

limbs, a function most readily measured by resistance to passive extension. Both these phenomena gradually passed away, with a certain tendency for the diminished flexor activity to persist for a time after the resistance to passive flexion had disappeared.

General. After unilateral operation, the contralateral limbs were chiefly but not exclusively involved. The homolateral hind limb was damaged to a lesser extent and the homolateral forelimb hardly at all. In general, also, the contralateral limbs were somewhat less disordered after unilateral, as compared with the bilateral operations. Marchi degeneration studies are in progress.

7000 C

Relative Utilization of Calcium from Calcium Carbonate and Calcium Gluconate by Chickens.

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Numerous investigations have indicated that the chicken may use a number of forms of calcium for its metabolic needs. Workers at the Kentucky Experiment Station¹ studied calcium carbonate, calcium lactate, calcium sulphate, tri-calcium phosphate and calcium chloride as sources of calcium for chickens and found that calcium carbonate was the most effective judged by the degree to which it was utilized in the production of eggs, its influence on the weight of egg contents and shells, and quantity of the salt consumed. Calcium sulphate was not so effective as the carbonate as shown by a smaller egg production and lower weight of shells and egg contents. Calcium lactate was readily utilized but the quantity consumed was variable and small as compared to the carbonate and sulphate. Only small quantities of calcium chloride were consumed and the precipitated tri-calcium phosphate was not a satisfactory source of calcium for egg production as compared to calcium carbonate. The above mentioned salts were fed as supplements to a wheat, yellow corn and skim milk ration.

Bethke² and his associates state that no difference was found in the availability of calcium in the carbonate, sulfate, lactate and

¹ Buckner, G. D., Martin, J. H., and Peter, A. M., *J. Agr. Res.*, 1928, **36**, 263.

² Bethke, R. M., Kennard, D. C., and Kick, C. H., *Poultry Science*, 1929, **9**, 45.

phosphate salts when fed to chicks on a basis of equal calcium intake on a minimum requirement basis.

As calcium gluconate is a readily soluble salt, it was thought desirable to study it as a source of calcium as compared to calcium carbonate, since the carbonate is the commonly used source of supplemental calcium for poultry. The following studies include both growing chicks and laying hens.

Two groups each consisting of 81 day-old Barred Rock chicks were placed in battery brooders and given rations that differed only as to the source of calcium. Group I received calcium carbonate as its supplemental source of calcium, while Group II received calcium gluconate.* These salts were incorporated with the ration which was fed in all-mash form and kept before the birds at all times. Table I gives the make-up of the starting and growing ration as well as the ration used for the laying birds in the second part of the experiment.

TABLE I

| | Starting and growing ration | | Laying ration | |
|--------------------|-----------------------------|-----------|---------------|-----------|
| | Ration I | Ration II | Ration I | Ration II |
| Ground yellow corn | 34.5 | 35.2 | 27.9 | 29.46 |
| " wheat | 15 | 15 | 15 | 15 |
| " oats | 20 | 20 | 20 | 20 |
| Alfalfa meal | 10 | 10 | 10 | 10 |
| Meat meal | 5 | 5 | 5 | 5 |
| Fish " | 5 | 5 | 5 | 5 |
| Dried Milk | 5 | 5 | 5 | 5 |
| Calcium carbonate | 1 | | 2.2 | |
| " gluconate | | 4.32 | | 9.5 |
| Sodium phosphate | | | .8 | .8 |
| Commercial sucrose | 4 | | 8.9 | |
| Cod liver oil | .5 | .5 | .2 | .2 |
| | 100 | 100 | 100 | 100 |
| % calcium | 1.38 | 1.40 | 1.86 | 1.88 |
| % phosphorus | 0.657 | 0.662 | 0.848 | 0.851 |

The sugar was added to the calcium carbonate rations to compensate for the gluconic acid carried by the gluconate rations.

