc. of the blood. The calcium in the blood was but little affected by the feeding of rations low in this element. This would indicate that the animal body is able to maintain the concentration of the blood fairly uniform when not receiving sufficient in the ration, by drawing upon the supply stored away. It has already been noted that the animals in Groups II and IV were not receiving sufficient calcium as denoted by their growth and development, but the amount in the blood was at all times ample. This agrees with the results of Meigs and co-workers (1919) and Halverson and co-workers (1917) who found that the concentration of calcium could not be altered greatly by feeding feeds rich in calcium.

It was found, however, that the inorganic phosphorus content of the blood could be easily affected by feeding rations low in phosphorus. This would indicate that the animals depend upon their feed to keep up the supply of inorganic phosphorus in their blood.

If 6 mgs. of inorganic phosphorus per 100 cc. of blood is considered the minimum concentration of phosphorus consistent with the well-being of an animal, then if less than 2 grams of phosphorus per hundredweight of the animal is fed per day, this concentration will not be maintained. It can be seen that the animals in Group I were fed a ration slightly below this limit. During the last period they consumed only 1.9 gms. of phosphorus per hundredweight of the animal in their ration, and the concentration of their blood dropped from 6.50 to 5.98 mgs. per 100 cc. This ration contained 0.22 percent phosphorus and was therefore considerably less than the optimal ration of at least 0.4146 percent as recommended by McCollum and associates (1922) for rats.

FIGURE 9.
—Number E
28, Showing
General Ap-
pearance at
Various
Stages of
Develop-
ment. Fed
Low - Cal-
cium - Low-
Phosphorus
Ration

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

Effect of the Rations upon the Bones of the Animals

Effect of Composition. Bones are useful in the animal body first, to act as a support and to give shape to the body; second, to protect some of the vital organs; and third, to act as a storehouse for calcium and phosphorus which might be drawn upon later as the animal finds need for it. It would seem reasonable, therefore, that the bones in animals which have been fed rations low in calcium or phosphorus would be lower in total ash than bones of animals fed normal rations.

Therefore, at the time of killing the animals in the experiment, samples were taken of representative bones of the animals. The bones used were the eleventh and twelfth ribs and the femur and humerus from the left side of each animal. Kruger and Bechdel (1928), studying the normal deposition of minerals in the bones of dairy calves, found that the ribs, femurs, and humeri were very suitable for such studies, more so than some other bones which they also studied.

Sampling the Bones. The bones were removed from the carcass very soon after slaughter, scraped free of all adhering flesh, and carefully weighed.

The ribs were sampled by taking pieces about two inches in length from near both ends and from the middle of each. These were carefully weighed and placed in a mason jar which had previously been weighed. They were kept sealed and in a cold room at a temperature of about zero until the time of analysis. The two ribs were then analyzed together.

The femurs and humeri were sampled the first two times by taking a piece $2\frac{1}{2}$ inches in length from the middle of the shaft of each bone. Upon realizing that this was not a fair sample of the entire bone, this method was changed and during the last two times a longitudinal medial section about $\frac{3}{16}$ inch thick was sawed out of each bone.* These were weighed, sealed in a mason jar, and kept in a cold room as were the ribs until the time of analysis. The femurs and humeri were analyzed together. All samples of bones were kept in this manner and analyzed at the same time.

Method of Analysis. At the time of analysis the jars containing the bones were weighed carefully and air-dried at room temperature. The bones were then broken up in an iron mortar and the remaining moisture was determined by drying for 48 hours in a vacuum oven maintained at 75°C . and at a reduced pressure of 27.5 inches of mercury. The dry broken bones were extracted with ether for 24 hours in a large soxhlet, dried, and ground to pass a 20-mesh sieve. Five grams of the ground bone was extracted for 24 hours with ether and then for 36 hours with 95 percent alcohol. The dry-extracted bone was transferred to a platinum dish and ashed in an electric muffle at a low red heat. The ash was dissolved in 4-molar hydrochloric acid.

*Suggested by Minnesota Agricultural Experiment Station.

The phosphorus was determined by the official method and the calcium by a slight modification of the McCrudden method. The non-extractable organic matter of the green bone was determined by subtracting the sum of the moisture, extractable material, and ash from 100. It seemed that a better picture of the bone could be obtained if the extractable material, which consists largely of fat and fat-like materials, is not combined with the non-extractable organic materials, as these are usually under the name of organic matter.

Analysis of Ribs. Table 13 gives the average percentage of the various constituents in the green bone by periods. This is also expressed in percentages of the normal-fed group.

It will be noted in a study of Table 13 that in general the percentage of the ash and the non-extractable organic matter is decreased, and the percentage of water and extractable material is increased in the bones of animals fed rations low in calcium or phosphorus as compared to the bones of normal-fed animals. The percentage of calcium and phosphorus in the bones, of course, was decreased as the ash decreased.

FIGURE 10.—
Number E 29,
Showing Gen-
eral Appear-
ance at Various
Stages of De-
velopment. Fed
Low - Calcium
and Low - Phos-
phorus Ration

Ages

A—5 mos.
B—13 mos.
C—2 yrs.

