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# Livability of Leghorn balut embryos stored under varying temperatures and storage times

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*Joyce Jong\* and F. Dustan Clark§*

## ABSTRACT

Baluts are fertile chicken or duck eggs that have been incubated and removed from the incubator prior to hatching for consumption. Chicken eggs are incubated for 11 to 14 days and duck eggs are incubated for 16 to 20 days. Baluts have an extremely specialized consumer market, with the majority of its consumers of Filipino decent. Current U.S. Department of Agriculture regulations for the storage of baluts prior to sale is 7.2°C, the same as for infertile commercial table eggs. Consumer preference is to purchase live baluts for consumption. Since exposure to 7.2°C causes embryo mortality within 8 hours of removal from the incubator, research was performed to assess mortality at various storage temperatures, which has not previously been established. Our study consisted of two identical trials to determine the livability of embryos when exposed to varying temperatures over predetermined storage times. Fertile Leghorn chicken eggs were incubated for 13.5 days and then removed from incubation, grouped, and placed in temperature-controlled environments corresponding to 15.6, 18.3, and 22.2°C. At predetermined times, eggs were opened to determine embryo viability. Random swab samples of the internal egg environment were also taken aseptically to determine the presence of microorganisms. Results demonstrated that the livability of embryos was longer when exposed to storage temperatures closer to incubation temperatures (37.5°C), and livability was shorter when storage temperatures neared refrigeration temperature (7.2°C).

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## INTRODUCTION

Baluts are fertile chicken or duck eggs that have been incubated and removed from the incubator prior to hatching for consumption. Chicken eggs are usually incubated for 11 to 14 days; whereas, ducks are incubated for 16 to 20 days. Under favorable incubation conditions, chicks hatch at approximately 21 days of incubation. Ducklings hatch between 26 and 30 days of incubation, depending on the breed.

Baluts have an extremely specialized consumer market, the majority of which are of Filipino descent. Doreen Fernandez, author of "The World of Baluts," is quoted as saying, "Whoever discovered balut stumbled onto the fact that food has changing excellence (taste, texture) as it evolves and develops. Thus between the egg and the full-grown duck [or chicken], there are stages that bear exploring—and eating. And the Filipino has explored them and evolved the culture of balut." (Magat, 1997) Others known to consume this product include Asian immigrant groups such as Vietnamese, Cambodians, Laotians, Thais, Malays, Indonesians, and Chinese.

Prior to consumption, baluts are boiled for 20 to 30 minutes. Edible parts include the embryo, yolk, and amniotic fluid (also referred to as the "soup") (Fig. 1). Baluts are believed to hold medicinal value and are often looked upon as an aphrodisiac. Consumers prefer to purchase live embryos, which have a better flavor, a "sweetness" to the "soup." Baluts containing dead embryos prior to cooking tend to have a more bland or slight bitter taste, depending on how long the embryos have been dead.

Current U.S. Department of Agriculture regulations mandate baluts be refrigerated at 7.2°C prior to purchase. "Baluts are potentially hazardous food and must be refrigerated upon removal from incubation and maintained at a refrigerated temperature of 7.2°C, or less, while transported, stored, or held for retail sale" (Besulieu, 1991).

In a pilot study we conducted, balut embryos removed from incubation after 13.5 days and immediately stored at 7.2°C resulted in embryonic death within 8 hours of storage (unpublished data). Before that study, the livability of embryos removed from incubation prior to hatch and exposed to various storage temperatures had not been established.

## Meet the Student-Author

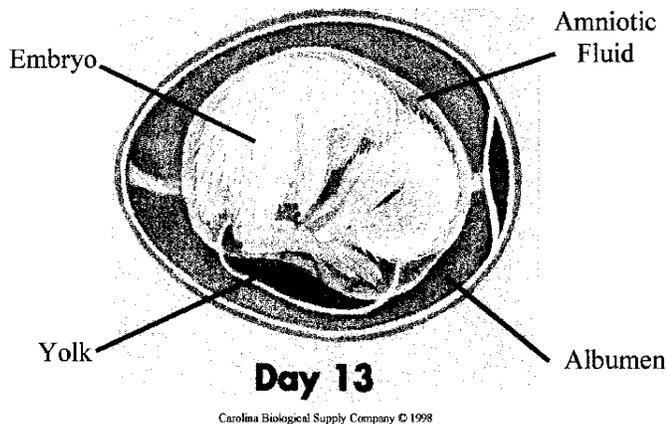
In May 2000, I received my B.S. degree in poultry science with a minor in agricultural business management. During my undergraduate years, I had a number of opportunities to participate in a variety of presentations. In 1998, I placed first in a student presentation competition hosted by the Pacific Egg and Poultry Association, in addition to receiving an award of merit. In 1999 and 2000, I won first place in the undergraduate category of the student paper competitions hosted by Gamma Sigma Delta. In addition, this year the Department of Poultry Science awarded me the Outstanding Undergraduate Award. In the fall, I will be attending the University of California, Davis, to begin work on a master's degree in agriculture and management. I am excited about the many opportunities the poultry industry offers and eager to pursue them after graduation.

