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Research Article

Spectrum of White Light During Incubation: Warm vs Cool White LED Lighting

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Abstract

Objective: Commercially chicken eggs are incubated in darkness, though naturally they would receive light exposure. Light has been shown to affect hatchability and post hatch development. **Methodology:** To determine if there is an effect of exposing embryos to light of warm (3900 K) or cool (5500 K), we incubated broiler chicken eggs ($n = 3096$) under either no light (Dark), warm LED (Warm) or cool LED (Cool) light; the light level was 550 lux. Dark eggs had lower hatchability (Dark $80.1 \pm 1.5\%$, warm $86.5 \pm 1.3\%$ $p = 0.01$, cool $84.9 \pm 1.0\%$ $p = 0.03$), less top quality chicks (Dark $56.6 \pm 4.7\%$, warm $82.1 \pm 1.7\%$ $p = 0.001$, cool $79.0 \pm 3.0\%$ $p = 0.002$), more pipped unhatched eggs (Dark $3.3 \pm 1.2\%$, warm $1.3 \pm 0.5\%$ $p = 0.01$, cool $1.3 \pm 0.4\%$ $p = 0.03$) and more chicks with unhealed navels (Dark $40.8 \pm 5.2\%$, warm $15.5 \pm 3.2\%$ $p = 0.002$, cool $19.1 \pm 2.5\%$ $p = 0.003$) when compared to either warm or cool. **Results:** A subset of each treatment ($n = 160$ chicks per treatment) was grown for 14 days. Stress susceptibility was assessed using a composite asymmetry score determined by middle toe length and metatarsal length and width. Dark chicks had a higher level of composite physical asymmetry (1.52 ± 0.09 mm) than did warm (1.17 ± 0.07 mm, $p = 0.004$) and cool (1.19 ± 0.09 mm, $p = 0.006$) broilers. Dark chicks were more fearful than warm and cool as they vocalized more (118.5 ± 8.5 vocal/3 min) than warm and cool (87.1 ± 7.4 and 92.4 ± 9.0 vocal/3 min, respectively; $p = 0.001$ and $p = 0.03$). There were no differences observed in 14 days growth or FCR ($p > 0.05$). **Conclusion:** The results indicate that LED light stimulation of chickens during embryogenesis results in increased hatchability, improved chick quality and has long-term effects on fear responses and stress susceptibility, furthermore they also indicate that warm vs cool light was not a factor.

Key words: Broiler, incubation, light, hatchability

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The greater demand for poultry products has led to a concurrent need for more fertile eggs and chicks hatched from those eggs. This has made maximizing the hatchability and chick quality a priority for the poultry industry. This leaves a few options to meet the increased demand: increase breeder numbers, increase breeder production or increase efficiency at the hatchery. While all are viable options increasing hatchery efficiency may be the most cost effective method.

Historically the primary focus to optimize hatchability of fertile poultry eggs has been on temperature, humidity, turning and even carbon dioxide concentrations during incubation. However, there is evidence that another environmental factor, light can have an effect on development of the embryo and hatchability as well as effects later in life¹⁻⁵. Providing light during incubation has been shown to result in a reduction in fear responses^{3,6} and a decrease in stress indicators^{1,2,7}. The addition of light during incubation has been shown to increase overall hatchability as well^{3,8-10}, though the degree of effectiveness has varied with the type of light or strain of bird. Differences in post-hatch growth as a result of lighted incubation have also been seen in previous studies. Results reported have been inconsistent with some papers reporting differences in growth and weight^{7,11} and others reporting no changes in performance¹.

Lighting spectrum can greatly affect birds. Red light has been shown to stimulate reproduction and activity while blue/green light has been shown to stimulate growth. However, it has also been observed that different spectrums of light can have an impact on embryogenesis¹². Furthermore, the pigment of the eggshell can influence which wavelengths of light pass through the shell and reach the embryo. Differences in hatch time have been seen when using different types of florescent lights and has attributed to the eggshell filtering certain light spectrums¹³. Ghatpande *et al.*¹³ concluded that only some of the light they were exposing the eggs to was reaching the embryo. Shafey *et al.*¹⁴ found that hatchability in lightly pigmented eggs was the highest at ~89% when exposed to low levels (900-1380 lux) of light, as opposed to medium and dark pigmented eggs that only reached ~81 and ~85% hatchability, respectively, when exposed to the same light. When exposed to high intensity (1430-2080 lux) light, the hatchability of lightly and medium pigmented eggs were reduced, while dark pigmented eggs saw no change¹⁴. Spectral analysis of pigmented and non-pigmented eggshells shows that on average 99.8% of light will be absorbed by the shell, with absorption in the near-ultraviolet spectrum being higher than the