TABLE 13.—Average Percentage of Various Constituents in Green Bone (Ribs) of the Various Groups, by Periods; Expressed also as Percentage of the Normal Group

Period Ending When Animals Were at Age of	Percent in Ribs of Group I (Normal)	Percent in Ribs of Group II	Percent of Normal Group	Percent in Ribs of Group III	Percent of Normal Group	Percent in Ribs of Group IV	Percent of Normal Group
MOISTURE							
8 mos.	15.22	16.27	106.9	14.90	97.9	14.71	96.7
13 mos.	18.45	19.57	106.1	22.70	123.0	19.90	91.6
19 mos.	17.33	15.10	87.7	18.41	106.2	18.55	107.0
25 mos.	15.08	15.52	102.9	17.68	117.2	20.93	138.7
EXTRACTABLE MATERIAL							
8 mos.	7.74	15.80	204.1	5.95	75.5	11.73	151.1
13 mos.	9.81	8.97	91.4	10.89	111.0	11.12	113.3
19 mos.	14.21	21.11	149.2	15.32	107.7	25.16	177.7
25 mos.	13.33	14.61	109.6	18.15	136.1	19.99	150.0
NON-EXTRACTABLE ORGANIC MATTER							
8 mos.	30.96	27.60	89.1	31.28	101.1	29.94	96.7
13 mos.	27.87	29.37	105.4	26.12	96.8	29.82	107.0
19 mos.	26.13	25.15	96.2	26.21	100.3	23.14	88.5
25 mos.	27.31	26.71	97.7	25.23	92.4	26.07	95.4
ASH							
8 mos.	46.08	40.31	87.5	47.96	104.1	43.56	94.5
13 mos.	43.87	42.08	95.9	40.29	91.8	41.65	94.5
19 mos.	42.36	38.63	91.2	40.04	94.5	33.14	78.2
25 mos.	44.27	43.15	97.4	38.93	87.9	33.01	74.5
CALCIUM							
8 mos.	18.25	15.87	86.9	19.07	104.5	17.20	94.2
13 mos.	17.51	16.74	95.6	16.16	92.3	16.59	94.7
19 mos.	16.72	15.37	91.9	16.26	97.2	13.57	81.2
25 mos.	17.68	17.20	97.3	15.68	88.7	13.42	75.8
PHOSPHORUS							
8 mos.	8.75	7.70	88.0	9.08	103.8	8.26	94.4
13 mos.	8.25	7.97	86.9	7.60	92.1	7.90	96.1
19 mos.	7.96	7.28	91.6	7.42	93.2	6.25	78.5
25 mos.	8.21	8.05	98.0	7.23	88.1	6.09	74.2

Extractable material and water seem to replace the ash and to a certain extent the non-extractable organic matter in the bones as the animal is deprived of normal rations. In the case of the animals in Group IV, which were fed a ration low in both calcium and phosphorus, at the end of the experiment the amount of ash and of calcium and phosphorus in the ribs was only about three-fourths of the amount in the bones of the normal-fed animals.

In Table 14 the average ash of the dry-extracted ribs and also the average percentage of calcium and phosphorus in the ash are given by periods and groups.

Table 14 shows that as the ration is limited in calcium and phosphorus, the amount of ash in the dry-extracted bone also is lowered in a very similar way as it is with the green bone, but since the water and extractable material had been removed the results were not as pronounced. The amount of calcium and phosphorus in the ash is affected but little by the ration fed.

Analysis of Femur and Humerus. Table 15 shows the average analysis of the various constituents of the bones for the different groups by periods.

The same general results were obtained by these analyses as by analyses of the ribs. Very little effect was shown at first when the ration was not reduced, but as soon as the rations were reduced there was an immediate effect upon the composition of the bones. This, in general, was a reduction in the ash and non-extractable organic matter and an increase in water and extractable material. The calcium and phosphorus also were reduced as the ash was reduced. The bones of the low-mineral-fed groups were affected, but the effects were more pronounced as to their calcium and phosphorus content.

FIGURE 11.—Showing the Legs of E 20. Note That They Are Slightly Bent. Taken at Time of Killing

There was an indication that the shaft of the bone is more stable in its composition than the ends of the bone. The bones of the low-mineral-fed groups during the first two periods when the samples were taken from the center of the bone were as high and in most cases higher in ash content and in their content of calcium and phosphorus than were the bones of the normal-fed group, while the ribs during the corresponding period showed some effect of the decreased mineral supply. When, however, the sampling was changed to include the entire length of the bone, the effect was just as pronounced with the femurs and humeri as with the ribs. This is as might be expected, however, since the growing parts of the bones are at the ends and these naturally would be the first affected.

The average amount of ash in the dry-extracted femur and humerus and the amount of calcium and phosphorus in the ash of the individual animals, averaged for groups by periods, is given in Table 16.

