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## Natural incubation patterns and the effects of exposing eggs to light at various times during incubation on post-hatch fear and stress responses in broiler (meat) chickens



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#### ABSTRACT

Although environmental conditions during incubation can affect poultry welfare, one factor often overlooked in the commercial incubation of eggs is light. Light stimulation during embryonic development is now known to affect the physiology and behavior of broiler (meat) chickens post-hatch, but little is known about the pattern of exposure needed to produce these effects. We determined how often naturally incubated eggs were exposed to light by giving 10 broody Junglefowl × New Hampshire Red hens a nestbox containing five fertile eggs and a light sensitive data logger which recorded nest attendance by determining whether light was reaching the eggs. On average hens stayed on the nest without leaving for 14.3 of the days of the 21-day incubation cycle, although they did leave periodically, particularly during the last week. Utilizing this information, we then investigated how the timing of light exposure during incubation affected fear and stress responsiveness posthatch in broiler chickens. Eggs were either not exposed to light (ODL), or exposed to light throughout incubation (21DL) or during only either the last 2 weeks (14DL) or the last week (7DL) of incubation. Lighting pattern had a significant effect on all parameters measured, with the most consistent differences found between ODL and 21DL. For the fear measures, the ODL birds had a longer latency to emerge during an emergence test (62.7 versus 37.2 s), a longer latency to right during a tonic immobility test (223.8 versus 107.2 s), wing-flapped more intensely during an inversion test (7.03 versus 6.4 flaps/s), and vocalized more during an isolation test (172.7 versus 127.1/3 min) than the 21DL. For the stress measures, 0DL had a lower IgG titer (52,683 versus 97,375 units) and greater corticosterone response (1.18 versus 0.55 ng/mL) to the crating stressor than 21DL, and showed more composite asymmetry (1.96 versus 1.49 mm). The 14DL and 7DL groups were generally intermediate. Unlike dark-incubated chicks, all light-stimulated groups showed lateralization of escape direction during the emergence test, suggesting that light-induced cerebral laterality could play a role in the observed effects. However, the direction of lateralization differed depending upon timing of exposure. These results confirm the importance of light stimulation during incubation on the later behavior and physiology of broiler chickens, but also indicate that providing light only during the last week of incubation, which would coincide with the maximum light stimulation provided by hens' excursions from the nest, is insufficient to produce these effects.

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1. Introduction

Many studies have shown that exposing chicken embryos to light can affect pre- and post-hatch development. Light exposure during incubation can increase

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productivity by increasing embryonic growth and hatchability (e.g. Shafey, 2004) and accelerating time to hatch (e.g. Shafey and Al-mohsen, 2002). It can also affect social behaviors (Rogers and Workman, 1989; Riedstra and Groothuis, 2004) and fearfulness (Dimond, 1968; Dimond and Adam, 1972; Dharmaretnam and Rogers, 2005) in chicks during the first few weeks of life. There is also increasing evidence that the effects of embryonic light stimulation can persist beyond this early post-hatch period. In a previous study (Archer and Mench, 2013), we found that 3-6 week old broiler chickens exposed to 12h of light daily throughout incubation exhibited less stress susceptibility than those incubated in complete darkness, as determined by composite asymmetry and corticosterone and immune responses to a stressor. They were also less fearful than dark-incubated birds, as determined by tests designed to elicit a variety of anti-predator fear responses (Mench et al., 2008).

Commercial poultry eggs are typically incubated in complete darkness, both to conserve electricity and because of concerns about potential negative effects on hatchability due to heat emanating from the light source. Under natural conditions, however, avian embryos would receive at least some light stimulation during development when the hen leaves the nest to feed (Mrosovsky and Sherry, 1980) or responds to distress calls from the chicks by rising to turn the eggs (Rogers, 1996). However, there is no information about the natural incubation pattern of domesticated fowl, which would potentially be useful information for determining an optimal lighting pattern for artificial incubation.

In the experiments reported here, we first determined the natural incubation pattern of hens from a Junglefowl/domesticated (New Hampshire Red) cross. We found that most of the hens remained on the nest continuously for days, and mainly left the nest to feed and drink during the last week of incubation. This period coincides with a developmental phenomenon involving the lateralization of the visual pathways in embryos that has been shown to affect various aspects of post-hatch behavior in young chicks (Rogers, 1996; Rogers et al., 2004). In the second experiment, we therefore provided light stimulation to chicken embryos at varying times during the incubation cycle and assessed the effects on several measures of fear and stress responsiveness post-hatch. Fear measures included latency to right after the induction of tonic immobility (Jones and Faure, 1981), wing flapping intensity after inversion (Newberry and Blair, 1993), distress vocalizations given when socially isolated from flockmates, and latency to emerge from a dark box (modified hole-in-thewall test; Jones and Waddington, 1992). During the last test we also assessed lateralization by recording the direction of box exit. Stress measures included the corticosterone and immune responses to a crating stressor (Kannan and Mench, 1996) and composite physical asymmetry (Archer et al., 2009), which is an index of developmental stress (Møller et al., 1995).

Our goal was to determine whether shorter periods of light stimulation, consistent with the overall pattern shown by the hens, would produce the same effects we found in our previous studies of incubation lighting effects



**Fig. 1.** Experimental nest box. Each nest box contained a clutch of eggs and data logger placed within a plastic ball for recording nest attendence.

on broiler chicken welfare, where we light-stimulated the embryos throughout the entire incubation cycle (Archer and Mench, 2013; Mench et al., 2008).

#### 2. Experiment 1

#### 2.1. Methods

We used Red Junglefowl × New Hampshire Red hens (N=10), which readily exhibit broodiness, to assess incubation patterns. The hens were housed individually in wire cages  $(0.6 \,\mathrm{m} \times 0.9 \,\mathrm{m})$  containing a cup waterer, feed trough, and a wooden open-top nest box. The nest box  $(0.3 \,\mathrm{m} \times 0.3 \,\mathrm{m})$  was filled with 8 cm of wood shavings, and the feeder and waterer were located within 0.3 m of the nest box. Hens were managed according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). They were given access to feed (Purina Mills Layena® SunFresh® Recipe, St. Louis, MI, USA, 16% protein) and water ad libitum throughout the study. The lighting in the room was fluorescent, with an illumination of 500 lux during the day and 0 lux at night. The lighting schedule was 16L:8D, with the lights off between 22:00 and 06:00 h. Ambient temperature was maintained between 21 and 24°C. All procedures in both this experiment and experiment 2 were approved by the University of California, Davis, Institutional Animal Care and Use Committee.

Each hen was allowed to lay eggs and accumulate a clutch. When she began to incubate the clutch, her eggs were replaced with a clutch of five fertilized White Leghorn eggs. Fertilized eggs were provided so that the embryos would develop and facilitate natural behavior by the hen, such as egg-turning in response to chick calls (Rogers, 1996). A data logger (HOBO, UA-002-64, Onset, Bourne, MA, USA) that measured light levels (monitoring capability ranging from 0 to 322,000 lux) was fixed inside of a clear plastic ball (5 cm in diameter). This plastic ball was affixed in the center of each nest box using bolts, such that it was exposed to light when the hen left the nest (Fig. 1). These loggers also provided information during the night, since they were sensitive to the infrared illuminators we used for night-time video recording. The logger was

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