This project provided a chance for me to conduct a research project from start to finish. It opened the doors for me to participate in a paper/presentation competition, as well as to write a paper for publication. As a result, I have gained a greater respect for the research process and will use what I have learned in various future applications.

My family is involved in the production of poultry products and was thinking about producing baluts. Prior to our initial production, I had the task of researching the regulations regarding them. In the process, I discovered that there are no available data on balut livability after they are removed from the incubator. This is an important factor in their marketability. The data from my research provided me with insight into how long embryos will live outside the incubator when exposed to various temperatures. My research also serves as a basis for future balut research. The ultimate goal is to provide scientific data for producers and regulatory agencies in order to influence current regulations to better reflect the product itself as well as the production and marketing.



*Joyce Jong*



**Fig. 1. Four parts of a balut embryo at 13 days of incubation.**

The purpose of this research was to determine livability of chicken balut embryos incubated for 13.5 days and stored at various temperatures and periods of time. In addition, the presence of microorganisms in the egg was determined. Based on knowledge gained from the pilot study, it was hypothesized that the livability of balut embryos would vary in relation to the storage temperature; livability would increase as storage temperatures increased and vice versa. In addition, it was hypothesized that few microorganisms would be detected.

### MATERIALS AND METHODS

Two identical trials were conducted. Both trials contained over 630 fertile Leghorn chicken eggs, which were obtained from a hatchery in Lincoln, Ark., with eggs supplied by Hy-Line International (West Des Moines, Ia.). These eggs were incubated for 13.5 days in a Jamesway incubator (Model 252B, Jamesway Incubator Co., Ltd., Cambridge, Ontario) at 37.5°C. Eggs were candled (a process in which a bright light is shone through the egg to view its internal contents) at the end of the 13.5 day incubation period. All unsuitable eggs (infertile eggs and eggs containing embryos that died at early stages of development) were removed and disposed of, and the remaining eggs containing live embryos were randomly divided into three groups consisting of 210 eggs per group. Each group of eggs was stored at temperatures corresponding to 15.6, 18.3, and 22.2°C. Thirty eggs from each storage temperature group were removed and opened to determine embryo livability by

visual observation (a process referred to as “breakout”) after 8, 12, 18, 22, 26, 30, and 34 hours of storage. In addition, 10 swab samples were aseptically taken from randomly selected embryos of each group at each breakout interval. Swabs were inoculated onto blood (5% sheep red blood cells) and MacConkey agar plates and incubated at 36.7°C for bacterial isolation attempts. The number of live versus dead embryos was documented for each temperature group and at each time interval of examination.

## RESULTS AND DISCUSSION

### Embryo Livability

In trial 1, the embryos stored at the target temperature of 15.6°C were actually stored at an average temperature of 16.6°C. In the first three breakout intervals, the percentage of live embryos exceeded the percentage of dead embryos. After the 18 hours of storage, no embryos survived.

Results of the second group of embryos, stored at an average of 18.3°C in the first trial, showed that embryos were alive throughout the entire storage interval from 8 to 34 hours. In the first four breakout intervals, the percentage of live embryos exceeded that of dead embryos, and in the last four breakout intervals the percentage of dead embryos exceeded that of live embryos.

In all breakout intervals of embryos stored at an average temperature of 37.5°C, the percentage of live embryos surpassed that of dead embryos, except at the breakout interval during the 22nd hour. During that interval, the percentage of dead embryos exceeded the percentage of live embryos.

In the second trial, embryos stored at 15.6°C were actually stored at an average temperature of 16.5°C. For the first breakout, percentage of dead embryos exceeded the percentage of live embryos. After 8 hours of storage, no embryos remained alive.

For all breakout intervals of embryos stored at an average temperature of 18.3°C in the second trial, the percentage of dead embryos exceeded that of live embryos. At the 18- and 34- hour breakout intervals, no live embryos were observed.

For the average storage temperature of 22.2°C in the second trial, live embryos were observed in all breakout intervals. The percentage of live embryos was less than the percentage of dead embryos at all breakouts except at 12 and 22 hours of storage, where the percentage of live embryos was the majority.

In both trials, there was a decreasing pattern of embryonic death. For both trials, the percentage of live embryos was greatest when stored at 22.2°C, followed by 18.3, 16.6, and 16.5°C, with the lowest percentage of live embryos observed in a storage time interval over 34 hours (Figs. 2 and 3).

### Bacterial Isolates

Two species of bacteria were isolated from the collected swabs of the first trial, *Staphylococcus epidermidis* and *S. aureus*. The bacteria were isolated from 12.9% of the eggs sampled that had been stored at an average temperature of 16.6°C. No bacteria were isolated from eggs sampled from groups stored at average storage temperatures of 18.3 and 22.2°C. In the second trial, *Staphylococcus* species were also isolated from samples taken from eggs stored at average temperatures of 16.5 and 22.2°C, 6.2 and 9.0%, respectively. No bacteria were isolated from swab samples taken from eggs stored at an average temperature of 18.3°C.