Table 16 shows again that in the dry-extracted bones the amount

TABLE 14.—Average Percentage of Ash in Dry-Extracted Ribs, and Average Percentage of Calcium and Phosphorus in Ash of Ribs, Given as the Average of All Groups by Periods; Expressed also as Percentage of the Normal Group

Period Ending When Animals Were at Age of	Average Percent in Dry-Extracted Ribs of Group I (Normal)	Average Percent in Dry-Extracted Ribs of Group II	Percent of Normal Group	Average Percent in Dry-Extracted Ribs of Group III	Percent of Normal Group	Average Percent in Dry-Extracted Ribs of Group IV	Percent of Normal Group
ASH (DRY-EXTRACTED)							
8 mos.	59.86	59.35	99.1	59.80	99.9	59.16	98.8
13 mos.	61.15	58.88	96.2	59.92	98.0	57.93	94.7
19 mos.	61.86	60.57	98.7	60.44	98.5	58.83	95.8
25 mos.	61.89	61.70	99.3	60.67	98.0	55.74	90.0
CALCIUM IN THE ASH							
8 mos.	39.61	39.56	99.3	39.77	100.4	39.48	99.4
13 mos.	39.91	39.79	99.7	40.12	100.5	39.83	99.8
19 mos.	40.03	39.80	99.4	40.60	101.4	40.96	102.3
25 mos.	39.93	39.87	99.8	40.28	100.8	40.64	101.8
PHOSPHORUS IN THE ASH							
8 mos.	18.98	19.09	100.5	18.97	100.0	18.96	99.9
13 mos.	18.91	18.94	100.1	18.88	99.8	18.97	100.3
19 mos.	18.80	18.87	100.3	18.53	98.5	18.56	98.7
25 mos.	18.56	18.61	100.3	18.55	100.0	18.45	99.4

of ash is lessened by feeding rations restricted in calcium or phosphorus. The same general results were obtained as with the green bones but the differences were not as pronounced. The composition of the ash is affected but little in regard to its calcium and phosphorus content.

Discussion of Results. The results of the analyses of the ribs and of the femur and humerus of the various animals in a general way are similar, except for the different methods of sampling used during the first two periods with the femur and humerus. As a rule, when the analyses of the ribs varied from normal, the analyses of the femur and humerus of the same group would vary in like manner. This would indicate that either type of bone could be used equally well in studying the effects of rations low in calcium or phosphorus upon the bones of animals. Both indicated that rations low in one or both minerals, but normal in regard to digestible protein and digestible nutrients, when fed to growing dairy animals, bring about a change in the composition of the bones of such animals. Such a change is characterized by an increase in the water and extractable material in the bone and by a corresponding decrease of the ash and non-extractable organic matter. The amount of calcium and phosphorus of the green bone also was decreased. The dry-extracted bone also shows less ash when such rations are fed, but not to the same extent as in the green bone. As far as the ash was concerned it contained practically the same percentage of calcium and phosphorus as did the bones of calves on normal rations.

It will be noted that the bones of the animals in the normal-fed group maintained fairly uniform composition at the different ages

TABLE 15.—Average Percentage of Various Constituents of Green Bone (Femur and Humerus) in the Various Groups, by Periods; Expressed also as Percentage of the Normal Group *

Period Ending When Animals Were at Age of	Average Percent in Femur and Humerus of Group I (Normal)	Average Percent in Femur and Humerus of Group II	Percent of Normal Group	Average Percent in Femur and Humerus of Group III	Percent of Normal Group	Average Percent in Femur and Humerus of Group IV	Percent of Normal Group
MOISTURE							
8 mos.	11.65	9.32	80.0	10.76	92.4	11.50	98.7
13 mos.	13.50	13.26	98.2	14.14	95.3	14.66	108.6
19 mos.	17.35	17.92	103.3	16.45	105.2	17.68	101.9
25 mos.	13.64	15.55	113.6	15.94	116.9	19.90	145.9
EXTRACTABLE MATERIAL							
8 mos.	3.27	4.77	145.9	4.88	149.2	1.50	45.8
13 mos.	1.81	0.60	33.1	0.51	28.2	0.89	49.2
19 mos.	28.73	29.40	102.3	29.49	102.6	36.69	127.7
25 mos.	31.60	30.68	97.7	34.16	108.1	39.40	124.3
NON-EXTRACTABLE ORGANIC MATTER							
8 mos.	26.72	25.57	95.6	25.35	94.4	26.30	98.4
13 mos.	27.91	27.98	97.0	27.19	97.4	26.65	95.5
19 mos.	19.21	20.10	104.7	19.39	100.9	16.47	85.7
25 mos.	19.04	20.19	102.8	18.29	92.6	17.94	86.3
ASH							
8 mos.	58.34	60.33	103.4	58.91	100.9	60.70	102.3
13 mos.	58.27	60.06	103.1	58.61	100.6	57.84	99.2
19 mos.	34.70	32.58	93.8	34.66	99.8	29.12	83.9
25 mos.	35.06	33.08	94.3	31.60	90.1	22.75	64.8
CALCIUM							
8 mos.	23.13	23.88	103.2	23.40	101.1	24.03	103.8
13 mos.	23.03	23.84	103.5	23.40	101.1	23.06	100.1
19 mos.	13.83	19.97	93.8	13.85	100.1	11.81	85.4
25 mos.	13.89	13.13	94.5	12.04	91.0	9.14	65.8
PHOSPHORUS							
8 mos.	10.89	11.23	103.1	10.10	92.7	11.30	103.7
13 mos.	10.91	10.79	98.9	11.00	100.8	10.91	100.0
19 mos.	6.45	6.15	95.3	6.44	99.9	5.39	82.9
25 mos.	6.61	6.41	96.9	5.94	89.8	4.28	64.7

during the entire experiment. There were slight variations from one period to another but the differences were not great.

