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Egg Storage and Incubation Temperatures Alter Parthenogenetic Development in Chinese Painted Quail Eggs

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Abstract: Parthenogenesis, embryos from unfertilized eggs, often exhibit delayed development at oviposition and throughout incubation at standard incubational temperature. Additionally, the first egg in a clutch sequence is more likely to exhibit parthenogenesis than subsequent eggs. Because the first egg in a clutch sequence stays in the hen's body longer, it is possible that the temperature of the hen's body accelerates parthenogenetic development. Increasing storage and incubational temperature may simulate the hen's body temperature and increase the incidence of parthenogenesis. Therefore, the objective of this study was to determine if storage and incubational temperatures impact parthenogenetic development in virgin Chinese Painted quail. Daily eggs were collected, labeled and divided among 3 different storage temperatures (20, 30 or 40°C). Eggs were incubated at 37°C for 10 d or 42°C for 48 h then returned to the standard incubational temperature of 37°C for the remaining 8 d of incubation. After 10 d of incubation, albumen pH and parthenogen size was measured for each egg. At an incubational temperature of 42°C, eggs stored at 20°C yielded the highest percentage of parthenogens. Also, eggs stored at 20°C and incubated at 42°C yielded the lowest albumen pH. As storage temperature increased, parthenogen size increased when eggs were incubated at 37°C. Also when eggs were stored at 30°C, embryo size was larger when incubated at 42°C for 48 h as compared to incubation at 37°C. In conclusion, elevated egg storage or incubational temperatures alter albumen pH, the incidence of parthenogenesis and the size of parthenogenetic embryos.

Key words: Parthenogenesis, egg storage, incubation temperature, egg albumen pH

INTRODUCTION

Parthenogenesis is defined as "the development of the egg cell into a new individual without fertilization" (Suomalainen, 1950). Unlike in many invertebrates, parthenogenesis in warm-blooded vertebrates often yields unviable embryos (Mittwoch, 1978). For example, in some species, such as domestic avian species, parthenogenesis can be a spontaneous and abortive form of embryonic development (Olsen, 1975), negatively affecting offspring production. Avian parthenogenesis has been studied in chickens (Kosin, 1945; Sarvella, 1970), turkeys (Cassar *et al.*, 1998; Olsen and Marsden, 1954) and Chinese Painted quail (Parker and McDaniel, 2009). In a normal fertilized chicken egg, the embryo is in the gastrula stage of development at oviposition (Fasenko, 2009) and is organized with an area opaca, area pellucida and marginal zone (Etches, 1996). However, parthenogenetic embryos have less embryonic development at the moment of oviposition. In fact, parthenogens are only in the early blastula stage of development (Haney and Olsen, 1958).

In Chinese Painted quail, Parker and McDaniel (2009) reported that the germinal disc of an egg exhibiting

parthenogenetic development after 10 d of incubation was similar to the germinal disc of a fresh fertilized egg. Both the parthenogen and fresh fertile egg exhibited an area opaca, area pellucida and periblastic ring. However, the germinal discs of fresh fertilized eggs was slightly larger (3.93 mm) than the germinal discs of infertile eggs exhibiting parthenogenetic development that had been incubated for 10 d (3.73 mm). The difference in embryo size may be explained by Olsen (1975) who reported that parthenogens develop at a slower rate than embryos from fertilized eggs. Olsen (1975) stated that the slower rate of development in parthenogens is due to a delay in cell cleavage and a lack of cellular organization.

Parker and McDaniel (2009) also reported that the first egg in a clutch sequence is more likely to exhibit parthenogenesis than subsequent eggs in a clutch sequence. Because the first egg in a clutch sequence stays in the hen's body longer (Fasenko *et al.*, 1992), it is possible that the hen's body temperature accelerates parthenogenetic development. However, research conducted by Parker and McDaniel (2009) only examined parthenogens that developed at the standard incubational temperature of 37°C with eggs stored from 0-3 d at 20°C prior to incubation.

After eggs stay in the oviduct for approximately 24 h at the hen's body temperature, oviposition occurs and at lay, eggs are exposed to environmental temperatures (Etches, 1996). In fact after oviposition, egg storage is a common practice that is used in the poultry industry. Due to different factors, most eggs are not incubated the same day that they are laid (Fasenko, 2007). Therefore, to preserve the physiological functions of embryonic cells for later incubation, fertile eggs are kept at a temperature that is known as physiological zero. Storing eggs at physiological zero reduces the growth rate of embryonic cells until the eggs are incubated (Edwards, 1902).

