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Parthenogenetic embryos from unfertilized Chinese painted quail eggs alter albumen pH, gases, and ion concentrations during incubation

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ABSTRACT

Parthenogenesis is a form of embryonic development that occurs without fertilization. Recently, parthenogenesis has been reported in Chinese painted quail eggs. In Japanese quail, it has been shown that albumen pH of incubated fertile eggs is lower than that of incubated infertile eggs. However, it is unknown if alterations, similar to those in incubated fertile eggs, occur in albumen pH, gases, or ion concentrations from unfertilized eggs exhibiting parthenogenetic development. Therefore, the objective of this study was to determine if any differences in pH, gases, or ion concentrations exist between incubated unfertilized eggs exhibiting parthenogenetic development versus unfertilized eggs with no development over incubation. In this study, eggs were collected daily from Chinese painted quail hens that were separated from males at 4 weeks of age, before sexual maturity. Eggs were stored for 0 to 3 days at 20 °C and incubated at 37.5 °C for 12 days. Eggs were weighed before and after incubation to obtain percentage egg weight loss. After incubation, embryo size and albumen O_2, CO_2, Ca^{2+}, Na^+, and Cl^- concentrations as well as pH were obtained from each incubated egg. Over incubation, albumen from unfertilized eggs exhibiting parthenogenetic development had a lower pH as well as less O_2 and Cl^-, yet a higher Ca^{2+} and Na^+ concentration as compared with the albumen of unfertilized eggs with no development. Also, eggs exhibiting parthenogenetic development had a higher albumen CO_2 concentration as compared with eggs without development. The rate of egg weight loss was much lower in eggs exhibiting parthenogenetic development as compared with eggs without development. Also, as parthenogen size increased, there was a decrease in albumen pH, O_2, and Cl^-, yet an increase in CO_2 and Ca^{2+}. In conclusion, it appears that, over incubation, parthenogenetic development from unfertilized eggs alters the composition of albumen as compared with the albumen from unfertilized eggs with no parthenogenetic development.

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1. Introduction

Parthenogenesis, embryonic development of an unfertilized egg [1], is a common form of reproduction in many invertebrates, such as scorpions [2,3], bees [4,5], and wasps [6]. In vertebrates, naturally occurring parthenogenesis has been reported in fish [7], amphibians [8–11], and reptiles [12,13]. Parthenogenesis has also been reported in birds, such as Beltsville Small White turkeys [14], chickens [15], zebra finches [16], and Chinese painted quail [17]. Interestingly, although parthenogenesis is a common form of successful invertebrate reproduction [1], for vertebrates such as birds, parthenogenesis most often results in an unorganized type of development that resembles early embryonic mortality in fertilized eggs [14].

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Several experiments have been conducted to study parthenogenesis in birds. However, little research exists that examines the impact of parthenogenetic development on internal components of the egg. For example, when using virgin quail hens, there was less incubational egg weight loss, presumably water, in quail eggs exhibiting parthenogenetic development at 10 days of incubation (DOI) as compared with eggs without development [18].

On the other hand, numerous studies indicate that embryonic development in fertilized eggs alters ionic composition of internal egg components. For example, Ono et al. [19] reported that albumen pH is lower in fertilized Japanese quail eggs as compared with unfertilized eggs when measuring albumen pH in fertile and unfertilized eggs at 24-hour intervals during the first week of incubation. Within 24 hours of oviposition, albumen pH was similar for both fertile and infertile eggs [20]. However, in fertile eggs after 2 DOI, the albumen pH decreased gradually over 7 DOI eggs; yet, during this same period, unfertilized eggs showed a constant increase in albumen pH from 2 to 7 DOI [20].

Additionally, egg albumen is the most dynamic component between the embryo and eggshell and plays an important role in gas exchange for the developing embryo [21]. For example, during the early stages of embryonic development in fertilized eggs, cells use O_2 to break down carbon components that consequently produces CO_2 that must traverse the albumen [22]. In fact, in fertile eggs, the utilization of O_2 increases over incubation because the embryo steadily consumes O_2, thus producing CO_2 during embryonic development [23].

