EFFECT OF AGE AND NUTRITION ON THE CALCIUM PHOSPHATE/CALCIUM CARBONATE RATION IN THE BONES OF CATTLE[1]

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INTRODUCTION

A knowledge of the changes induced in the skeletal structure of cattle by different planes of mineral nutrition should aid in determining the optimum mineral intake for the development of the skeleton and its maintenance in normal condition. The proportions as well as the total quantity of the mineral constituents may be altered through nutrition. Only in recent years have methods been devised for the study of these changes. Kramer and Howland (*7*) [3] provided such a method, and its adaptation for the analysis of the skeletons of cattle has been described in a previous paper (*11*).

This method of analysis permits the determination of calcium, magnesium, phosphorus, and carbonate on unashed bone. Since these comprise the constituents of the mineral matter, except for minute amounts of some other elements, it is possible to make deductions concerning some of the most probable salts and their proportions. By assuming that the carbonate is all present as calcium carbonate and the magnesium as trimagnesium phosphate, it was found that the remaining calcium and phosphorus were generally in nearly the same proportion as for tricalcium phosphate. Using this result in support of the assumption that the calcium phosphate is tricalcium phosphate, the ratio of tricalcium phosphate to calcium carbonate may be calculated.

In spite of the fact that only small variations may be secured in the Ca/P ration of bone ash (*4, 5, 9, 10, 12*), Howland, Marriott, and Kramer (*6*) and Kramer and Shear (*8*) found significant variations in the composition of calcification by analyzing unashed samples of bone. They reported the calcium phosphate/calcium carbonate ratio for the bones of normal rats to be 10.8 and 10.6, and for a rachitic rat 6.7. A similar reduction of the ratio was observed in rachitic human bones. A constant ratio would not be expected. The concentration of the ions in the body fluids surrounding the ossifying tissue is not constant and this should be reflected in the resulting ossification.

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[2] Resigned June 30, 1929.

[3] Reference is made by number (italic) to Literature Cited, p. 121.

EXPERIMENTAL DATA

So far as the writers are aware, no analyses of unashed bones of cattle have ever been reported. It was believed that such a study performed on the skeletons of cattle reared or maintained on rations of known calcium and phosphorus content would aid in the elucidation of the requirement for these elements. Normal mature cattle were taken from the University Farm herd where there has never been any evidence of mineral deficiency. Mature cattle that had received a phosphorus-deficient ration were available from the experiments of Eckles, Becker, and Palmer (*3*). Other animals used in the same project had received a phosphorus-deficient ration which was later supplemented with sodium or calcium phosphate. The younger animals were all from a project on the mineral requirements for growth. These latter animals were grouped according to the amount of calcium and phosphorus received.

Whether the supply of calcium and phosphorus was considered as low, medium, or high, was based on the computation made by Armsby (*1*) from the analyses of Lawes and Gilbert showing that the average daily storage is 3.2 gm. of calcium and 1.7 gm. of phosphorus per hundred pounds live weight. An animal receiving a moderate excess over these amounts was considered to be receiving a normal mineral ration. An average ration of mixed hay and grain supplies a slight excess of calcium and a liberal amount of phosphorus. Lack of data on the percentage assimilation of calcium and phosphorus by growing cattle precludes a definite statement as to the absolute adequacy of such a ration.

Natural feeds were used in all the rations and were assumed to supply sufficient vitamin D. Regardless of the quantitative intake of this vitamin, it was sufficiently uniform to prevent its being the determining factor in the composition of the mineral of the skeletal structure.

The ratio of residual calcium to residual phosphorus is not shown in this paper because in all cases it closely approximated the value for tricalcium phosphate, and so was not of additional significance.

Twenty animals were included in the normal group. The composition of the skeletons of the younger animals varied. Lack of absolute uniformity in the rations, individuality of the animals, and differences in their early history are probable causes of this variation. The average amount of calcium and phosphorus supplied was more than enough to care for the usual storage. All animals were normal in size and appearance.

Average ratios of the calcium phosphate to calcium carbonate obtained, as shown in Table 1, were: 7.32 for those slaughtered at 6, 9, and 12 months, 7.11 for those at 18 and 24 months, and 6.57 for mature cows. The decrease in the second year is very slight, but its occurrence is substantiated by the further decrease in mature cows—probably due to a more uniform mineral intake. These results for normal cattle serve as a standard of comparison for the cattle on abnormal mineral rations.

The cows on a known phosphorus-deficient ration received prairie hay grown in the phosphorus-deficient area in western Minnesota, ground oats, common salt, and water. The ratios for five such animals are presented in Table 2, the average for the low four being

5.30. E–37 was omitted from this average because she was not in milk, and no longer showed symptoms of mineral deficiency at the time of slaughter. A very liberal proportion of ground oats in her ration apparently had supplied her phosphorus requirement.

