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The effect of environmental condition before incubation on the growth and immune system of native chicken (*Gallus gallus domesticus*)

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Abstract

Climate variability can influence avian reproductive success by altering environmental factors critical to egg viability and embryonic development. In avian species such as *Gallus gallus domesticus*, the period before incubation exposes eggs to fluctuating conditions that may affect their survival and the offspring's physiological performance. This study evaluated the effects of pre-incubation environmental conditions on the growth and immune response of native chickens. Fertilized eggs were subjected to different temperature and humidity treatments before incubation to simulate natural environmental variations. The findings revealed that neither low-temperature exposure nor excessive moisture significantly reduced egg viability or embryonic development. Post-hatching observations, including biometric parameters and tonic immobility tests, showed no significant behavioral differences among treatment groups. Although all chicks exhibited daily weight gain, those from the control group displayed more stable growth patterns, indicated by lower standard error values. Measurements of innate immune response, inferred from body temperature fluctuation, were comparable across groups. These results suggest that native chicken eggs possess physiological plasticity that enables them to maintain normal embryogenesis and immune development under variable environmental conditions.

Keywords: avian reproduction, climate change, embryonic plasticity, survival

Introduction

Climate change is already negatively affecting bird populations worldwide by altering their distribution, abundance, and seasonal behaviors, such as migration and breeding. While it can be difficult to directly link a change in one species to climate, the consistent global trends are undeniable. Although there are numerous case studies demonstrating the effects of climate change on bird life, the available data are not yet fully capable of understanding the ecological mechanisms or physiological adjustments underlying them (Gowaty, 2008; Klemming, 1998; Ricklefs et al., 2017). In an effort to understand these mechanisms, we attempt to explain the phenomenon of reversible plasticity, where organisms are able to undergo short-term physiological changes in response to environmental changes that occur over a short period of time (Ahmad & Li, 2023; Jankowski et al., 2020). The symptoms exhibited by a number of animal species clearly demonstrate varying levels of plasticity (Hoffman et al., 2023).

One of the main environmental factors influencing energy use and physiological processes in an organism is temperature (Bertin et al., 2018; De Morita et al., 2016; Flores-Santin & Burggren, 2021). This topic is particularly interesting in birds due to their strong connection to their environment at every stage of development (Ipek et al., 2015; Kou et al., 2024; Sauve et al., 2021). Bird embryos are naturally exposed to temperature changes during incubation, particularly when the parents are away from the nest foraging or when predators disturb them. In previous research, we have studied embryonic responses to various environmental challenges during incubation (Lelono et al., 2025).

Here, we performed an experiment using local chicken embryos to study this phenomenon. We manipulated the early thermal conditions of the eggs to mimic environmental fluctuations found in nature. We then measured how the embryos responded via their survival, heartbeat beat and development to temperature changes and analyzed the relationship between these responses and their subsequent growth and other biological traits. To understand the development and their immune responses, we challenge the chick with Lipopolysaccharides (LPS) injection and measure the body temperature increase. LPS is commonly used to investigate the innate immune system of the chicken (Lelono & Surya, 2023; Noviyani & Lelono, 2025). This research aims to provide new insights into how and when reversible plasticity develops in birds.

Materials and Methods

Materials

This study used fertile free-range chicken eggs from a breeder specializing in chicken breeding. These chickens were raised in enclosed cages with a ratio of one male to four females to ensure fertilization. Fresh eggs, no more than two days old, were weighed, cleaned, and labeled with a pencil to avoid contact with toxic materials. The selected eggs were then randomly divided into three treatment groups. The treatment durations were based on the natural principle that a hen needs 8 to 15 days to lay an egg before the incubation period begins. The study included three types of treatments: Treatment 1: Eggs were left at room temperature (23-28°C) with 75% humidity and were not manipulated. Treatment 2: Eggs were submerged in room-temperature water for 10 minutes to simulate excess moisture. This was repeated daily at the same time from day 0 to day 10. Treatment 3: Eggs were placed in a 12°C cooler for 60 minutes. This was repeated daily at the same time. On day 8, all eggs were placed in an incubator at 39.5°C and 70% humidity. They were checked daily to monitor for embryonic development.