The bones of the animals in Group II, which was fed a ration low in calcium throughout the experiment, showed the typical variation in composition. In general, from the first they were high in moisture and in extractable material and low in non-extractable organic matter and in ash. The ash seemed to increase toward the latter part of the experiment. This checks with observation, since the animals in this group seemed to improve in general appearance and thrift toward the end of the experiment. They, however, were fed the same ration at all times. The only explanation for this is that the requirements for calcium were lowered as the animals approached maturity and were not growing so fast as previously, and that, while the ration was not sufficient when the animals were younger and growing fast, it was sufficient when this period of rapid growth was

*It must be borne in mind in studying Table 15 that the first two analyses given in the table cannot be compared directly with the last two analyses, since different methods of sampling were used. The first two and the last two can be compared, and in a general way all four can be considered together.

over. At any rate, the bones of the animals in this group from the analyses seemed to be approaching normal toward the end of the experiment.

The bones of the animals in Group III, the low-phosphorus group, after the first period, when their ration approximated normal, showed the same general composition as the low-calcium group. The ash and non-extractable organic matter was found decreased while the water and extractable material was increased. Here, again, the composition of the bones correlated with the general appearance of the animals. Individuals in this group, as was seen, had developed abnormal characteristics during the last period, and the analyses show that the bones of this group at the last killing contained over 10 percent less ash than those of normal-fed animals.

FIGURE 12.—Showing E 28. Note the Sway in Her Back. Taken at Time of Killing

The bones of the animals in Group IV showed the greatest difference in their composition. This group was fed a ration low in both phosphorus and calcium. The ration, however, was not low in phosphorus until almost the time of the second killing; consequently during the first two periods the bones should compare with those in Group II. The analyses show a fair degree of comparison between the two groups. During the last two periods the ration of Group IV was very low in phosphorus and consequently its bones were much reduced in ash. The ribs contained scarcely 75 percent and the femur and humerus scarcely 65 percent as much ash as the normal bone at the time of the final killing. The animals in this group at that time were growing slowly and had developed abnormal characteristics, shown by the sway in the back of individual animals. The bones of these animals contained more moisture and were very much higher in the amount of extractable material than the bones of normal-fed animals.

The results found in this study were very similar to those obtained by other investigators, since many have found that the ash content

TABLE 16.—Average Percentage of Ash in Dry-Extracted Femurs and Humeri, and Average Percentage of Calcium and Phosphorus in Ash, Given as the Average of All Groups, by Periods; Expressed also as Percentage of the Normal Group

Period End- ing When Animals Were at Age of	Average Percent in Dry-Extracted Femur and Humerus of Group I (Normal)	Average Percent in Dry-Extracted Femur and Humerus of Group II	Percent of Normal Group	Average Percent in Dry-Extracted Femur and Humerus of Group III	Percent of Normal Group	Average Percent in Dry-Extracted Femur and Humerus of Group IV	Percent of Normal Group
ASH							
8 mos.	69.24	70.25	101.4	70.13	101.3	69.76	100.7
13 mos.	68.79	69.72	101.3	68.67	99.8	69.49	101.0
19 mos.	64.36	61.85	96.2	64.13	99.6	61.75	95.9
25 mos.	64.08	62.95	98.2	63.15	98.5	57.09	89.1
CALCIUM							
8 mos.	39.65	39.58	99.8	39.72	100.2	39.58	99.8
13 mos.	39.53	39.70	100.4	39.92	100.9	39.87	100.8
19 mos.	39.86	39.82	99.9	39.96	100.2	40.54	101.7
25 mos.	39.63	39.68	100.1	39.99	100.8	40.19	101.4
PHOSPHORUS							
8 mos.	18.62	18.61	100.0	17.12	91.9	18.63	100.0
13 mos.	18.73	18.79	100.3	18.76	100.1	18.87	100.7
19 mos.	18.75	18.88	100.7	18.57	99.0	18.50	98.6
25 mos.	18.85	19.10	101.3	18.81	99.8	18.84	100.0

of bones is lowered when animals are fed rations low in minerals. Voit (1880) found that the skeleton of pigeons and of puppies became weakened and porous on rations low in calcium. He described the condition as rachitic. Weiske and Wildt (1873) found that with lambs fed rations low in calcium and phosphorus, the ratio of the two minerals in the compact part of the bone remained the same as in the normal bone but that the percentage of calcium and phosphorus was reduced.

Maynard, Goldberg, and Miller (1925) found that the bones of stiff pigs, fed a calcium-deficient diet, were markedly lower in dry matter, ash, calcium, and phosphorus than litter mates which received adequate rations. Increasing the amount of calcium in the basal ration by adding bonemeal or limestone produced bones much higher in calcium and phosphorus and saved the animal from stiffness. There was a striking tendency for the calcium and phosphorus ratio to remain constant in the bone ash.