To avoid the negative impact that egg storage has on embryonic development, some researchers believe that preincubation of eggs, either prior to or during storage, will improve embryonic development and viability. For example, Reijrink *et al.* (2010) stored eggs for 15 d at 16°C and observed that eggs which were preincubated for 7 h prior to the 15 d storage period and eggs which were preincubated 6 times for 30 min at 37.8°C during storage had a higher number of embryonic cells than eggs without preincubation treatment. Because there was a decrease in the number of embryonic cells in eggs without preincubation treatment during storage, they suggested that embryonic development in the untreated eggs was suppressed.

The practice of egg storage has also been reported to affect the length of incubation. It has been suggested that stored eggs extend the incubational period because these embryos have a slower rate of development during the first week of incubation. As a result of egg storage, embryos are not able to begin development immediately when normal incubational temperatures are provided (Robinson *et al.*, 2003). Therefore, high incubational temperatures have been examined to possibly decrease the effects of egg storage. For example, Christensen *et al.* (2003) hypothesized that using high incubational temperature on stored eggs could accelerate embryonic development, possibly resulting in more viable embryos. Christensen *et al.* (2003) stored eggs for 3 or 15 d at 12.8°C prior to incubation at a higher incubational temperature of 37.8°C. During the first 2 wk of incubation, they reported a 4.3% increase in embryonic livability as compared to eggs incubated at the standard incubational temperature of 37.5°C. They also reported that, during the first week of the higher incubational temperature, embryonic livability was even greater (6%). They concluded that embryos from the stored eggs receiving the higher incubational temperature are more likely to survive due to an accelerated growth rate during the early stages of development. It is possible that some of the accelerated growth reported at the higher incubational temperature could actually be

parthenogenetic development because the higher incubational temperature is closer to the hen's body temperature.

The stage of embryonic development at oviposition has also been reported to affect the ability of the embryo to survive storage (Butler, 1990; Bakst and Akuffo, 2002) as well as affect the number of embryonic cells and cell activity (Reijrink *et al.*, 2010). Coleman and Siegel (1966) examined embryonic development in two different lines of birds during egg storage. They found that embryos which were more advanced at the time of oviposition could withstand storage when compared to embryos with less cellular development.

In the research of Coleman and Siegel (1966), it may be that some of the embryos with less cellular development could be a type of retarded development similar to parthenogenesis (Olsen, 1975). When examining the influence of incubational and storage temperature on parthenogenetic development, Sarvella (1974) observed no differences for the incidence of parthenogenesis when eggs were incubated within an hour of oviposition as compared to eggs stored at 12.8°C for 3 d. Yet in another study, Schom *et al.* (1982) reported that storing turkey eggs for 3 or 6 d at 17°C prior to incubation yielded a 12.1% of incidence of parthenogenesis. However when eggs were stored at 6°C, the incidence of parthenogenesis was only 3.2%.

Also, research has shown that the albumen pH of fertile eggs is altered during incubation, (Romanoff and Romanoff, 1967) and parthenogenetic development can also alter the albumen pH (Santa Rosa, 2014; Santa Rosa *et al.*, 2014). Therefore, the objective of this study was to determine if a combination of storage and incubational temperatures impacts the incidence and size of parthenogenetic development as well as egg albumen pH in Chinese Painted quail eggs.

MATERIALS AND METHODS

Housing and environment: Sexually mature virgin Chinese Painted quail hens 78 were obtained from a population of quail selected for parthenogenesis. Prior to sexual maturity, females were separated from males at 4 wk of age, based on plumage differences (Parker and McDaniel, 2009). At 6 wk of age, virgin hens were placed into individual cages to record egg production. All birds were fed a commercial quail breeder diet *ad libitum* and were exposed to 17 h of light. All birds were treated in accordance to the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (NRC, 1996).

Egg storage and incubation: Daily, eggs from each virgin hen were collected, labeled, weighed and divided into 1 of the 3 different storage temperatures: 20, 30 and 40°C. Eggs (918) were stored from 0-3 d in one of the 3 storage temperatures. For each storage temperature,

there were 3 egg storage chambers representing 3 replicates per storage treatment. After storage, eggs from each group were subdivided into 2 incubational groups. The first group of eggs were incubated at 37°C for 10 d and the second group of eggs were incubated at 42°C for 48 h then subsequently returned to a standard temperature of 37°C for the remaining 8 d of incubation. There were 6 incubators (n = 6) for eggs incubated at 37°C and 4 incubators at 42°C (n = 4). Also, storage and incubational temperatures were recorded daily to monitor temperature alterations. To ensure there was no statistical bias due to hen, over time, eggs from each hen were exposed to each storage temperature and each incubational temperature. After incubation, eggs were opened and egg albumen pH was measured by placing a pH strip (VWR North American) directly into the albumen (Santa Rosa, 2014; Santa Rosa *et al.*, 2014). Also, under a magnifying lamp, the incidence of parthenogenesis and embryo size was determined as described by Parker and McDaniel (2009).