Embryo development and chick's growth

Embryonic development was first detected on day 3, and a black spot surrounded by a faint network of blood vessels. This marked a developing embryo, which became clearer on day 5 and beyond. Candling—shining a light through the egg to see the embryo—was performed daily to confirm its growth and presence. If an embryo wasn't visible or was identified as dead, the egg was specially marked, and checks were repeated for three consecutive days to be sure. Beginning on day 11, when candling was no longer effective, daily detection included measuring the heartbeat. The number of beats per minute for each embryo was recorded. Eggs with no detectable heartbeat were categorized as dead and noted in the observation log. Embryonic development was monitored until day 20, when the embryos were ready to hatch. After hatching, the chicks' biometrics were recorded. These measurements, including body weight, tibia-tarsus length, head length, and wing length, were taken daily to track early growth and health. For eggs that failed to hatch, the eggs were opened, and the embryos were measured. The final developmental state of these embryos was then documented.

The measure of tonic immobility

Predator-prey relationships in animals have led to the development of various survival instincts, such as running away, camouflaging, or playing dead. The act of "playing dead," or tonic immobility, is a common defense mechanism used to trick predators that prefer to eat prey they have killed themselves rather than scavenge a carcass. To measure tonic immobility, each chick was individually placed in a separate, quiet room, away from the main coop, to avoid any disturbances. Researchers first gently immobilized the chick to initiate the state of tonic immobility, and then measured the amount of time it remained motionless. Each measurement lasted a minimum of 10 seconds and a maximum of 60 seconds, with three repetitions per chick. This test was conducted when the chicks were between 5 and 10 days old.

Temperature measurement after LPS injection

The body temperature of the chicken was measured before LPS injection, which was used as the control temperature (0^{th} hour) of the chicken before being infected with *E.coli* LPS. Following the LPS injection, temperatures were continuously monitored every hour for 9 hours, beginning at the first hour post-injection. To measure a chicken's rectal temperature using a Thermo One thermometer, gently stretch the chicken. Apply lubricant to the thermometer's tip to reduce pain before inserting it into the chicken's rectum. Wait for the reading on the thermometer to stabilize before removing it.

Data analysis

In this study, data on the growth and development of chicken embryos and chicks are presented in graphs. These graphs show a decline in the number of eggs that did not survive at various stages of development. This visual representation enables a comparison between different treatment groups, from the first detection of an embryo to the day before hatching. For embryos that did not hatch, images illustrate their final state on day 23 when they were confirmed to be dead. Additionally, data on the heartbeat, competitiveness, and tonic immobility of the chicks were analyzed using a mixed model. In this model, the treatment was a fixed factor, and the chick's identity was a random factor. The increase in temperature after the LPS (lipopolysaccharide) injection was also analyzed using a mixed model. This analysis compared the normal temperature at the critical point (three to five days after the injection) with the temperature of the LPS treatment groups. In this analysis, the LPS treatment was a fixed factor, and the chick's identity was a random factor. All data analyses were conducted using SPSS 22 software.

Results and Discussion

We found that eggs from three different treatment groups showed a decline in their chicks' growth, indicated by the survival of the embryo (Figure 1). Another data came from the heartbeat of the group of treatment, which showed no significant difference in the treatment ($F = 0.622$; $p = 0.553$). We also check the embryonic development of three individuals that fail to hatch from different groups, which shows the relativity the similar condition between groups 1 and 2 (figure 2). This indicates that the chicks were unable to complete the final stage of their embryonic development.