Hart, McCollum, and Fuller (1909) found that on rations extremely low in phosphorus the percentage of ash in the skeleton of pigs was reduced to nearly one-half that of pigs receiving normal rations. They also found that the calcium and phosphorus tended to remain in the same proportion as in the normal pig. This indicates that when an animal is starved for phosphorus it draws this element from the skeleton but removes calcium and phosphorus in the proportion found in tri-calcium phosphate.

In studies of bone growth Hammett (1925, 1925a, 1925b) found that the humerus and femur decreased in water content and increased in ash content as the animal grew older. The organic matter con-

tinued about the same. The calcium in the bones increased as the animal grew older and the phosphorus and magnesium percentage decreased. The change in the composition was practically complete at 75 days of age, the end of puberal period.

Kruger and Bechdel (1928) experimenting with dairy calves under 6 months of age found that the percentage of phosphorus and calcium in the bone ash is nearly constant and varies only slightly with the age of the animal. The percentage of water decreases, while the percentage of ash and organic matter increases with the age of the animals. The ash replaces the greater part of the decrease in the water percentage.

Effect on Appearance. Figures 13 and 14 show the appearance of the femurs of animals from each group at the time of the last killing.

The appearance of the femurs and humeri of the different groups at that time were quite noticeably different.

The femurs and humeri of the animals in the normal group were dense at the end and hard in the shaft. The walls of the shaft were thick and the amount of marrow was only 8.2 percent of the weight of the bone. The entire bone was well calcified.

FIGURE 13.—Cross-section of Femur of Animals E 5, from the Normal Group, and E 20, from the Low-Phosphorus Group

The femurs and humeri of the animals in Group IV, the low-calcium-low-phosphorus group, were spongy and soft at the ends. The shaft while hard was not as thick as the normal bone, and there was a larger amount of marrow. Upon weighing it was found that 10.3 percent of the weight of the bone was marrow. The ends of the bones were not well calcified but took of the nature of a rachitic

bone as described by McCollum and Simmonds (1927). They state that "in the rachitic bone all the processes of growth and the maintenance of equilibrium are abnormal. Calcium salts are not deposited in the normal way. In a normal bone the junction of the cartilage with the shaft forms a straight regular line. In the rachitic bone it is ragged because there is no provisional zone of calcification and the cartilage is invaded by large tufts of blood vessels from the shaft which destroy it irregularly. At the same time masses of cartilage persist where they should be destroyed, and islands remain cut off from the main body of the tissue. Osteoid is not calcified, as it is formed either in the trabeculae or by the periosteum, so that the bone becomes soft. The animal attempts unsuccessfully to compensate for the weakness due to the lack of lime salts by producing osteoid tissue in abnormal amounts, especially at points subject to stress and strain. . . . This overproduction of osteoid and cartilage causes irregular enlargements of the bones, and their weakness consequent upon the lack of lime salts results in bowing and fracture."

FIGURE 14.—Cross-section of Femur of Animals E 12, from the Low-calcium Group, and E 29, from the Low-calcium-low-phosphorus Group

When these bones were allowed to remain for a few days in a room of ordinary temperature, they became dark in color and expelled a considerable amount of moisture. After allowing the tarsal bones to remain for several days in this manner, it was found that the shaft had split longitudinally at two or three places and had expelled a large amount of moisture.

The femurs and humeri of the bones in Group II, the low-calcium group, were very similar to those of Group IV. They were soft and spongy at the ends but in this respect were not so bad as were those

of Group IV. The shaft was dense but the walls were thin. It was found that 10.4 percent of the weight of the bone was marrow. The bones upon standing showed the same general characteristics as those of Group IV.

TABLE 17.—*Average Breaking Strength of Tarsal Bone of Animals in the Various Groups*

Item	Group I		Group II		Group III		Group IV	
	19 mo.	25 mo.	19 mo.	25 mo.	19 mo.	25 mo.	19 mo.	25 mo.
Average weight of animals (<i>pounds</i>)	764	966	688	833	721	924	645	782
Breaking strength of bones (<i>pounds</i>)	1135	2020	942	1490	1165	2030	915	1010
Breaking strength of bones per cwt. of animal (<i>pounds</i>)	148.5	209.1	136.9	178.9	161.6	218.5	141.8	129.1
Average breaking strength (<i>pounds</i>)	1572		1261		1597		962	
Average breaking strength per cwt. of animal (<i>pounds</i>)	178.8		157.9		190.0		135.4	
Percent of normal group..	100.0		89.4		106.2		75.7	

The femurs and humeri of the animals in Group III, those fed the low-phosphorus ration, were somewhat different from the ones already described. While the ends of the bones were soft and spongy and not well calcified, the center of the bone was hard and the walls were as thick as those of the animals in the normal group. The amount of marrow also was about the same as in the normal bones, as 8.3 percent of the bone was marrow. These bones seemed to be affected more at the ends than in the shaft.