In general, the results of both trials demonstrated that embryos held at 22.2°C tend to have the highest percentage of livability, followed by 18.3°C with the next highest livability, and finally 16.6 and 16.5°C, which had the lowest livability.

The graphic patterns of decreasing embryonic death for all storage groups in both trials may be the result of how the eggs were physically stored and the insulating capacity of the egg flats. In both trials, the height and

number of flats were identical.

An observation of the eggs and embryos of the second trial revealed that both egg size and embryo size were considerably smaller. Since the stage to which the embryos developed was not different in either trial, it is possible that the small embryos were the result of smaller egg sizes, which may account for the higher percentage of death resulting from their inability to retain heat at the same rate as the larger embryos of the first trial. "Low temperatures slow the development process as embryos are not completely homeothermic even by hatching time. Thus lowering environmental temperatures also lowers the embryo's temperature..." (Card and Nesheim, 1972).

An important factor that determines embryo livability outside of the incubation environment is referred to as "physiological zero," which is the "temperature below which embryonic growth is arrested, and above which it is initiated" (often cited in the range of 21.1 to 26.7°C) (North and Bell, 1990). "Developing embryos are extremely sensitive to [the] temperature of the environment. Some eggs will hatch if eggs are continuously maintained at a temperature between 35 and 40°C. Outside this range, essentially no eggs can be expected to hatch." Since development of the embryo will cease, heat production will cease, resulting in embryo death (Card and Nesheim, 1972).

*S. epidermidis* and *S. aureus* were isolated from a small percentage of the eggs sampled. It is likely that the presence of these organisms was the result of environ-

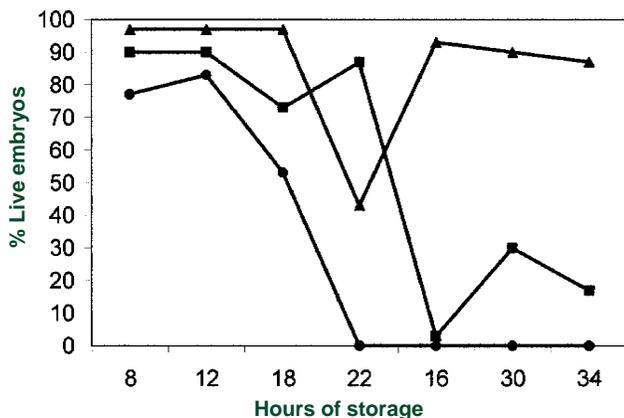


Fig. 2. Trial 1: comparisons of the percentage of live embryos at average storage temperatures of 16.6 (circles), 18.3 (squares), and 22.2°C (triangles).

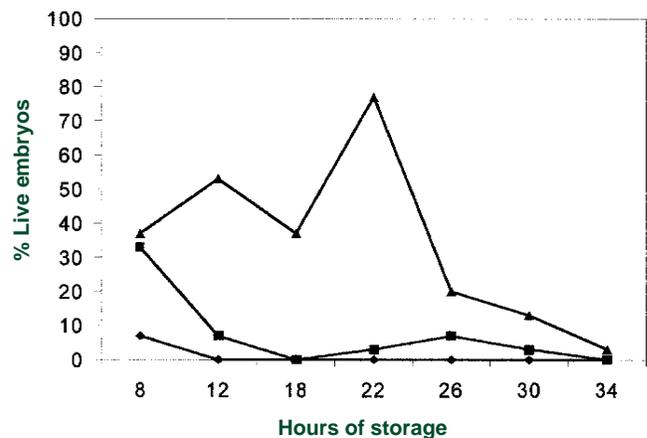


Fig. 3. Trial 2: Comparisons of the percentage of live embryos at average storage temperatures of 16.5 (diamonds), 18.3 (squares), and 22.2°C (triangles).

mental contamination when the eggs were opened. Prior to incubation, if an egg is contaminated with bacteria, the heat from the incubation environment and the yolk (an enriched medium) provide ideal conditions for bacterial proliferation. This proliferation would lead to an adverse affect on the embryo in the early development stages. In both trials, eggs containing “early dead” embryos were removed in the candling process.

In conclusion, livability of chicken embryos is correlated to the temperature at which they are stored. As the temperature nears that of refrigeration (7.2°C), embryo livability declines. Storage temperatures closer to incubation temperature (37.5°C) result in greater embryo livability. To prolong the life of the embryo after removal from the incubator, baluts need to be stored at temperatures greater than 7.2°C. Bacterial presence inside the eggs and/or baluts appears not to be problematic.

Subsequent trials are planned to determine a storage temperature for maximum livability. In addition, the

effects of the bacteria (such as *Staphylococcus* species and *Salmonella enteritis*) on the embryo during incubation and prior to cooking need to be further investigated. This information and that from subsequent research can be utilized by regulatory agencies and balut consumers to determine product safety.

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