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Cover Page Footnote

Hannah Feuerborn is a May 2022 honors program graduate with a major in Poultry Science. Sara Orłowski, the faculty mentor, is an Assistant Professor in the University of Arkansas System Division of Agriculture's Cooperative Extension Service.

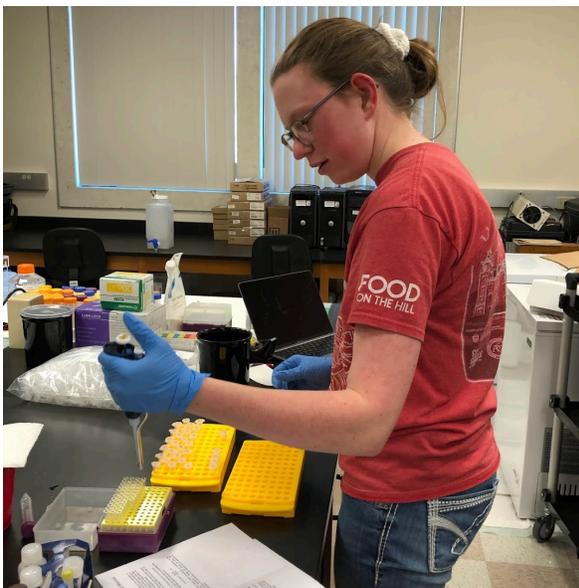
Effects of Sunrise/Sunset Lighting on Corticosterone Levels in Coturnix Quail (*Coturnix coturnix*)

Meet the Student-Author



Hannah Feuerborn

Growing up in Edmond, Oklahoma, I was not involved with poultry until high school when I raised some chicks for my FFA State Agriscience fair project. I had the opportunity to compete in the state Agriscience fair, finishing 2nd in my division each year. I won the State FFA Agriscience: Animal Systems Proficiency award in 2017 and was a national finalist at National FFA Convention in 2017. At the University of Arkansas, I was on the Dean's and Chancellor's list for multiple semesters. I was also on the Poultry Judging team and won 1st high individual at the LSU National Poultry Judging contest in Spring 2022. I have been privileged to be an intern at Cobb-Vantress in the R&D department, conducting, analyzing, and presenting research to improve the genetics of our food supply. I would like to thank my mentor, Dr. Sara K. Orłowski, for her help and guidance, my thesis committee, Dr. Sami Dridi and Dr. Gisela F. Erf, for their time and support, Dr. Liz Greene for her guidance with lab analysis, and my coworkers for helping me raise my quail and helping me collect blood samples.



Hannah in the lab getting a new pipette tip between moving blood serum samples from their storage tubes into the tubes to prepare them for data collection.

Research at a Glance

- Sunrise/sunset lighting is better overall than traditional on/off lighting.
- Corticosterone is the primary stress hormone in poultry, and increased levels can lead to less muscle and egg production, as well as lower organ weights.
- Quail in sunrise/sunset lighting took longer to come into lay but laid more eggs than quail in on/off lighting.

Effects of Sunrise/Sunset Lighting on Corticosterone Levels in Coturnix Quail (*Coturnix coturnix*)

Hannah M. Feuerborn* and Sara K. Orlowski†

Abstract

Both genetics and environment play important roles in the growth, performance, and overall welfare of poultry species. Current commercial production practices typically do not mimic the natural environmental conditions of ancestral poultry species, specifically lighting. The current study aimed to evaluate the impact of genetics and the effect of sunrise/sunset lighting on the stress response of 4 genetic lines of Coturnix quail. The 4 lines utilized in this study included high stress (H), low stress (L), stress control (R), and Arkansas randombred (A) quail lines. Day old chicks from these lines were placed in one of two environmentally controlled rooms. All conditions were kept similar between the rooms until week 4. At week 4, half of the quail in each room were relocated to the other room, and the experimental conditions began. One room was subjected to sudden on/off lighting, while the other room was subjected to a 1-hour long sunrise/sunset treatment. At 8 weeks of age, blood was collected from one bird in each line of quail at 5 time points: before lights on, 3 points during sunrise lighting, and after lights were on at full intensity. The subsequent plasma samples were evaluated for their corticosterone concentrations. Quail housed in the sunrise/sunset lighting room had numerically lower corticosterone concentrations overall, and transferred quail had numerically elevated corticosterone concentrations. In conclusion, sunrise/sunset lighting has the potential to improve overall production, regardless of the genetic line.