At time of slaughter it was found that there was a slight enlargement at the costochondral junction of the ribs of the animals in Groups II and IV. These could not be described as "beads," but at the point of junction there were enlargements surrounding the junction which seemed to consist largely of fat or fat-like material. This is shown in Figure 15 for the animal E 28. The ribs of these two Groups, especially the twelfth, were not well calcified and upon standing turned dark and emitted moisture. In the case of the animals in group IV it was found that the twelfth rib was composed largely of cartilage. This was not shown as pronounced in the animals of the other groups.

Effect of Breaking Strength. The breaking strength was determined of the tarsal bone taken from the left leg of the animals at the ages of 19 months and 25 months. These were all determined at the same time after the first set of bones had dried out for some time. The two therefore are not strictly comparable.

Since the animals were not all of the same size the breaking strength of the bones was calculated per hundredweight of the animal. The results are given in Table 17.

It will be noted that whereas the breaking point of the bones of Groups II and IV was below that of the normal-fed group, that of Group III was above.

The breaking strength of the bones was taken on the shaft. The bones of the animals in Group III, as shown by a study of their composition, were affected just as much or even slightly more than were those of the animals in Group II, but only ends of the bones were affected. The shaft of the bones was just as thick as shown by measurements, by breaking strength, and by the amount of marrow, as was the shaft of the bones of the animals fed normal rations. This would seem to substantiate the indication already mentioned that, while the animal has the power to draw upon the storage of calcium in the bone in order to maintain the normal amount in the blood, it does not have the power to draw upon the reserve of phosphorus sufficiently to keep the inorganic phosphorus of the blood normal.

The breaking strength of the bones of the animals in Group II, the low-calcium group, was only 89.4 percent, and that in Group IV, the low-calcium-low-phosphorus group, was only 75.7 percent of the breaking strength in the normal-fed group.

Summary. Feeding rations to growing dairy animals which are low in calcium or phosphorus or in both these minerals results in changes in the bones as shown by analysis, breaking strength, and appearance.

FIGURE 15.—Showing Enlargements Surrounding Junctions of Ribs in Animal E 28

In general the analyses of such bones show that the ash and non-extractable organic matter are reduced while the moisture and extractable material are increased. The ash of the bones of the animals of Group IV, the group fed the ration low in both calcium and phosphorus, was more than 25 percent lower than that of the group fed the normal ration, while the ash of the animals in Groups II and III was lower than that of the normally-fed animals.

The bones of the low-mineral-fed animals were not calcified as well as those of the normal-fed group, and the breaking strength, with the exception of Group III, was considerably less than that of the animals fed the normal ration.

TABLE 18.—Average Weight of Internal Organs on Basis of Hundredweight of Animals, and Average Dressing Percentage of Each Group, by Periods

Period Ending When Animals Were at Age of	Group I	Group II	Group III	Group IV
HEART				
	(gms.)	(gms.)	(gms.)	(gms.)
8 mos.	189	179	164	185
13 mos.	163	162	158	199
19 mos.	148	154	151	171
25 mos.	153	148	150	189
LIVER				
8 mos.	610	548	560	531
13 mos.	484	564	490	624
19 mos.	441	446	472	468
25 mos.	393	416	394	403
BRAIN				
8 mos.	70	67	63	73
13 mos.	70	69	62	75
19 mos.	42	52	44	54
25 mos.	38	47	37	43
SPLEEN				
8 mos.	72	72	63	76
13 mos.	79	92	63	82
19 mos.	69	58	63	81
25 mos.	64	65	71	89
DRESSING PERCENTAGE				
8 mos.	47.5	43.5	44.7	44.2
13 mos.	49.2	48.5	50.8	49.0
19 mos.	52.9	51.6	56.0	50.1
25 mos.	59.1	50.7	58.5	55.8

The results would indicate that a study of the bones of growing dairy animals is a very good index as to whether the animals are receiving sufficient minerals, and, furthermore, that any of the long bones can be used with equal success for such study.

Effect of Rations upon Internal Organs and Dressing Percentage

At the time of slaughtering the animals an examination was made of their internal organs. These were removed, examined, and carefully weighed. Since the weight of the animals varied considerably in each group, the weights of the different organs were converted to the live weight basis of the animals. In Table 18 the average weights of the heart, liver, spleen, and brain of the groups are given for the various periods on the basis of a hundredweight of the animals. This table also gives the dressing percentage of each group by periods.

Table 18 shows that as the normal-fed animals grow older the weight of their internal organs increases, but not in proportion to their live weight. When it is expressed on the basis of a hundredweight of the animal, the weight of the heart, liver, brain, and spleen all decrease as they grow older. In general this also seems to be the case with the animals in the other groups. The spleen, however, of the animals in Groups III and IV seems to increase at a faster rate than the live weight of the animals as they grow older. This was especially noticeable in the case of the animals in Group IV. The animals in this group on the hundredweight basis seemed to increase in the size of all the organs in comparison with the animals in the

normal-fed group. This was true but less pronounced of the animals in Group II and was least shown by those in Group III.

The animals in Groups I and III averaged highest in dressing percentage, which increased as the animals grew older. It was also observed that the animals in these two groups were in better condition as to flesh than those in Groups II and IV. This would indicate that the difference in the size of the internal organs might be due largely to the difference in the mineral content of the ration.