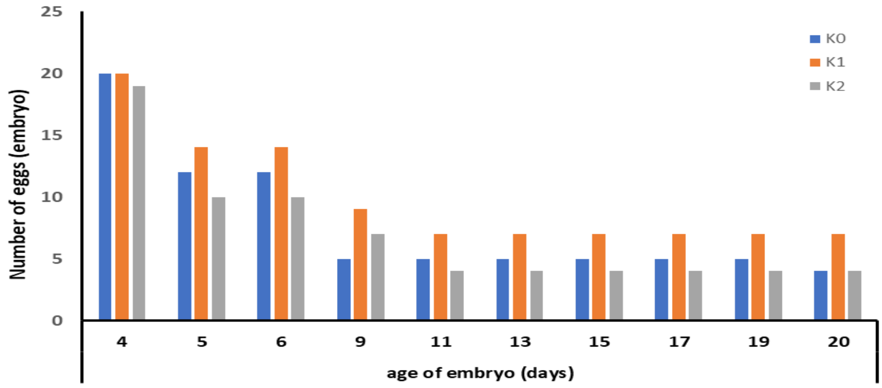

Figure 1. Illustrates the embryonic development of a chicken from day 0 to day 19, just before hatching (K0=normal; K1=watering; K2=low temperature/17 °C). The figure highlights key indicators of viability, specifically embryonic activity and heart rate, to demonstrate the embryo's survivorship during this period.

There was an increase in the chick's body mass during the experimental period (Figure 3), which shows the relative similarity of the growth between the three different treatment groups. However, we couldn't find the differences in chicks' tonic immobility ($F= 1.991$; $p=0.199$).

Figure 2. Embryonic development of three individual embryos from two different groups (Group 1 and Group 2). The embryos exhibit incomplete formation, indicating they did not reach the normal developmental stages. The variation in their size corresponds to differences in the initial egg mass, which in turn affects the size of the resulting chicks.

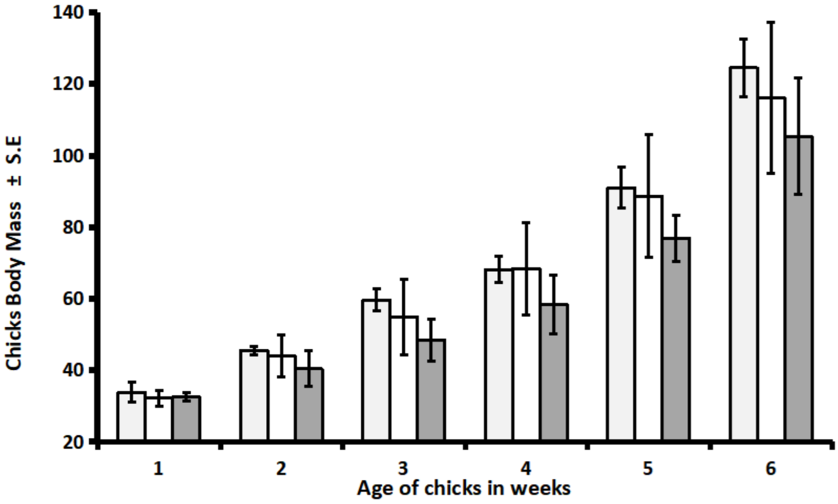

Figure 3. The chicks' growth from day 1 to day 6 in three different groups of treatment, as indicated by their gradual increase in body mass

On the final measurement point, we found a significant increase and decrease in body temperature after LPS injection (Figure 4). There were two different slopes (increase and decrease) of the temperature, which were significantly different compared to the normal body temperature ($F=22.248; p=0.001$).

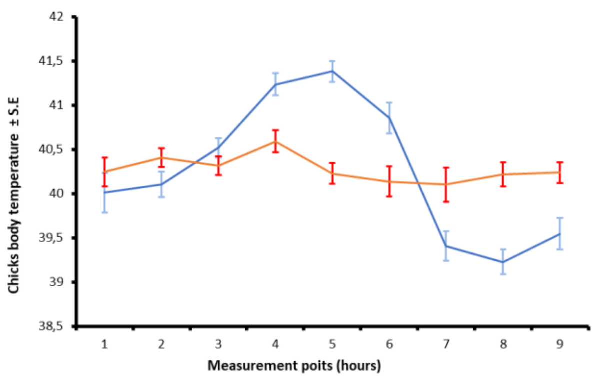

Figure 4. The curve of the chicks body temperature (blue line) after LPS injection and the normal body temperature (red line) when the chick is in normal condition.