The males were discarded at the end of 6 weeks, and at 8 weeks of age, 8 pullets were removed from each group, the left tibia was dissected from each and bone ash determinations made on the oven dry fat-free tibiae. Group I exhibited an average bone ash of 49.8%, Group II a bone ash value of 48.9%. Since vitamin D had been supplied in ample quantity it was thought advisable to study the bone ash of birds that had been fed the same rations as were

* The calcium gluconate used in the study was obtained through the courtesy of Röhm and Haas Co., Inc., of Bristol, Pa.

fed to Groups I and II but to restrict the cod liver oil to 0.10% of the total ration. Previous observations had shown that when this level of cod liver oil was administered, many birds became rachitic at an early age. Two groups of 15 chicks each were used in this study and when bone ash determinations were made at 8 weeks, the birds receiving calcium carbonate had an average bone ash of 37.6 and the ones receiving calcium gluconate had an average bone ash of 39.2. No further studies were made with these birds.

When the birds that were started on the original experiment were 22 weeks old, they were taken from the batteries and 12 birds from each group were placed in individual compartments of laying cages. At this time the average weights of the birds in Groups I and II were 1,450 gm. and 1,535 gm., respectively. The results with growing chicks are in agreement with those of Hart³ and associates, who compared calcium carbonate, calcium sulfate, bone meal, rock phosphate, dicalcium phosphate and calcium gluconate as sources of calcium for the growing chick in the presence of ample quantities of vitamin D. They state that: "These findings indicate clearly that it cannot be assumed that one form of calcium is better than another for animal feeding because it is more soluble in water. The rate of solution of calcium salts in the animal intestine is sufficient, even in case of the more insoluble compounds to make all forms equally effective to the animal provided adequate vitamin D is present."

The rations fed to the birds after they were placed in the laying cages differed only slightly from the rations they received during the growing period. It was necessary, however, to add more calcium to the ration to take care of the increased demand for calcium for the production of egg shells. The phosphorus content of the rations was also raised, by the addition of sodium phosphate. It was felt that in this study any differences that might result from the use of the 2 salts would be more apparent if vitamin D were supplied in limited amounts, consequently 0.2% of cod liver oil was supplied in the ration which was fed as all mash and kept before the birds at all times. This amount of cod liver oil furnished vitamin D in quantity less than that required for good egg production from birds denied access to sunlight. The laying birds in this experiment were housed in a basement room and any sunlight reaching them was filtered through window glass. The composition of the ration used for egg production is given in Table I.

³ Hart, E. B., and Deobald, H. J., Wisconsin Bulletin 421.

After egg production started the following data were collected: number and weight of eggs produced, weight of shell, weight of shell ash, and in some cases the calcium level of egg contents was determined. Limited studies were also made on the hatchability of eggs and livability of the resultant chicks. The birds continued on the study for 12 months after egg production started and egg shell studies were made except for 2 months during which time the eggs were used for hatchability studies. The determination of calcium in egg contents was not started until after the birds had been in production for 3 months and was discontinued while the eggs were being used in hatchability studies.

Eggs were collected daily and were weighed the following day, at which time the shells were removed, washed free from adhering contents, dried to constant weight and ashed. For the most part the shells from a number of eggs were pooled for the ash determinations. Table II gives the shell and shell ash determinations by months, the percentages being based on the weights of the original eggs. Table II also includes results of calcium determination on egg contents.

TABLE II

| Month | Shell | | Shell Ash | | Ca in egg contents | |
|---------|--------------|---------------|--------------|---------------|--------------------|---------------|
| | Group I % | Group II % | Group I % | Group II % | Group I % | Group II % |
| 1 | 7.9 | 8.8 | 4.1 | 4.7 | | |
| 2 | 8.3 | 8.5 | 4.3 | 4.5 | | |
| 3 | 7.6 | 8.5 | 3.9 | 4.4 | .051 | .053 |
| 4 | 8.0 | 8.9 | 4.1 | 4.5 | .050 | .053 |
| 5 | 8.1 | 9.0 | 4.3 | 4.7 | .051 | .053 |
| 6 | 8.0 | 9.1 | 4.2 | 4.8 | .050 | .053 |
| 7 | 8.3 | 8.8 | 4.5 | 4.7 | .050 | .052 |
| 10 | 8.4 | 8.9 | 4.6 | 4.9 | .050 | .052 |
| 11 | 8.3 | 8.8 | 4.4 | 4.6 | .050 | .052 |
| 12 | 8.3 | 8.7 | 4.3 | 4.5 | .051 | .052 |
| Average | 8.13 | 8.79 | 4.27 | 4.62 | .050 | .053 |

Calcium determinations were made on pooled samples of eggs from the 2 groups and in order to obtain a representative sample, the eggs were broken and stirred for 10 minutes with an electric stirrer before samples were taken for analysis. The contents of eggs produced by Group I had an average analysis of .050% calcium and the eggs produced by Group II had an average of .053% calcium for the 8 months during which these determinations were made.

