CALCIUM AND PHOSPHORUS IN THE DEVELOPMENT OF THE TURKEY EMBRYO*

By

W. M. INSKO, JR. AND MALCOLM LYONS

(*From the Kentucky Agricultural Experiment Station, Lexington, Kentucky*)

Received for Publication—October 31, 1932

PROGRESS in the determination of causes of embryo mortality seems to be dependent in a measure upon accurate knowledge of the metabolism of the developing embryo. A review of the literature of this subject is given by Needham (1).

EXPERIMENTAL METHOD

The Bronze turkey eggs used in this experiment were produced at the Western Kentucky Experiment Substation at Princeton, Kentucky. They were collected for a period of thirteen days, shipped to Lexington, Kentucky, and placed in an electric (Petersime) incubator. The average temperature was 99.75°F. throughout the period of incubation with a relative humidity of 64 per cent. All eggs were turned four times daily. At twenty-four hour intervals, six eggs weighing 80 to 90 grams each were removed and stored in a refrigeration room at 28 to 30°F. This effectively stopped embryonic development and preserved the eggs until analyzed at a later date. The embryos were removed from the eggs, compared with previously standardized specimens, freed of all adhering yolk, including that within the body cavity, dried on filter paper, transferred to silica dishes, weighed, and dried to a constant weight in an electric oven at 100C.° The dry embryos were burned to a white ash, which was dissolved in hydrochloric acid, made to a definite volume with distilled water, and calcium and phosphorus determined in separate aliquots. Calcium was determined by the McCrudden volumetric method (2) and phosphorus by the volumetric method of the Association of Official Agricultural Chemists (3).

RESULTS AND DISCUSSION

The present study of the Bronze turkey embryo is concerned with: 1.—embryonic growth as measured by wet weight, dry weight, and ash content; 2.—percentage of moisture; 3.—growth cycles; and 4.—calcium

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

and phosphorus content. Comparisons of these studies will be made with those reported on the chick embryo.

The data covering the growth of the turkey embryo as measured by wet weight, dry weight, per cent moisture, and ash content, are given in Table I and Figure 1. The daily increase in wet weight, dry weight, and ash content is very small until the twelfth day after which the increments of growth noticeably increase. As shown in Figures 1 and 2, embryonic growth is divided into three distinct phases or cycles with definite periods of retardation between the ninth and tenth days and between the nineteenth and twentieth days. These periods of retardation appear at approximately the same relative time as those appearing on the ninth and sixteenth days for the chick embryo. Needham (*loc. cit.*) has shown that during the early period of incubation up to and including the seventh day, the chick embryo uses carbohydrate as the source of energy. This period corresponds to the first nine days of the turkey embryo's life and it will

TABLE I

GROWTH, MOISTURE AND ASH CONTENT OF THE TURKEY EMBRYO

Day	Average weight of eggs	Number of embryos	Wet weight grams	Dry weight grams	Per cent moisture	Ash content grams
5	84.2	5	.0156	.0050	67.95	
6	84.5	2	.0330	.0104	68.48	
7	83.5	4	.0797	.0204	74.40	.0004
8	83.7	3	.1903	.0279	85.34	.0013
9	84.5	2	.5121	.0586	88.56	.0044
10	86.3	3	.5823	.0576	90.11	.0043
11	82.5	2	.9492	.0719	92.43	.0085
12	82.5	4	1.7670	.1583	91.04	.0138
13	83.0	5	3.9664	.2719	93.15	.0269
14	82.7	6	4.4310	.3715	91.62	.0319
15	83.7	3	5.6975	.6530	88.54	.0523
16	85.6	5	6.2538	.9141	85.38	.0669
17	84.8	6	7.0468	1.2354	82.47	.0865
18	81.0	2	12.1482	2.2035	81.86	.1501
19	83.7	3	15.4144	3.1686	79.44	.1923
20	84.5	4	16.5000	3.2900	80.09	.2175
21	83.5	4	23.8900	4.8200	79.81	.3250
22	84.0	4	27.5100	5.8200	78.86	.3950
23	85.8	5	33.2100	6.8600	79.35	.4900
24	83.4	5	36.9300	7.8900	78.63	.5840
25	85.0	5	44.3600	9.2900	79.05	.6900
26	84.2	5	49.3600	11.3700	76.96	.7920

