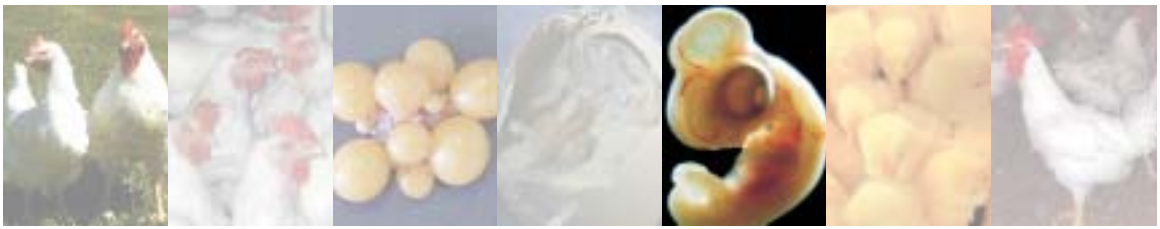

47 Examining the Embryonic Metabolism of Short and Long Term Stored Eggs

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The Problem

Although cool storage of fertile hatching eggs prior to incubation is a common practice, problems occur when the length of storage exceeds one week. As shown in Chapter 46, embryos of broiler eggs that are stored for 14 days have a different developmental rate then embryos of eggs stored for 4 days; the data proved that the embryos from 4 day stored eggs developed faster than embryos from 14 day stored eggs. This being the case, the embryos from 4 day stored eggs may have a different metabolic rate during incubation. Thus, longer incubation times may be required for eggs that are cool stored for extended periods (Mather and Laughlin,

Mather, C M and K F Laughlin. 1976. British Poultry Science 17:471-479.

Romanoff, A L. (1967) Biochemistry of The Avian Embryo. John Wiley & Sons, New York.

Vleck, C M, D F Hoyt, and D Vleck. 1979. Physiological Zoology 52:363-377.

1976).

Oxygen consumption can be used as a means of measuring the metabolism of an avian (bird) embryo (Vleck *et al.*, 1979). Romanoff (1967) stated that "the consumption of oxygen may actually be considered as a measure of embryonic life". Oxygen consumption values have often been used for calculating the metabolic rates and the physical activities of the embryo. Romanoff (1967) used a respiratory quotient of 0.84 to relate oxygen consumption and carbon dioxide production during metabolism of the chick embryo.

Metabolism: The sum of all the biochemical reactions in an organism's cells and tissues that allow the organism to keep growing and functioning. These reactions include processes that build and maintain the body of the animal through energy formation (anabolism) and processes that release energy by breaking down substances (catabolism). When the cell utilizes nutrients (mostly compounds containing carbon (C)), oxygen (O_2) is required to break down these compounds into carbon dioxide (CO_2), water (H_2O), and energy. This is also known as cellular respiration.

Our Objectives

- To design a monitoring system capable of measuring the carbon dioxide (CO_2) production of broiler embryos during incubation.
- To compare the embryo output of carbon dioxide to determine if there are metabolic differences between the embryos of eggs stored for 4 and 15 days.

Our Approach

Egg Collection and Storage

A total of 80 fresh eggs were collected from a commercial flock of broiler breeders. All the eggs were weighed and stored for 14 days. At 10 days of storage, a second group of 80 freshly laid eggs were obtained from the same breeder and treated in the same manner. This group was stored for 3 days. After storage of the two groups of eggs was complete, the eggs were again weighed and 16 eggs per each storage treatment group were selected. The fresh egg weight range for the 4 day stored eggs was 64.7-66.7 g, while the weight range for the 15 day stored eggs was 64.4-66.8 g.

Four of the metabolic chambers that held individually incubating eggs.

Embryonic Developmental Staging

A sub-sample of eggs (n=40) from each storage treatment group was set aside so that the embryonic development of 20 embryos could be established using the staging technique described by Eyal-Giladi and Kochav (1976).

Egg Incubation

Ten of the eggs per each storage treatment group were set in individual 350 mL airtight metabolic chambers that were housed inside an incubator (Figure 1). Two infertile eggs (within ±1 g of the experimental eggs collected) from virgin Leghorn hens were also placed into two of

the metabolic chambers. The CO_2 produced from these two eggs were to be used as control values. Two of the metabolic chambers were also kept empty so that the CO_2 levels of the air inside the incubator could be measured.

Respiratory Quotient: The ratio of the volume of carbon dioxide produced to the volume of oxygen taken up. During the breakdown of food to produce energy, oxygen is needed and carbon dioxide is produced. The respiratory quotient (RQ) is usually about 0.8 because more oxygen is taken up than the amount of carbon dioxide produced during the break down of food.

Measuring Carbon Dioxide Production

Figure 1 illustrates the equipment used in the present study. Each chamber was connected to a CO_2 /H_2O analyzer by tubing. The CO_2 analyzer was calibrated and a small pump was used to move the air from the metabolic chambers through the CO_2 analyzer. The air flow through the analyzer was set at 0.3 L/minute. An airflow meter was used to calibrate the airflow through each metabolic chamber and a vacuum/compressor pump drew air through the metabolic chambers.