near-infrared⁹. Hluchy *et al.*¹² tested monochromatic lighting during incubation of broiler eggs and found red light produced a higher hatchability than blue, with white light having the highest overall hatchability. However, even white light is not all the same with cool and warm white light having differing color components. Therefore, it is possible that different types of white light may have differing effects on hatchability, chick quality and development.

While it has been shown that white light exposure during incubation can increase hatchability and post-hatch behavior and stress susceptibility it is still not known if the white light color temperature is important. The objective of this study was to determine if there was a difference in the hatchability, embryo mortality and chick quality of broiler eggs exposed to either cool or warm white LEDs during incubation. In addition, data was collected to determine if treatments had differential effects on stress, fear and growth. It is hypothesized that eggs incubated under either lighted conditions will result in greater hatchability and lowered stress susceptibility when compared to dark incubated eggs, with possibility that the warm LEDs may be superior to the cool LEDs as red light has been shown to improve hatch over blue light.

MATERIALS AND METHODS

Two trials were conducted to investigate the differential effects of warm and cool LED light and no illumination during incubation on hatchability, chick quality and post hatch fear, stress and growth of broiler chickens. All methods were approved by the Texas A and M Institutional Animal Care and Use Committee (AUP# 2012-211).

General procedures: There were two trials conducted using Cobb500 broiler eggs (n = 3096) from 58 weeks old breeder flocks. Six GQF 1500 incubators and six GQF 1550 hatchers (GQF Manufacturing, Savannah, GA) were used in each trial and their front windows were blacked out with cardboard to prevent light intrusion into the machines. Two incubators were operated with the traditional dark method of incubation (0L:24D, dark), while two others were outfitted with cool white (7500 K) LED strips (Superbrightleds WFLS-X3 Saint Louis, MO; cool) and the final two incubators were outfitted with warm white (3250 K) LED strips (Superbrightleds WFLS-X3 Saint Louis, MO; warm). Treatments were randomly rotated between trials. The LED lights were on each level, with 2 strips running the length of the racks. The strips were attached to metal frames, which were in turn attached to the bottom of the rack above them. For the top rack, light strips were held up by a metal frame made to rest on the top rack. The lights were

very low profile as to not interfere with airflow within the incubator and produced negligible heat which did not affect incubator function or egg shell temperatures. The lights were operated by a timer with a 12L:12D light schedule at 250 lux at egg level as measured using a light meter (Extech 401027, Extech Instruments, Nashua, NH). Two egg trays were set on each rack with each tray holding 43 eggs for a total of 6 trays over 3 levels equaling 258 eggs per incubator. The incubators were maintained at standard temperature and humidity levels of 99.5°F and 55% relative humidity. The eggs were incubated for 18 days at which time they were moved into the hatchers of the same treatment. The lights were outfitted similarly to the incubators, except the metal racks rested on top of each hatch tray instead of being attached to the frame above. Again the lights were kept at a 12L:12D schedule. The eggs were transferred with all room lights off to avoid unneeded light exposure. Each egg was candled with a handheld flashlight and any non-viable eggs were removed and broken out after all eggs were transferred. For each incubator, the number of broken, infertile, early dead, mid dead and late dead eggs were recorded during the breakout. The remaining eggs were incubated in the hatchers for the remaining 3 days of the incubation period. All of the chicks were weighed and counted at hatch. The quality of the live chicks was assessed and they were categorized and counted as either no defect, having an unhealed navel, having leg abnormalities, weak, dirty, having traits a hatchery would cull or having any other abnormality. The remaining unhatched eggs were broken out and counted as pipped, broken, infertile, early dead, mid dead and late dead.