Since it was observed that the calcium level of the egg contents was somewhat higher in the group receiving calcium gluconate, it

was thought desirable to determine the relative amounts of thick and thin albumen present in the eggs produced by the 2 groups of birds. This was done using the method and apparatus described by Holst and Almquist.⁴

Group I produced an average of 105 eggs per hen during the year, with an average weight of 50.1 gm. per egg, while Group II in the same time produced an average of 115 eggs with an average weight of 50.5 gm.

Seven birds out of the 12 in Group I that were started in the second part of the experiment survived and 8 birds of the 12 that were started in Group II were alive at the completion of the study. All hens were individually mated and observations made as to the fertility and hatchability of eggs produced by the 2 groups. The fertility in both groups was quite low but no difference between the groups was noted. The hatchability of fertile eggs was satisfactory for both groups. Because of the small number of birds involved, the hatchability data are not presented.

In the presence of ample vitamin D, calcium gluconate seems to function as well as calcium carbonate as a source of calcium for the growing chick. The same is true when these supplements are supplied in rations where vitamin D is limited. The birds receiving calcium gluconate grew slightly better than those receiving calcium carbonate. In rations fed to the laying hen, where vitamin D was supplied in quantity less than the optimal, calcium gluconate appears to be somewhat more efficient than calcium carbonate in the formation of egg shell, as evidenced by greater weight of egg shell and a higher percentage of shell ash. Calcium gluconate under the conditions involved in these experiments also caused a greater deposition of calcium in the inner parts of the egg. It has been generally believed that the calcium content of the interior parts of the egg is constant and not influenced by feeding or other treatment of the hens, but Hughes and associates⁵ were able to raise the calcium level of the egg contents by irradiating the hens producing the eggs.

During the first few months that observations were made on the amount of thick white present, it appeared that the eggs produced on the calcium gluconate ration had a higher percentage of thick white than those produced on the calcium carbonate ration. This difference tended to lessen as the study proceeded and the average

⁴ Holst, W. F., and Almquist, H. J., *Hilgardia* 6, 48, 1931.

⁵ Hughes, J. S., Payne, L. F., and Latshaw, W. L., *Poultry Science*, 1923, 3, 151.

percentage of thick whites from the 2 groups was not substantially different when calculated for the entire period.

Due to differences in the calcium content of the 2 salts studied, it was necessary to use more than 3 times as much gluconate as carbonate to maintain an equal calcium level. The use of calcium gluconate in such amounts as were used in this study is likely to be impractical from a cost standpoint, but it would be desirable to determine whether or not the gluconate has a supplemental effect when the major part of the calcium in the ration is furnished by calcium carbonate or some other source of calcium. The level of vitamin D was decidedly lower than the optimum level and it is possible that the birds could function as well as they did under the conditions of this experiment, if lower levels of calcium were used in the presence of ample vitamin D.

The number of birds involved in the egg production studies was quite small but the differences observed in shell, shell ash and calcium level of the egg contents seem to be significant.

Summary. Calcium carbonate and calcium gluconate seem to function with equal efficiency as sources of calcium for the growing chick when equivalent amounts of calcium are supplied. With the laying hen the calcium gluconate seems to function slightly more efficiently than the carbonate, as evidenced by increased egg shell, shell ash, and an increase of calcium in the egg contents.

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The in vivo Action of Staphylococcus Bacteriophage in Presence of Staphylococcus Antitoxin.

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In a previous report¹ it was shown, as it has been by others, that normal rabbit serum in broth inhibits the action of staphylococcus bacteriophage on staphylococci. However, when rabbit serum, immune to staphylococcus toxin was used in the place of normal serum, the bacteriophage remained active and subcultures taken from the higher concentrations of bacteriophage were sterile. This finding suggested that a combination of staphylococcus antitoxin

¹ Burky, E. L., *J. Immunology*, 1933, **24**, 513.