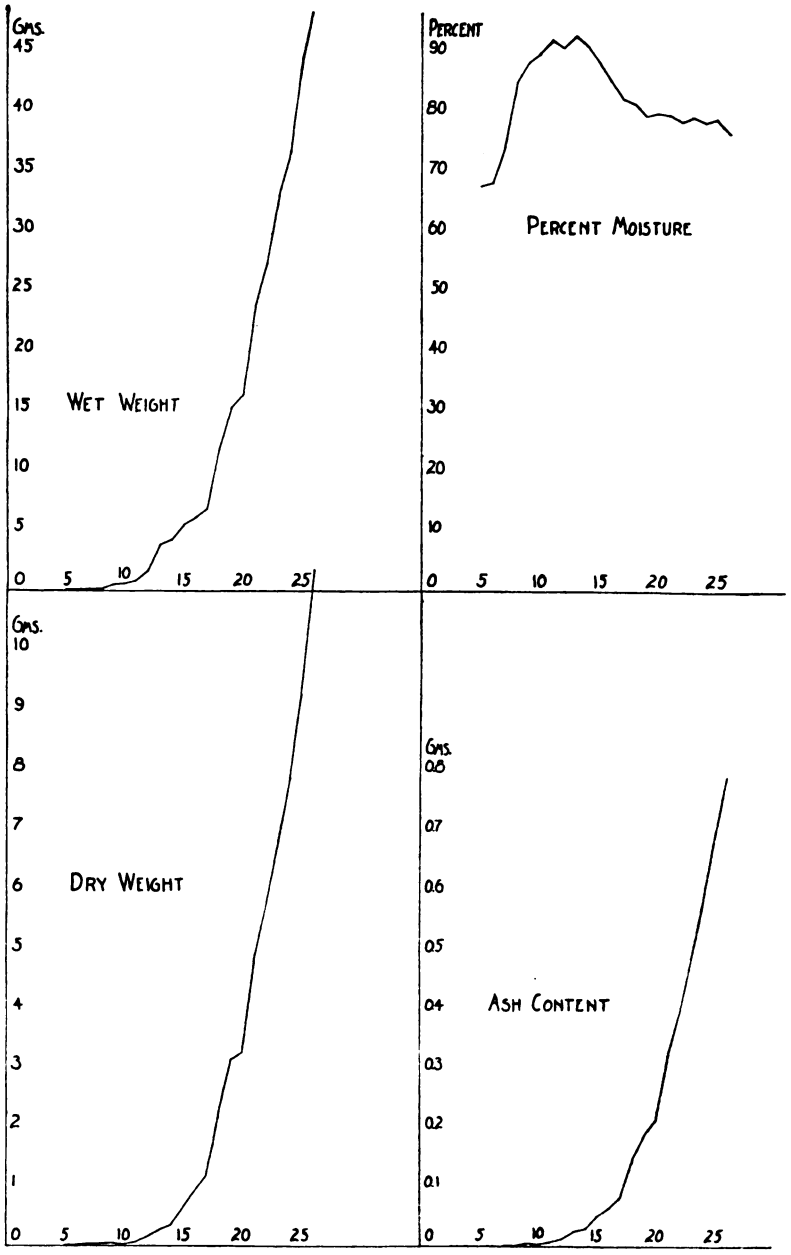

FIG. 1.—The Growth of the Bronze Turkey Embryo as Measured by: Wet Weight, Ash Content and Per Cent of Moisture.

be noted that the retardation of growth between the ninth and tenth days probably marks the limit of this phase of embryonic metabolism. It is especially noticeable that the percentage rate of gain in dry matter (Figure 2) is 71.0 from the eighth to the ninth day and −1.7 per cent from the