Statistical analysis: Because eggs from every egg storage chamber were placed in each incubator, data were analyzed as a split plot design with incubators as whole plots and storage temperatures as split plots. Fisher's protected least significance differences was used to separate means at p<0.05 (Steel and Torrie, 1980).

RESULTS

The interaction for eggs exhibiting parthenogenesis for the 3 storage and 2 incubational temperatures is presented in Fig. 1. When eggs were incubated at 37°C there were no differences for the incidence of parthenogenesis due to storage temperature. However, when eggs were incubated at 42°C for 48 h prior to incubation at 37°C, eggs stored at 20°C exhibited the highest incidence of parthenogenesis when compared to egg stored at either 30 or 40°C. Yet when comparing eggs stored at 30 or 40°C that were incubated at 42°C for 48 h prior to incubation at 37°C for 8 d, there was no difference in the incidence of parthenogenesis. Similar to the incidence of parthenogenesis, there was a difference in pH with storage and incubational temperature (Fig. 2). When eggs were incubated at 37°C there were no differences for albumen pH due to storage temperature. However, when eggs were incubated at 42°C for 48 h prior to incubation at 37°C, eggs stored at 20°C exhibited a lower pH when compared to eggs stored at 30°C but a similar pH to eggs stored at 40°C. For eggs stored at 30 or 40°C and incubated at 42°C for 48 h prior to incubation at 37°C, there were no differences for albumen pH. However, when eggs were stored at 20°C, the pH was lower in eggs incubated at 42°C for 48 h when compared to incubation at 37°C for 10 d.

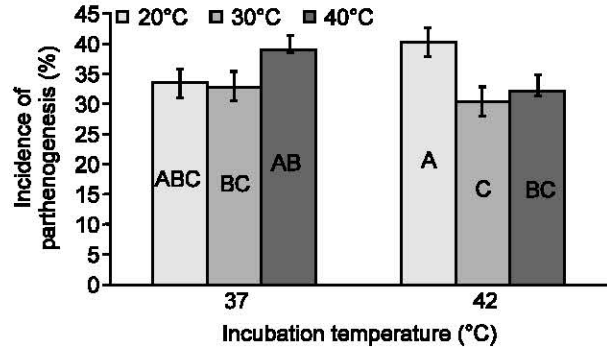


Fig. 1: Interaction of different storage and incubational temperatures on the incidence of parthenogenesis. Each bar represents the average storage temperature: p = 0.004. A-C Means with different letters are significantly different at p<0.05

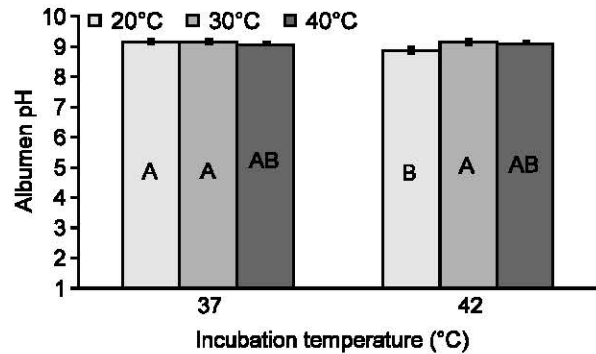


Fig. 2: Interaction of different storage and incubational temperatures on the albumen pH. Each bar represents the average storage temperature: p = 0.10. A-C Means with different letters are significantly different at p<0.05

The interaction of the 3 storage and 2 incubational temperatures for parthenogenetic embryo size is presented in Fig. 3. Eggs that were stored at 40°C and incubated at 37°C had a larger embryo size than did eggs stored at 20°C but were similar to eggs stored at 30°C. For eggs incubated at 37°C, embryo size was similar for eggs stored at 20°C and 30°C. However, when eggs were incubated at 42°C for 48 h prior to incubation at 37°C, parthenogens were larger for eggs stored at 30°C when compared to parthenogens from eggs stored at 40°C. There were no differences in embryo size between eggs stored at 20°C or 30°C and incubated at 42°C for 48 h prior to incubation at 37°C for 8 d. When comparing eggs stored at 30°C, parthenogen size was larger for eggs incubated at 42°C for 48 h versus eggs incubated at 37°C for 10 d.

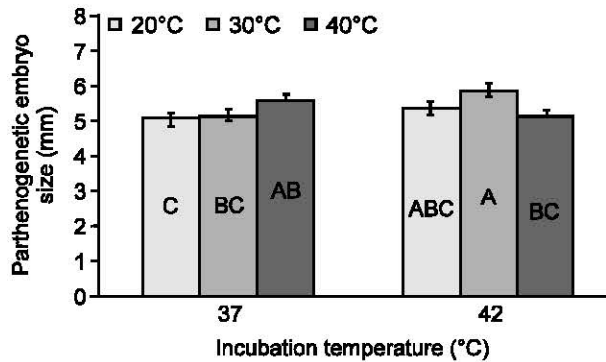


Fig. 3: Effect of different storage and incubational temperatures on parthenogen size. Each bar represents the average storage temperature: $p = 0.05$.