After hatch analysis, 80 chicks per trial from each treatment were set aside and reared for 14 days. The birds were managed according to the guidelines set forth in the Guide for the Care and Use of Agricultural Animals in Research and Teaching¹⁵. They were housed in pens measuring 1 × 2 m with 20 birds per pen and placed in a random-block design within the house. They were fed *ad libitum* a standard starter feed milled at the Texas A and M Poultry Research Center. Water was provided through nipple drinkers. The house was illuminated by incandescent bulbs and dimmed to an average of 20 lux at chick level using a light meter (Extech 401027, Extech Instruments, Nashua, NH) and set to a 20L:4D light schedule. All feed was weighed (Ohaus Champ CD-11, Pine Brook, NJ) when added to the feeders and the residual was weighed and subtracted from the total at the end of the 14 days trial to quantify total feed consumed per pen. The chicks were weighed when placed into the pens, at one week of age and at the end of the growout. Pen weight and feed conversion ratio was calculated using these numbers.

Fear tests: To test the fear response of chicks an isolation test was performed. The isolation tests were performed at 10 days of age by randomly collecting 10 birds from a pen, bringing them to a separate room and placing them in a 133 L uncovered plastic container. The birds were then individually placed in an unlidded 19 L bucket. A timer was set for 3 min and the number of vocalizations produced by the bird during this time were counted. Afterward, the bird was placed in a separate holding container. After all 10 birds had been tested, they were returned to their pen and 10 birds from the next pen were collected and tested. More vocalizations were considered to indicate more fearfulness.

Stress measures: Physical asymmetry of each bird was measured as per¹ at 14 days immediately after they were euthanized. In brief, using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL), the middle toe length, metatarsal length and metatarsal width were measured for both the right and left legs. The composite asymmetry score was calculated by taking the sum of the absolute value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be $(|L-R|_{MTL} + |L-R|_{ML} + |L-R|_{MW})/3 = \text{composite asymmetry score}$.

Spectrum analysis of eggs: Twenty brown broiler eggs were obtained and the contents emptied, making sure the large half of the egg remained intact. After the shells air dried for 10 min, they were individually placed over the sensor of an MK350 (UPRTek, Jhunan Taiwan) LED meter and illuminated with a LED strip, either cool or warm (Superbrightleds WFLS-X3 Saint Louis, MO, LED) held 5 cm over the sensor. The spectrum was measured for light passing through all 20 eggs. A final measurement of unfiltered light was taken as a control and all duplicated readings were averaged (Fig. 1).

Statistical methods: One-way ANOVAs were used to investigate treatment effects on hatchability, embryo mortality, chick quality, composite asymmetry, isolation, weight gain and feed conversion. The least significant difference test was used to test all planned comparisons. All of the assumptions of ANOVA were tested (Shapiro-Wilk test for normality, Levene's test for homogeneity of variance). No transformations were needed to meet assumptions. All analyses were performed using SAS 9.3 for windows (SAS Institute Inc.). Significant differences were determined at $p < 0.05$.

RESULTS AND DISCUSSION

Effects of warm vs cool LED: This study sought to evaluate the effects of warm vs cool LED lights used to illuminate eggs during incubation. The hypothesis was that the different spectral outputs by these lights could result in differential effects on hatchability, chick quality and post-hatch fear, stress and growth. Previously, it has been observed that using LED lighting during incubation can improve hatchability, chick quality and alter stress and fear responses⁵, however by Huth and Archer⁵ only cool LED lighting was used. Hluchy *et al.*¹² observed that monochromatic red lighting during incubation produced a higher hatchability than blue which could mean that white light with more blue or red could show differences in hatchability. This study observed no differences ($p>0.05$) between cool and warm LED in embryo mortality, chick quality, hatchability, fear or stress responses (Table 1 and 2). Ghatpande *et al.*¹³ concluded that not all the light they were exposing the eggs to was reaching the embryo which could explain why the cool and warm LEDs did not differ. Analysis of the light filtering of the eggs used in this study illustrated this fact. The warm and cool LEDs were in fact filtered similarly by the shells (Fig. 1) making them basically the same light. This is likely why they had similar effects on hatchability, chick quality, pipped chicks and stress and fear responses.