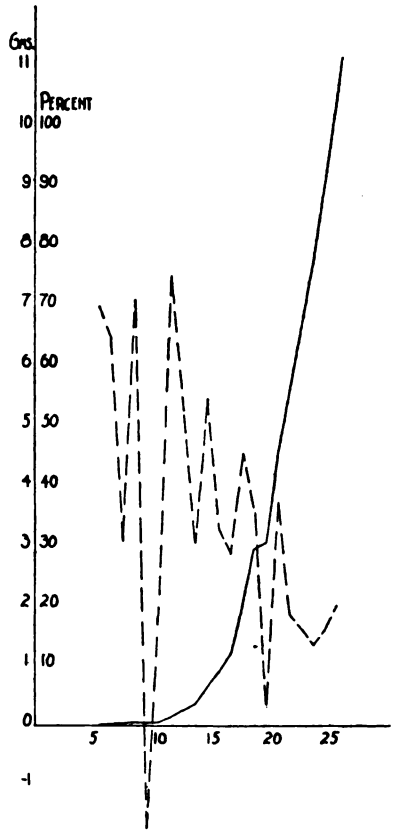

FIG. 2.—Cumulative Gain and Percentage Rate of Gain Turkey Embryo.

ninth to the tenth day. The formula used for the calculation of the percentage rate of growth was a modification of Minot's formula proposed by Brody (4). Percentage rate of gain $= 100 \frac{(W_2 - W_1)}{\frac{1}{2}(W_1 + W_2)}$ in which W_1 = weight at the beginning of the period and W_2 = the weight at the end of the period.

The second period of retardation which occurs between the nineteenth and twentieth days gives a definite decrease in percentage rate of gain, and a decided pause in dry weight, wet weight, and ash content. Needham has discussed the utilization of protein during the period from the seventh

to the sixteenth day of incubation and the metabolization of fat thereafter. The break in the curve of the turkey embryo corresponds to the change in materials metabolized. This break in the curve of the chick coincides with the peak of embryonic mortality as shown by Payne (5). Mortality curves have not as yet been given for turkey embryos.

The percentage of moisture in the turkey embryo was found to be the lowest between the fifth and seventh days of incubation. The moisture content at this period was approximately that of the content of the egg. The moisture content of the embryo increases rapidly from the sixth to the eleventh day of incubation and remains fairly constant from the eleventh to the fourteenth day. After this time the percentage of moisture declines steadily to the nineteenth day and remains rather constant until the twenty-fifth day.

The data on the calcium and phosphorus content of the turkey embryo are presented in Table II and Figure 3. The total amount of calcium gives a somewhat similar picture to that presented by the growth curves of the embryo. The increase in calcium content is relatively slow until the seventeenth day, when the growth becomes more rapid with the exception of the

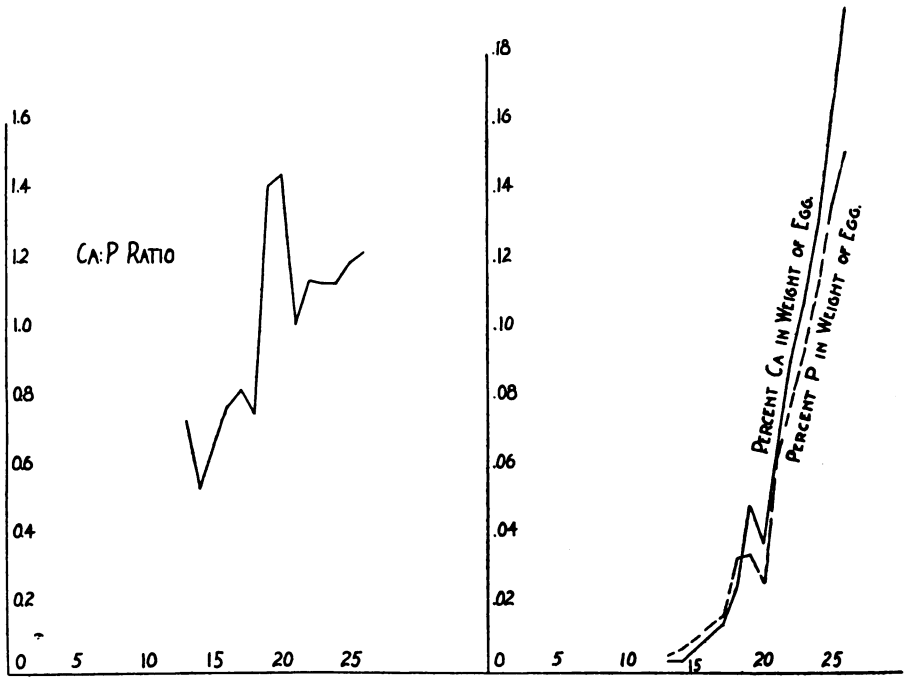

FIG. 3.—Calcium and Phosphorus in the Developing Turkey Embryo.