Solenoid activated valves were use to switch the airflow between the metabolic chambers and the CO_2 analyzer. Every valve directed the metabolic chamber exchange air to the CO_2 analyzer once an hour.

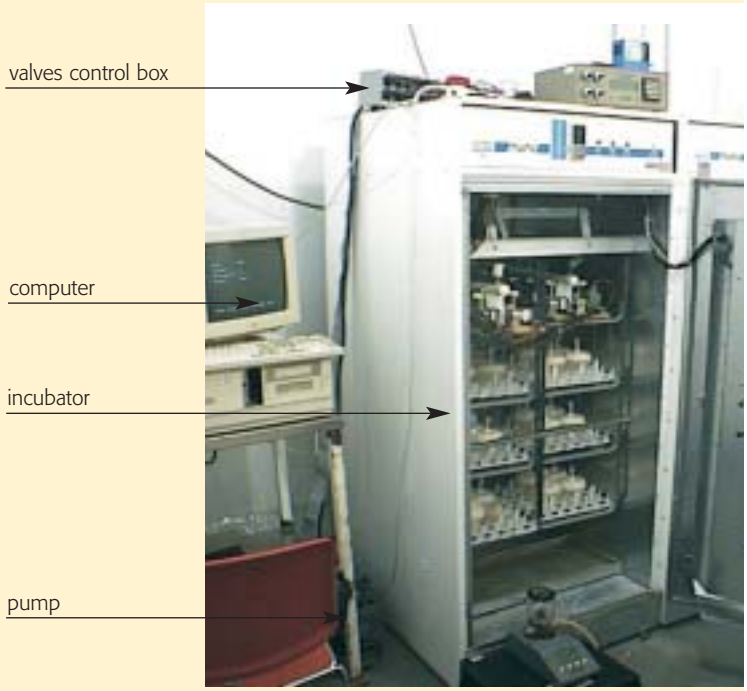
The units of the CO_2 analyzer readings are µ-mole/mole or parts per million (ppm). To facilitate the calculations, averages of the CO_2 (in ppm) that were given off (respired) each day per egg were made from the difference between values of the measurements from the metabolic chambers, and the CO_2 values from the empty metabolic chambers. In order to determine oxygen consumption, a respiratory quotient (RQ) of 0.84 was used. The oxygen consumption was calculated by dividing the CO_2 output by the respiratory quotient.

Embryo and Yolk Sac Weights

After 448 hours of incubation all eggs were removed from the metabolic chambers and weighed. The eggs were broken open, the embryos euthanized via decapitation and then detached from the yolk sacs. The wet yolk sac and embryo weights were obtained prior to placement of the embryos and yolk sacs in a drying oven at 64°C. After 5 days in the drying oven the embryos and yolk sacs were re-weighed to determine dry matter content. The experiment was repeated beginning 2 weeks after the completion of the first trial.

How do chick embryos breathe?
Although the eggshell appears to be a solid structure, there are microscopic holes called pores in the eggshell. Oxygen travels from the environment outside of the egg through the pores of the shell, to the inside of the egg where the concentration of oxygen is not as high. This process is called diffusion. Carbon dioxide and water, which are in high concentrations inside the egg, diffuse through the pores of the shell into the environment outside of the shell where these compounds are not as plentiful. A membrane that surrounds the inside surface of the eggshell (chorioallantoic membrane) acts like the lung of the embryo exchanging oxygen for carbon dioxide and water vapor. (See Rahn, H., Ar., A. and Paganelli, C. 1979. Scientific American 240(2):46-55 for a review.)

Figure 2
Carbon dioxide monitoring equipment



Our Observations

Embryonic Development

There was no effect of the storage treatments on embryonic stage of development immediately after storage (data not shown). This result, that embryonic development, as measured by the staging technique of Eyal-Giladi and Kochav (1976), does not advance during storage. This was an important factor to verify prior to incubation to establish that embryos from both the 4 and 15 day storage treatments were entering the incubator at the same stages of embryonic development.

Eyal-Giladi, H and S Kochav. 1976. Developmental Biology 49:321-337.

Embryo Weights

Wet embryo weight at the time of sampling was greater in embryos of eggs stored for 4 versus 15 days (Table 1). This was expected and was in agreement with the results of the study presented in chapter 45. Dry embryo weight did not differ between the two storage treatments (although significance was approached at P=0.07). This result showed

Table 1
Egg, embryo, and yolk sac weights from eggs stored for 4 or 15 days prior to incubation.[1]

Parameter measured	Eggs stored for 4 days[2]	Eggs stored for 15 days[2]
Fresh egg weight (g)	65.6[2a]	65.7[a]
Egg weight at embryo sampling[4] (g)	59.1[a]	58.8[a]
Egg weight loss during incubation (%)	9.7[a]	9.9[a]
Wet embryo weight at sampling (g)	27.0[a]	25.0[b]
Wet yolk sac weight at sampling (g)	12.8[a]	12.7[a]
Dry embryo weight (g)	4.9[a]	4.5[a]
Dry yolk sac weight (g)	6.9[a]	6.9[a]

[a,b]Means within a row with no common letter differ significantly (P<0.05).
[1]n=20 eggs per each storage treatment group.
[2]Least square mean.