No difference was noted in the general appearance of the organs of the several groups.

GENERAL DISCUSSION OF RESULTS

In bringing together the results of the experiment it is evident that the animals in Group I, the group which was fed a ration typical of those fed under practical conditions, obtained sufficient calcium and phosphorus for normal growth and development. They did not grow as well as might have been expected during the first year, but this probably was due to the poor start which they received and not to any deficiency in their ration. At the time of the last analyses the inorganic phosphorus in their blood was slightly low. This may have been due to the low phosphorus content of the ration, which during the last periods contained only 0.22 percent phosphorus and was providing only 1.9 gms. of phosphorus per day per hundredweight of the animal.

The optimum requirement for laboratory animals as found by McCollum and coworkers (1922) was 0.4146 percent phosphorus. This ration contained only a little more than one-half of this amount; in fact none of the rations fed after milk-feeding was discontinued contained as much phosphorus as this amount. It would hardly be expected, since the dairy animal is not as fast a growing animal as the rat, that it would require as much phosphorus in the ration as does the rat.

Armsby (1917) calculated that during the first year the normal animal retains 1.7 gms. of phosphorus daily per hundredweight of the animal. Since the animals in the normal group were receiving only a little more than is retained by normal animals, it can be seen that the animals in this group were being fed a ration dangerously close to the lower limits of phosphorus requirements.

Since this ration was being used as one typical of those being fed under practical conditions, the danger of underfeeding of phosphorus is evident. The animals in this group, however, were normal in weight and height at withers after the first year, were in good physical condition, and showed no abnormal physical characteristics throughout the experiment. Hence they must have received sufficient calcium and phosphorus.

The animals in Group II were fed a ration low in calcium but containing a little more phosphorus than was contained in the ration fed to the animals in the normal group. Any abnormal condition in

the animals of this group can be attributed to the low calcium content of the ration, since all animals were fed according to their requirements for digestible protein and digestible nutrients.

The ration fed during the first year contained 0.286 percent calcium and during the second year 0.249 percent. This during the first year was about 3.5 gms. and during the second year about 2.75 gms. per hundredweight of the animal. McCollum and coworkers (1922) found that the optimum requirement of calcium for growing rats was 0.641 percent, and Armsby (1917) calculated that the amount retained by normal cattle during the first year was 3.2 gms. of calcium per day per hundredweight of the animal. The ration fed contained in proportion considerably less than one-half of the optimum requirements for rats and during the first year just a little more than is retained by normally growing cattle. During the second year the ration contained a little less than is retained by normally growing cattle. It is evident then that these rations were low in their calcium content. The rations contained a little more phosphorus than did those fed to Group I and therefore they should not be lacking in phosphorus.

The animals in Group II did not grow so well, especially during the second year, as did those in Group I. This was shown both by weight and by height at withers. Neither were they in as good physical condition at any time, as shown by general appearance and dressing percentage. The blood, however, remained normal in its calcium and inorganic phosphorus content. Analyses showed that the green bones of the animals in this group were lower in ash and consequently lower in both calcium and phosphorus than were the bones of the normal group. The bones were slightly higher also in moisture and extractable material. This condition was true during the entire experiment, but it seemed to be improving toward the end of the trial. The circumstance would indicate that the animals as they grew older did not require as much calcium as they did when they were younger and making faster gains.

These animals averaged about $1\frac{1}{2}$ months older at the time of the first signs of oestrus than those in Group I, and they were quite irregular thereafter. However, other than being in not quite as good condition and not growing quite as fast as Group I, Group II showed no abnormal physical characteristics. Their bones indicated, however, that they were being fed a ration too low in calcium for best development, but since they were kept for so long a time without very striking results, it would indicate that the ration was only slightly low in this element.

The animals in Group III during the second year were fed a ration containing only 0.131 percent of phosphorus. This provided about 1.43 gms. of phosphorus per day per hundredweight of the animals. The amount of calcium in the ration was about the same as in the ration fed to the animals in the normal group. Any abnormal condition in the animals of this group can be attributed to the lack of phosphorus in the ration. It must be borne in mind, however, that

the ration fed to the animals in this group was normal in its amount of phosphorus during most of the first two periods. Hence the effects of the ration fed could not be expected before this time, as it was then equal in both calcium and phosphorus to the ration fed to the normal group. Therefore only the difference noted during the latter part of the experiment is significant.

The animals in Group III grew almost as well as did those in Group I, both in height at withers and in weight during the first year, but during the last year they did not make quite as good gains. Their growth curves show them to have made much better gains than those in either Groups II or IV but this may have been due largely to the better start which they obtained because of the normal ration which they received during the first year. They were in better condition than those in Groups II and IV, as shown by dressing percentage and general appearance, but this again may have been due to the better start which they received. The first period of oestrus occurred at about the same time as that of the normal group, but that also would be as expected, since they were receiving a normal ration up to about that time.

When their ration was changed to a low-phosphorus ration, however, within a week the inorganic phosphorus in the blood had dropped to almost one-half what it had been previously and it remained there throughout the rest of the experiment.

Toward the end of the experiment one of the animals in this group became stiff in the hind legs, and the legs became slightly bent. The other animal became slightly sway-backed.