TABLE 1.—*Effect of age on the calcium phosphate/calcium carbonate ratio in the skeletons of dairy cattle on normal rations*

Animal No.	Age	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
	Months		
E–106	6	7.41	
E–114	6	7.10	
B–163	6	7.09	
E–102	9	7.73	
E–79	12	7.27	
Average		7.32	±0.237
E–55	18	6.92	
E–85	18	7.14	
E–88	18	7.20	
WVa–3 [a]	18	6.78	
WVa–6	18	7.23	
E–86	24	7.37	
E–78	24	7.47	
E–98	24	6.66	
WVa–4	24	7.02	
WVa–5	24	7.30	
Average		7.11	± .236
E–25	(b)	6.59	
E–23	(b)	6.76	
310	(b)	6.50	
101	(b)	6.59	
19	(b)	6.41	
Average		6.57	± .116

[a] Samples of the bones of cattle numbered with the prefix WVa were furnished by H. O. Henderson of the West Virginia Agricultural Experiment Station.
[b] Mature.

TABLE 2.—*Calcium phospate/calcium carbonate ratio in the skeletons of mature dairy cattle fed rations containing different levels of phosphorus*

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION

Animal name or No.	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
Daisy	4.84	
E–36	5.36	
E–37	[a] 6.22	
E–73	5.66	
E–90	5.34	
Average	5.30	±0.294

ANIMALS ON A PHOSPHORUS-DEFICIENT RATION SUPPLEMENTED WITH SODIUM OR CALCIUM PHOSPHATE

E–34	6.30	
E–58	6.31	
E–59	6.20	
E–61	6.29	
E–62	6.77	
Average	6.37	±0.202

[a] Omitted from the average.

The animal called Daisy showed symptoms of extreme phosphorus deficiency. When she was slaughtered her ribs were found to be extremely soft and were readily pierced with a knife. E–90 was very emaciated, showed depraved appetite, and her joints creaked when she walked. The inorganic phosphorus in her plasma as determined by the Briggs (*2*) method was nearly 1.00 mg. per 100 c. c. of plasma. E–36 was in a similar condition. E–73 did not show such acute symptoms of phosphorus deficiency, but would chew bones. The inorganic phosphorus in her plasma averaged 2.00 mg. per 100 c. c. of plasma for the six months previous to slaughter.

Daisy, E–90, and E–36 showed the lowest ratios, E–73 somewhat higher, and E–37 a nearly normal ratio. This was in direct agreement with the degree of deficiency exhibited. The average ratio, 5.30, is almost the same as that reported for rachitic rats *(6)*. The relative decrease in the amount of calcium phosphate indicates that there is a selective absorption during periods of deficiency. The animal in drawing on its mineral reserve in the skeleton for phosphorus either did not withdraw a proportionate amount of carbonate, or even replaced some of the calcium phosphate with calcium carbonate. The result may also indicate that the metabolism of the bone carbonates is independent of that of the phosphates.

It has been shown (*3*) that the feeding of the phosphorus-deficient ration supplemented with either sodium or calcium phosphate will result in normal reproduction, increased lactation, and gain in body weight. The bones of five animals so fed were analyzed. Before the feeding of the supplement the condition of these animals was comparable to that of the phosphorus-deficient group. E–62, which received the supplement longest, showed the highest ratio, 6.77. (Table 2.) All the ratios were within the range of the normal, and from this it is concluded that the skeletal structures of the animals had recovered their normal proportion of calcium phosphate and calcium carbonate. These are believed to be the first analyses reported from cattle after a period of phosphorus-deficiency followed in turn by adequate rations, and they indicate that recovery from such a condition can be complete.

Less is known of the effect of phosphorus deficiency on growing cattle than on mature ones. Henderson and Weakley (*5*) found an increase in the moisture content and a decrease in the breaking strength of bones from cattle raised on a low phosphorus ration. Bone samples from eight of their animals on low phosphorus rations are included in this study. (Table 3.) During the first year these animals received an ample quantity of phosphorus, but during the second year they were provided with only 60 to 75 per cent of the phosphorus usually retained.