Statistical Analysis
Embryonic developmental data were analyzed using Fishers Exact Test (SAS Institute, 1992. The SAS® System for windows 3.10 Release 6.08., Cary NC.) The egg and embryo weight data were analyzed using the General Linear Models procedure on SAS (SAS Institute, 1992). All percent data were transformed using Arc-Sine Transformations prior to analysis (SAS Institute, 1992). Least square means and standard error of means were calculated for egg and embryo weights. Significance was measured at $P < 0.05$. When the model established significant differences, the means were separated using the Pdiff procedure on SAS (SAS Institute, 1992). Means shown in tables with different superscripts are significantly different.

that the moisture content, and not the dry matter content, made up the difference between the embryo weights from the two egg storage treatments (Table 1). Both wet and dry yolk sac weights were not significantly different between the two storage treatment groups (Table 1).

Carbon Dioxide Production

The mechanical equipment that measured CO_2 in the current study functioned well, and the values for both CO_2 (Figure 3) and O_2 (data not shown) compared well to values previously published by Romanoff (1967).

All eggs showed relatively low CO_2 production during the first 6 days of incubation (Figure 2). After day six, the CO_2 production increased linearly until 10 days. This trend was the same for incubating eggs from both treatments and the increase in CO_2 production is a result of the increased tissue production of the embryo during these periods. After day 11, the slope of the CO_2 production curve began to increases sharply until day 16. Eggs that were stored for 4 days had a significantly higher average CO_2 production from incubation days 4 to 18 when compared to the eggs that were stored for 15 days (Figure 2). These differences in CO_2 production continued to increase until 18 days of incubation. At day 18 of incubation the average CO_2 output was 473 mL/day of CO_2 for the 15 day stored eggs versus 487 mL/day for the eggs stored for 4 days (Figure 2).

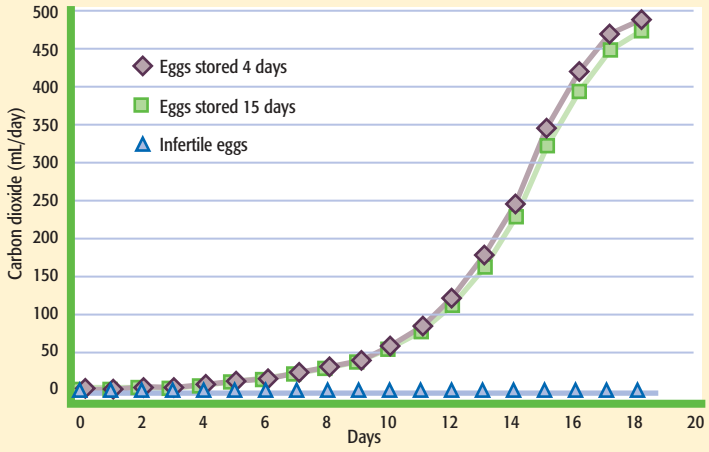
Romanoff, A L. 1967. Biochemistry of The Avian Embryo. John Wiley & Sons, New York.

Lewis, P D, T R Morris, and G C Perry. 1999 Poultry Science 78: 1227-1231.

Renema, R A, F E Robinson. 2001. Poultry Science 80:1112-1120.

Renema, R A, F E Robinson, J J R Feddes, G M Fasenko, and M J Zuidhof. 2001. Poultry Science 80:1121-1131.

Figure 2
Carbon dioxide production of incubated infertile eggs and fertile eggs stored for 4 or 15 days before incubation. From incubation days 4 to 18, carbon dioxide production was significantly higher in the 4 versus the 15 day stored eggs.



What Does It Mean?

- Even though the embryonic development of the two storage treatment groups was the same at the time the eggs were placed into the incubator, after 448 hours of incubation, the embryo weights of eggs stored for 15 day were significantly lower than the embryo weights of eggs stored for 4 day.
- The equipment used in the present study to measure CO_2 production worked well and the data recorded on CO_2 production compared well to values previously published.
- There are significant CO_2 production differences, beginning as early as 4 days of incubation, between eggs stored for 4 or 15 days. As expected, the eggs stored for 4 days had higher CO_2 output levels than eggs stored for 15 days. This means that embryos from eggs stored for 4 days had a higher metabolic rate and were growing faster than the embryos from eggs stored for 15 days.
- Future research examining possibilities to accelerate the metabolism of embryos of eggs stored for long periods may help alleviate the dilemma of egg storage and the extended incubation times associated with storage.