The amount of ash in the green bone also fell and continued low until the end of the experiment. This as usual was accompanied by a rise in the moisture and extractable material of the bones. The breaking strength of the bones remained normal in this group and the amount of marrow in the bones also was normal.

A study of these results indicates that the growing dairy animal must depend for her phosphorus supply largely upon her feed, especially when she is obtaining sufficient calcium in her feed. This is indicated first because of the almost immediate decrease in the inorganic phosphorus of the blood when rations below the needs of the animal were fed; and second, whereas the total ash in the bones was decreased below normal, the shaft of the bone was not decreased in strength and there was no more marrow in it than in the normal bone.

While the ash content of the bones in the animals fed the low-calcium ration was no less than it was in Group III, nevertheless the breaking strength of the bones was reduced, and the marrow increased in amount, notwithstanding the fact that the calcium content of the blood was not decreased. This would seem to indicate that, whereas the animal may draw upon the bone for its calcium in order to keep its blood supply normal, it cannot do so for phosphorus, especially if there is sufficient calcium in the ration. If this is true it might explain one of the reasons why the ratio of these elements should be kept within certain limits.

The results also indicate that it is much easier to affect the phosphorus supply of the blood than the calcium supply in growing dairy animals. This is shown in a comparison of Groups II and III. The bones of both of these groups were affected in comparison to about the same degree, but whereas the blood of Group IV was lowered materially in phosphorus, that of Group III was maintained at normal in calcium.

Group IV was fed a ration low in calcium and after the first two periods low also in phosphorus. During the first two periods the ration compared with that fed to Group II, and the results were very similar. They grew at about the same rate as indicated by weight and height at withers. The results of the analyses of the bones also were very similar.

At about the end of the second period the ration fed was low also in phosphorus, containing only about 0.133 percent of that mineral. The inorganic phosphorus content of the blood immediately dropped to about one-half that of normal. The calcium content of the blood remained normal throughout the entire experiment, however. This group showed more serious symptoms than any of the others. The composition of the bones was greatly affected. The ash was lowered to less than three-fourths that of normal bones and contained much more moisture and extractable material than normal bones. The calcium and phosphorus were correspondingly lowered. The breaking strength of the bones was not much over one-half that of normal bones. The animals also took on abnormal appearance toward the end of the experiment. They became sway-backed and one individual became slightly bent in the legs.

This ration was very similar to Ration II in its calcium content (slightly higher) and to Ration III in its phosphorus content (slightly higher), but the effects were much worse than in either of these groups. This would show that both calcium and phosphorus were too low in these rations for the normal well-being of the animals.

SUMMARY AND CONCLUSIONS

Four groups of growing dairy animals were fed in such a way that all were receiving approximately the same amount of digestible crude protein and total digestible nutrients. Group I was fed a supposedly normal ration in so far as its content of phosphorus and calcium was concerned. Group II was fed a ration low in calcium but with sufficient phosphorus. Group III was fed a ration low in phosphorus but with sufficient calcium, and Group IV was fed a ration low in both calcium and phosphorus. No effort was made to supply the anti-rachitic vitamin, and the animals were kept in a building where the sunlight came in only through common window glass.

The effects of the ration were studied by weighing and measuring the animals at weekly intervals, by analyzing the blood for inorganic phosphorus and calcium, and by analyzing the bones for moisture, extractable material, ash, and the ash for calcium and phosphorus. Data were also taken as to condition of animal, abnormalities, time of showing oestrus, and any other factors which could be attributed to the ration.

Under the conditions of the experiment as outlined, the following conclusions were reached:

Growing dairy animals fed rations low in calcium (0.286 percent or less) or low in both calcium (0.331 percent or less) and phosphorus (0.298 percent or less), but otherwise ample in nutritive value, grow nearly as well as normal-fed animals for some time, but over a period of two years do not make as good gains as measured by weight and height at withers. Animals fed a ration low in phosphorus (0.131 percent) during the second year continued to grow almost as well as did normal-fed animals, even though the inorganic phosphorus content of their blood was much below normal.

The inorganic phosphorus in the blood of growing dairy animals can be more easily reduced than can the calcium content by feeding rations low in these minerals.

Rations containing less than 0.20 percent of phosphorus will decrease the amount of inorganic phosphorus in the blood.

Rations containing as little as 0.25 percent of calcium will not affect the calcium content of the blood.

Growing dairy animals when fed rations low in phosphorus cannot draw sufficient phosphorus from their bones to maintain the normal supply of inorganic phosphorus in the blood.

Rations which contain less than 0.35 percent of calcium or less than 0.20 percent of phosphorus give rise to a bone which is low in ash and consequently low in calcium and phosphorus but high in moisture and extractable material.

The ash of bones contains approximately the same percentage of calcium and phosphorus regardless of the rations fed.

Either the ribs or femurs and humeri can be used for analysis with the same general results.

Rations which are fed under practical conditions may be dangerously near the lower limits of phosphorus requirements.

The analyses of the bones of the animals in the normal group give figures which are fairly representative of the composition of the bones of normal dairy animals at various ages.

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