Because egg albumen characteristics of fertilized eggs are altered during embryonic development, perhaps albumen from unfertilized eggs containing developing parthenogens are also altered. For example, it is possible that, over incubation, parthenogens could lower albumen pH below that of unfertilized eggs without development. Furthermore, the albumen gases and ions in eggs containing parthenogens may be different in comparison to unfertile eggs that do not exhibit embryonic growth. Therefore, the objective of the present study was to determine if changes occur in albumen pH, gas exchange, and ion concentration over 12 DOI in eggs exhibiting parthenogenetic development versus eggs with no embryonic development.

2. Materials and methods

2.1. Housing and environment

Parthenogenetic virgin hens were obtained from mating hens selected for the parthenogenetic trait to males whose sisters exhibited parthenogenesis. Fertilized eggs from these mated quail were incubated to produce chicks for the examination of parthenogenesis. At hatch, chicks were fed a commercial quail starter diet ad libitum for the first 4 weeks of age. At 4 weeks of age, before sexual maturation, males and females were separated using differences in feather color and were moved into colony cages to adapt to the nipple drinker system. Beginning at 4 weeks of age, birds were then fed a commercial quail breeder diet. At 6 weeks of age, quail hens were placed into individual cages to record individual egg production. All birds were exposed to 17 hours of light and received feed ad libitum. Birds were treated within the approved guidelines of the Institutional Animal Care and Use Committee of Mississippi State University, before the experiment was performed (10-096).

2.2. Albumen characteristics

Daily, eggs from 132 virgin quail hens were collected, labeled, and incubated from 0 to 12 DOI. Eggs (832) were weighed before and after incubation to obtain egg weight loss. Eggs were incubated at 37.5 °C and 55% relative humidity. After incubation, eggs were examined for the incidence of parthenogenesis on each DOI [17]. Egg albumen pH was measured by pH strip (VWR North American). Also, albumen O_2, CO_2, Ca^{2+}, Na^{+}, and Cl^{-} concentrations were measured using an ABL77 gas and electrolyte analyzer (Radiometer, Copenhagen, Denmark). All pH, gas, and ion concentrations were determined immediately after opening eggs. For each individual egg, approximately 50 μL of albumen was aspirated to determine O_2, CO_2, Ca^{2+}, Na^{+}, and Cl^{-} concentrations. Next, eggs were examined under an illuminated magnifying lamp to determine the existence of parthenogenetic development, and the size of the parthenogen was measured across the widest part of the germinal disc to the nearest millimeter [17].

2.3. Statistical analysis

Data were analyzed using a completely randomized design with a 2×12 factorial arrangement: parthenogenesis (yes or no) and DOI (0–12). Regression analyses were used to examine the relationships of egg weight loss, albumen pH, and Ca^{2+} concentration over DOI. Correlation analyses were used to determine the relationship of parthenogen size with albumen gas and ion concentrations. Means were separated using Fisher's protected least significant differences ($P < 0.05$) [24].

3. Results

There was an interaction for albumen O_2 concentration between eggs exhibiting parthenogenetic development versus eggs with no parthenogenetic development from 0 to 12 DOI (Fig. 1). With the exception of 8 DOI, albumen O_2 in eggs exhibiting parthenogenesis was lower in eggs without development over incubation. There was no interaction over DOI for albumen CO_2. However, the main effect of parthenogenetic development ($P < 0.0001$) showed an increase in CO_2 concentration from 0.210 μmol/mL in eggs without parthenogenetic development to 0.935 μmol/mL in eggs exhibiting parthenogenetic development.

Also, eggs exhibiting parthenogenetic development had a lower pH over every DOI as compared with eggs without parthenogenetic development (Fig. 2). However, pH increased from 0 to 2 DOI in eggs not exhibiting parthenogens but remained more stable during this period for eggs containing parthenogens. Additionally, pH declined linearly in eggs exhibiting parthenogenesis over DOI.