TABLE 3.—*Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of phosphorus* [a]

Animal No.	Age	$Ca_3(PO_4)_2$ / $CaCO_3$	Standard deviation
ANIMALS ON A LOW PHOSPHORUS RATION			
	Months		
WVa-27	18	5.94	
WVa-30	18	6.12	
WVa-19	18	6.49	
WVa-22	18	6.11	
Average		6.16	±0.201
WVa-28	24	6.86	
WVa-29	24	6.39	
WVa-20	24	6.92	
WVa-21	24	6.51	
Average	24	6.67	±.225
ANIMALS ON A HIGH PHOSPHORUS RATION			
E-87	6	8.04	
E-96	6	7.65	
Average		7.85	

[a] The bone samples from animals on a low phosphorus ration were furnished by Doctor Henderson and Professor Weakley of the West Virginia Agricultural Experiment Station and represented samples used by them in another study (*5*). We acknowledge their kindness in supplying us with this material.

The calcium phosphate/calcium carbonate ratio was reduced in these animals. The normal ratios for cattle 18 and 24 months old were 7.05 and 7.16, respectively, as compared with 6.16 and 6.67 for these animals. Henderson observed some improvement in their condition during the last six months, and attributed it to a lessened phosphorus requirement which made a partial recovery possible. The ratios observed support this explanation of their improvement.

One-half of the animals of this group received a ration that was low in both calcium and phosphorus. There was no apparent ill effect from the low calcium, possibly because the deficiency of phorphorus reduced the requirement for calcium by preventing the usual amount of new calcification. The rations used were not so low in phosphorus as those employed at University Farm, nor were the ratios obtained so low. However, considering the difference in the normal ratios for the two ages, comparable reductions were secured in both cases. The higher mineral requirement of the young animal for skeletal growth makes it possible to secure a deficiency on rations carrying appreciable quantities of phosphorus.

It was planned to study the effect of low, medium, and high calcium and phosphorus intake on the growth and development of dairy cattle. Only two animals were available in the high phosphorus group, and none in the medium group when these data were obtained. (Table 3.) Both of the animals in the high phosphorus group were slaughtered at 6 months of age, and the high ratio of 7.85 was secured from the analyses of the bone samples. This raises the question whether an abnormally high reserve of phosphorus can be stored in the skeleton, and if so, would it not aid in meeting the phosphorus requirement during later lactation? It seems improbable that there should be any deleterious effects from high phosphorus intake in

view of the quantity of supplement (100 gm. of sodium or calcium phosphate daily) fed to cows on phosphorus-deficient rations (*3*).

The necessity of feeding milk to calves made it impossible to secure satisfactory low calcium rations for the first six months. Although the usual calcium intake was close to the amount generally retained, some departure from the normal was observed in three of the calves slaughtered at 6 or 12 months. (Table 4.) Three of those slaughtered at 18 and 24 months showed an increase in the calcium phosphate/calcium carbonate ratio, while the fourth was practically normal. However, there were too few animals and the calcium intake was not sufficiently low to warrant any definite conclusions. Indications are that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency.

TABLE 4.—*Calcium phosphate/calcium carbonate ratio in the skeletons of young dairy cattle fed rations containing different levels of calcium*

Animal No.	Age	$\frac{Ca_3(PO_4)_2}{CaCO_3}$	Standard deviation
ANIMALS ON A LOW CALCIUM RATION			
	Months		
E-103	6	6.60	
E-104	6	6.91	
E-109	12	6.85	
E-110	12	7.23	
Average		6.90	±0.224
WVa-11	18	7.75	
WVa-14	18	7.76	
WVa-12	24	7.61	
WVa-13	24	7.27	
Average		7.60	±.198
ANIMALS ON A HIGH CALCIUM RATION			
E-95	12	7.58	
E-89	12	7.25	
Average		7.41	

There were but two animals on a high calcium ration. The ratio of calcium phosphate to calcium carbonate was normal when they were slaughtered at 12 months of age.

CONCLUSION

The residual calcium/residual phosphorus ratio of cattle bone approximates the calcium/phosphorus ratio of tricalcium phosphate. The residual calcium/residual phosphorus ratio of the bone is not affected by calcium or phosphorus deficiency in the ration. This is the ratio between the calcium and phosphorus which do not occur in the bone as calcium carbonate or trimagnesium phosphate; it always closely approximates the value for tricalcium phosphate.

The calcium phosphate/calcium carbonate ratio of the bones of dairy cattle is affected by age and nutrition. This ratio decreases with age. Phosphorus deficiency causes a decreased ratio, but feed-

ing a phosphorus supplement permits the recovery of a normal ratio. Rations high in phosphorus may produce a high ratio.

There were indications that either an increase or a decrease in the proportion of phosphate in the skeleton may occur as a result of calcium deficiency, although the results did not warrant definite conclusions. The ratio of calcium phosphate to calcium carbonate was normal in two animals on a high calcium ration when slaughtered at 12 months of age.

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