Effects on embryo mortality, hatchability and chick quality:

The addition of light into the incubator (cool or warm) affected the percent of pipped eggs, the percent of unhealed navels and the overall percent of no defect chicks and the hatchability of fertile eggs. The percent of early, mid and late dead embryos was not affected by providing light during incubation ($p>0.05$, Table 1), however, the percent of chicks that pipped but failed to hatch was affected by providing light

over no light during incubation. There were fewer pipped eggs in the both the warm ($1.3\pm0.5\%$, $p = 0.01$) and cool ($1.3\pm0.4\%$, $p = 0.03$) treatments when compared to the dark treatment ($3.3\pm1.2\%$, Table 1). It is possible that this is because the chicks in the light treatments were synchronized to hatch out in a tighter window than the dark treatment. It has been demonstrated that light stimulation during incubation can cause birds to develop circadian rhythms in hormones and body temperature. So it may be possible that the embryos all synchronized to hatch closer together when light stimulated though this needs further research to confirm.

The hatch of fertile percentage was also increased by providing light during incubation (Table 1). The warm ($86.5\pm1.3\%$, $p = 0.01$) and cool ($84.9\pm1.0\%$, $p = 0.03$) treatments both had higher hatch of fertile rates than the dark treatment ($80.1\pm1.5\%$). This improvement in broiler egg hatchability has been previously observed^{5,8-10}. This improvement again illustrates the importance of light to the

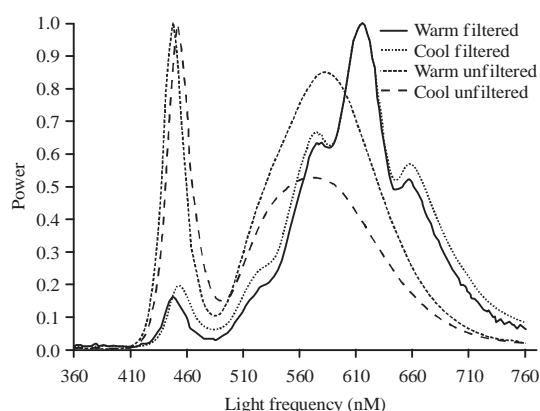


Fig. 1: Comparison of spectrum readings through the shells (filtered) of warm and cool LEDs and unfiltered spectrum of the LEDs used in the incubators

Table 1: Embryo mortality (%), chick quality (%), weight (g) and hatch of fertile (%) of eggs incubated under either warm or cool white LED light or no light (Dark)

Treatments	Early dead	Mid dead	Late dead	Pipped	Dead chick	Total dead	Un-healed navel	Leg or weak	Dirty feather	Cull	No defect	Weight	Hatch of fertile
Warm	6.43	1.24	4.04	1.28 ^A	0.18	13.13	15.52 ^A	0.16	1.28	1.00	82.05 ^A	46.95	86.47 ^A
Cool	7.26	1.19	4.88	1.32 ^A	0.36	15.00	19.13 ^A	0.60	0.89	0.41	78.96 ^A	47.16	84.90 ^A
Dark	8.73	1.25	5.51	3.34 ^B	0.22	19.04	40.76 ^B	0.54	0.86	0.56	56.55 ^B	48.78	80.56 ^B
SEM	0.76	0.57	0.96	0.72	0.17	1.25	3.46	0.24	0.56	0.32	9.92	0.07	1.25

Different letters within column indicate significant differences ($p<0.05$)

Table 2: Broiler 14 days growout results

Treatments	14 day weight	FCR	Isolation vocalizations	Composite asymmetry
Warm	0.37 ± 0.06^a	1.19 ± 0.03^a	87.1 ± 7.4^a	1.17 ± 0.07^a
Cool	0.39 ± 0.08^a	1.23 ± 0.03^a	92.4 ± 9.0^a	1.19 ± 0.09^a
Dark	0.39 ± 0.11^A	1.21 ± 0.07^A	118.5 ± 8.5^B	1.52 ± 0.09^B

Significant differences between treatments of $p<0.05$ designated by differing superscripts within measure, comparison of final bird weight (kg), feed conversion ratio, isolation test (No. vocalizations/3 min) and composite asymmetry scores (mm) of eggs incubated under either warm or cool white LED light or no light (Dark), (Mean \pm SE)

optimum development of the avian embryo. Previous study which saw depressions in hatchability can likely be attributed to the lighting source. Incandescents out put too much heat and the light produced by a fluorescent fixture may not be optimum. The LED lights do not produce heat and can provide a precise spectrum of light to expose the eggs to during incubation.