^{A-C}Means with different letters are significantly different at $p < 0.05$

DISCUSSION

In the present study, regardless of egg storage temperature, there were no differences in the incidence of parthenogenesis when eggs were incubated at 37°C. These results are similar to what was reported in other research. For example, Sarvella (1974) found no differences in the incidence of parthenogenesis due to storage temperature. Even though the incidence of parthenogenesis was not impacted due to egg storage when eggs were incubated at 37°C, storage temperature affected parthenogenesis when eggs were incubated at 42°C for 48 h prior to incubation at 37°C for 8 d. For instance, the incidence of parthenogenesis was highest for eggs stored at 20°C which is close to physiological zero (Edwards, 1902). In the current study, apparently for eggs stored at 20°C, cellular activity was arrested resulting in parthenogen preservation. For eggs stored at 20°C, possibly the higher incubational temperature provided an environment similar to the hen's body (Fasenko *et al.*, 1992) allowing more parthenogenetic cells to develop and thus increasing the incidence of parthenogenesis. Parthenogenetic development using different egg storage and incubational temperatures did affect internal egg components as well. According to Romanoff and Romanoff (1967) fresh fertilized eggs exhibit low pH due to the high concentration of CO₂ concentration from the hen's body. In the second day of incubation the CO₂ is lost to the environment due to a pressure difference. However, the pH decreases after the third day of incubation due to CO₂ production by embryo respiration. Also, in virgin quail hens, Rosa *et al.* (2012), Santa Rosa (2014) and Santa Rosa *et al.* (2014) reported a lower albumen pH in eggs exhibiting parthenogenesis as opposed to eggs with no parthenogenetic development. In that study, Rosa *et al.* (2012) only examined eggs that

were stored at 20°C and incubated 10 d at 37°C. In the current study, albumen pH was lower for eggs stored at 20°C and incubated at 42°C as opposed to eggs stored at 20°C which received the lower incubational temperature. Apparently, the greater development of the parthenogen when eggs are stored at 20°C and then incubated at the higher incubation temperature resulted in more CO₂ production and therefore a lower pH.

Additionally, for eggs exposed to the lower incubational temperature, parthenogen size increased as storage temperature increased. Also, it appears that when the higher incubational temperature was applied to eggs stored at 30°C, embryonic growth was accelerated thereby advancing parthenogenetic development. It is possible that this increase in parthenogen size for eggs stored at the higher temperature is due to preincubation of the eggs. For example, Reijrink *et al.* (2010) reported that when eggs were preincubated for 7 h at 37.8°C prior to storage, there was an increase in the number of embryonic cells. Because parthenogen size was greater for eggs stored at 40°C and incubated at 37°C, possibly the higher storage temperature allowed the parthenogen to reach a more advanced stage of embryonic development thus allowing the parthenogen to survive incubation. However, when eggs were stored at 40°C and incubated at 42°C for 48 h there was a decline in parthenogen size as compared to eggs stored at 30°C. When eggs were stored at 40°C prior to incubation at 37°C, parthenogen size was numerically greater as opposed to eggs stored at 40°C and incubated at 42°C. It is possible that the combination of higher temperature during both storage and incubation, sequentially, inhibits parthenogenetic development. Christensen *et al.* (2003) reported that when using a higher incubational temperature after storage at lower temperatures, embryonic development was accelerated resulting in an increase in embryonic livability. In this study, parthenogens that received the higher incubational temperature also exhibited accelerated growth in the early stages of development after storage at a lower temperature. However, for eggs exposed to both the high storage and incubational temperature, it is possible that the parthenogenetic cells underwent cellular degradation resulting in the death of the parthenogens. In fact, the pH was approximately 9.1 for eggs stored and incubated at the higher temperatures which is similar to the pH Rosa *et al.* (2012), Santa Rosa (2014) and Santa Rosa *et al.* (2014) reported for infertile eggs that did not contain embryos.

From this study, it is concluded that storage and incubational temperatures alter parthenogenetic development. Elevated storage or incubational temperatures increase parthenogenesis and decrease albumen pH. However, exposure to both high storage and incubational temperatures appears to be detrimental to parthenogenetic growth. Because the data

has shown that storage and incubational temperature alters parthenogenesis and albumen pH in Chinese Painted quail, research should be conducted to study if the manipulation of albumen pH could alter the degree of parthenogenesis.

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