decrease from the nineteenth to the twentieth day. The rapid increase in calcium content is resumed after the twentieth day. The phosphorus curve follows very closely that of calcium, with the same decrease from the nineteenth to twentieth day. The calcium-phosphorus ratio with phosphorus remaining constant at 1.0 is shown in Figure 3 and Table II. Until the

TABLE II

CALCIUM AND PHOSPHORUS CONTENT OF TURKEY EMBRYOS

Day	Calcium (Ca) grams	Phosphorus (P) grams	Ca:P ratio
13	.0031	.0042	.74:1
14	.0031	.0057	.54:1
15	.0057	.0085	.67:1
16	.0092	.0118	.78:1
17	.0119	.0143	.83:1
18	.0207	.0274	.76:1
19	.0409	.0289	1.42:1
20	.0321	.0222	1.45:1
21	.0543	.0534	1.02:1
22	.0764	.0664	1.15:1
23	.0928	.0812	1.14:1
24	.1081	.0948	1.14:1
25	.1388	.1160	1.20:1
26	.1632	.1327	1.23:1

nineteenth day the value of calcium is below 1.0 while after that time the value is more than 1.0. The calcium-phosphorus ratio during the latter period of incubation (approximately 1.2 Ca:1.0 P) may be an indication of the proper ratio in the diet of newly hatched turkeys.

The ratio between the calcium and phosphorus in the embryo may suggest the manner in which these elements are combined as the embryo develops. For example, the ratio between the calcium and phosphorus in monocalcium phosphate ($CaH_4P_2O_8$) is 0.65:1. This closely approximates the ratio in the embryo up to the nineteenth day. On and after the nineteenth day the ratio is more nearly that of dicalcium phosphate ($CaHPO_4$) or 1.29:1. In this connection it is interesting to note that Romanoff and Romanoff (6) found a marked lowering of the pH value of egg yolk on the sixteenth day of incubation of the hen's egg. This corresponds approximately to the nineteenth day of the life of the turkey embryo. This abrupt change from an alkaline to an acid state and the return to alkalinity is explained by them as follows, "The above-mentioned sudden drop in pH

value under both natural and artificial incubation is possibly related to the natural depression of growth in the life span of the embryo." It is possible, however, that this change of pH is ascribable to a sudden change in the form of the calcium-phosphorus compound present, as discussed above.

Summary

1. A study was made of 1.—embryonic growth as measured by wet weight, dry weight and ash content; 2.—per cent of moisture; 3.—growth cycles; 4.—calcium and phosphorus content of the Bronze turkey embryo.

2. The daily increase in wet weight, dry weight, and ash content was very small until the twelfth day, after which time increments of growth noticeably increase.

3. The increase in calcium and phosphorus content was relatively slow until the seventeenth day.

4. The calcium-phosphorus ratio was less than 1.0 until the nineteenth day of incubation, after which time it exceeded 1.0.

5. Turkey embryo growth is divided into three distinct phases or cycles, with definite periods of retardation between the ninth and tenth and between the nineteenth and twentieth days. These periods of retardation were also noted in the calcium and phosphorus content of the embryo. Certain correlations between these periods of retardation and the change in the type of food metabolized are suggested.

6. It is suggested that the calcium content of the embryo may be used as a measure of growth provided the yolk material drawn into the body cavity is removed before analysis.

References

1. Needham, Joseph, Chemical Embryology, New York, 1931.
2. McCrudden, F. H., *Jour. Biol. Chem.* 1909, **7**, 83.
3. Official and Tentative Methods of Analysis of the A. O. A. C. 1920, 3.
4. Brody, S., *Missouri Agr. Exp. Sta. Res. Bull.* **97**, 1927, 13.
5. Payne, L. F., *Amer. Assn. Instr. and Inves. in Poultry Husb.* 1919, **6**, 9.
6. Romanoff, A. L., and Romanoff, A. J., *Biol. Bull.* 1929, **57**, 300.