Discussion

Our study shows there is no significant effect of environmental manipulation before incubation on embryonic development and hatchability (Figures 1 and 2). In the poultry industry, storing eggs before incubation is a common practice. This allows hatcheries to better coordinate their activities and manage the time between when eggs are laid and when they arrive. It also gives them the flexibility to meet demand and synchronize hatches, making the process more efficient (Adriaensen et al., 2022; N. Pokhrel et al., 2021). In general, birds have the remarkable ability to pause the development of their embryos until the conditions are right for incubation. This happens when the temperature is too cold or when the mother has laid enough eggs to form a full clutch. This developmental pause is called embryonic diapause, and it's achieved by stopping cell growth in the G phase of the cell cycle and preventing programmed cell death (apoptosis). Storing eggs for more than a week significantly reduces their ability to hatch (Adriaensen et al., 2022; N. Pokhrel et al., 2021; Rukmiasih et al., 2016; Tong et al., 2013). This is because long-term storage harms the blastoderm, which is the part of the egg that develops into the embryo. Essentially, the longer eggs are stored, the more their quality declines, leading to higher rates of embryo death and lower overall hatchability (Rukmiasih et al., 2016; Willemsen et al., 2010). For both turkey and chicken breeders, it's common knowledge that storing eggs for too long has negative consequences (Flores-Santin & Burggren, 2021; Schilling et al., 2018). However, due to logistical challenges, it can be hard to avoid. The problem is that extended storage alters the quality of the egg and can damage the blastoderm, the part that develops into the embryo, to a point where it can't recover and begin development once incubation starts (Adriaensen et al., 2022).

Here, we exposed the eggs to the lower temperature (17 °C), and another treatment was soaked in the water at room temperature, which represents the harsh environmental condition. We found that some chicks were unable to hatch. This is likely because the stress from those conditions harms egg quality and leads to early embryonic mortality, meaning embryos die early in the incubation process (Jankowski et al., 2020; Kou et al., 2024; Rukmiasih et al., 2016). While prolonged storage primarily increases the rate of early mortality, it can also negatively impact embryos later in development, leading to late mortality just before hatching (Gao et al., 2017; Hu et al., 2024). This is often caused by many factors, such as improper storage conditions, incorrect temperature or humidity, or poor management during the incubation process (Figure 2).

Chicks that hatch after being exposed to harsh environmental conditions often have lower biological quality, including stunted growth, compared to those from a normal environment (Figure 3). The biological quality of the chicks was influenced by conditions before and during the incubation periods. The stability of the ideal temperature during hatching would ensure the embryo would complete its growth normally. The embryonic development of animals plays a critical role in determining individual phenotypes and is also a vulnerable stage susceptible to environmental changes (Chang et al., 2013; Rukmiasih et al., 2016; Schilling et al., 2018; Wijnen et al., 2020). Developmental plasticity is the irreversible change in phenotype caused by changes in environmental conditions during development. Animals are inevitably exposed to changes in environmental factors during different life stages (De Morita et al., 2016; Narayan Pokhrel et al., 2022).

We also found that chicks responded to the LPS injection, which increased body temperature at 3 hours after injection and decreased respectively in the last 3 hours (Figure 4). We found no differences in response to lipopolysaccharide injections across all treatment groups, demonstrating the chicks' ability to withstand immunological challenges. Our previous studies demonstrated that chicks are capable of an innate response with an increase in body temperature. Injection of lipopolysaccharide, a substance commonly found in the outer walls of bacteria, significantly impacts the chicks' immune systems during development (Lelono et al., 2024). This substance can stimulate widespread inflammation and trigger a chain reaction of various changes. These changes include shifts in the number and population of different immune cells and changes in the production of cytokines, proteins responsible for the bird's immune system. This can ultimately negatively impact the overall health and growth of the chicks (Slawinska et al., 2016; Wei et al., 2018).

This study concludes that varying environmental conditions applied to eggs before incubation negatively impact hatchability during embryonic development. Despite this, the embryos demonstrated remarkable adaptability, maintaining development even when conditions generally deteriorated. Further testing of chick profiles at early growth stages demonstrates the importance of pre-incubation conditions in ensuring healthy embryonic development.

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