Chick quality also does not appear to be strongly affected by the difference in light spectrum, as it was improved in all trials simply by addition of light (Table 1). The percent of unhealed navels was lower in both the cool ($19.1 \pm 2.5\%$, $p = 0.003$) and warm ($15.5 \pm 3.2\%$, $p = 0.002$) treatments when compared with the dark ($40.8 \pm 5.2\%$) treatment. Furthermore, the overall percent of no defect chicks was higher in both the cool ($79.0 \pm 3.0\%$, $p = 0.002$) and warm ($82.1 \pm 1.7\%$, $p = 0.001$) treatments when compared to the dark treatment ($56.6 \pm 4.7\%$). This agrees with several previous studies, which found light to increase chick quality^{1,5,7} when birds were exposed to light during incubation. The difference in navel tag percentage could be related to the faster growth rate seen in previous lighted incubation experiments^{10,16}, as it may result in the chick internalizing the yolk and healing its navel more quickly than birds incubated in darkness⁹. Ozkan *et al.*⁷ also concluded that lighted incubation better primed the chicks to deal with novel environments.

Effects on fear and stress: The results of this study also reproduce and expand upon previous findings in Archer *et al.*¹ and Archer and Mench², which showed that lighting of broiler eggs during incubation resulted in lower stress susceptibility post hatch. As seen previously, physical composite asymmetry scores were significantly lower in the light incubated birds than the dark (Table 2). Dark chicks had a higher level of composite physical asymmetry (1.52 ± 0.09 mm) than did warm (1.17 ± 0.07 mm, $p = 0.004$) and cool (1.19 ± 0.09 mm, $p = 0.006$) broilers. Since a greater physical asymmetry score indicates the bird underwent some form of stress¹⁷, this suggests that lighting during incubation can reduce the effects of stress on a growing bird. Again, Ozkan *et al.*⁷ concluded lighted incubation made the chick better able to handle novel stimuli. This could be a major reason that the chicks exposed to light during incubation appear to be less susceptible to stress.

The dark chicks were more fearful than warm and cool chicks as they vocalized more (118.5 ± 8.5 vocal/3 min) than warm and cool chicks (87.1 ± 7.4 and 92.4 ± 9.0 vocal/3 min, respectively; $p = 0.001$ and $p = 0.03$, Table 2). While the isolation test has not been used often in poultry as a method for determining fear it has in many other species. When done in other animals it has been concluded that decreased

vocalization frequency is a behavior that correlates with reduced fear¹⁸. Archer and Mench³ saw this same correlation of decreased vocalization to decreased fear in broilers, indicating that it is a viable test of fear in chickens. This reduction in fear response demonstrates improved welfare and supports the findings of Ozkan *et al.*⁷. This decrease in fear may be associated with developmental changes in the brain such as visual lateralization which is associated with fear responses in birds.

Effects on growth and feed conversion: The feed conversion ratio and overall weight of the birds was not significantly different ($p > 0.05$) at the end of the study (Table 2) as has been seen in other light incubation studies¹¹. This could be related to the fact that Zhang *et al.*¹¹ used monochromatic light while this study used full spectrum white lights. As there was no difference between the types of white light used in this study possibly a white light containing more green light would improve growth. However, it should be noted that Zhang *et al.*¹¹ did not see an improvement in FCR until after 35 days of age. Therefore, it is possible if the birds in this experiment were grown for a longer period the same effect might have been observed.

CONCLUSION

There were no differential effects of using warm and cool white LED lights during incubation as both are filtered similarly by the egg shell. Exposing embryos to white light during incubation improves hatchability, chick quality and decreases stress and fear responses post hatch in broilers compared to traditional dark incubation. This study's results can be used to improve hatchery efficiency by utilizing lighted incubation to increase hatchability and chick quality and improve broiler welfare post hatch by decreasing the fear and stress response.

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