* Hannah Feuerborn is a May 2022 honors program graduate with a major in Poultry Science.

† Sara Orlowski, the faculty mentor, is an Assistant Professor in the University of Arkansas System Division of Agriculture's Cooperative Extension Service.

Introduction

Poultry species, including chickens, turkeys, and quail, are typically grown in controlled environments to maximize both genetic potential and economic gains. In these controlled environments, nearly every aspect of management, including temperature, humidity, day length, light intensity, water pressure, and diet, is regulated. Characterizing the best environment to grow poultry is dynamic since the commercial bird is constantly changing due to genetic selection. As mentioned, lighting is controlled during production but fails to emulate the natural habitat from which their ancestors evolved (Manci et al., 1992).

Current production practices continue to utilize traditional on/off lighting, though the technology for gradual sunrise/sunset lighting is available (Manci et al., 1992). Hypothesized benefits of the gradual lighting system include the creation of a low-stress environment as indicated by reduced corticosterone (Majer et al., 2019). This leads to an increased growth rate, better metabolism, and improved overall bird welfare through a lessened stress response.

In North America, natural day length is not practical for sustained commercial egg production. Light supplementation that is balanced between natural and artificial day length is necessary to maintain peak production (Rubinoff, 2016) and persistence of lay. For housing with solid sidewalls, artificial lighting is the only light available. Consistent day length is critical for synchronization of lay. Achieving synchrony of lay is important for the consistent performance of the birds in a poultry house. Since breeder management changes with the age of the bird, it is important to establish and maintain flock uniformity by managing nutrition and lighting requirements. A uniform flock is easier to manage because special attention to small or oversized breeders would not be required.

Currently, research has focused on the effects of light intensity and spectrum and its impact on hormone production, metabolism, and other aspects of avian physiology and reproduction (Ibrahim et al., 2012), but there is minimal research investigating how turning lights on and off affect hormonal stress response, specifically concentrations of corticosterone. One study focusing on gradual lighting changes on fish was found (Ryu et al., 2019). Ryu and colleagues (2019) determined that plasma concentrations of cortisol were greater in the control group when compared to the group subjected to dimming lighting. Evaluating the impacts of light intensity changes on birds will help advance the optimal environmental conditions required for maximized poultry production and improved bird welfare.

The objective of this experiment was to determine the impact of gradual sunrise/sunset lighting changes on hormonal stress levels in quail as compared to traditional on/

off lighting. In addition, lines of quail, known to differ in stress response, were exposed to the various lighting treatments to evaluate potential genotype by environment response. It was hypothesized that quail in the experimental sunrise/sunset lighting system would have lower corticosterone concentrations than the quail in the control on/off lighting system. The sunrise/sunset lighting system would better mimic what their ancestors would be exposed to, and the control group would be less synchronized than the experimental group.

Materials and Methods

Since this study involved the use of vertebrate animals, Institutional Animal Care and Use Committee approval was obtained prior to the initiation of this study (Approval #18083-General Rearing of Selected Chicken and Quail Populations).

Four genetic lines of quail were utilized in this study. These included high stress (H), low stress (L), stress control (R), and Arkansas random (A). The H, L, and R lines were originally selected as described in Satterlee and Johnson (1978) and have been maintained as randombred populations at the University of Arkansas since the 1990s. The A line was developed as a randombred control of quail by Dr. Nick Anthony. At hatch, straight run chicks from each of the 4 lines were tagged with an identifying wing band. At placement, each genetic line was split evenly and randomly into one of two rooms using stratified sampling (Imbens, 1996) by genetic line and subjected to either traditional sudden on/off lighting (OO) or gradual sunrise/sunset lighting (SS). In the sunrise/sunset treatment, lights were programmed to come on gradually over a 1-hour period and shut off gradually over a 1-hour period, mimicking the natural rising and setting of the sun. At 4 weeks, a subset of birds from each room was switched between rooms. This created 4 environmental treatments. The environmental treatments included OO-N for chicks placed initially in OO and not transferred, OO-T for chicks initially placed in room 1 but transferred to room 2, SS-N for chicks initially placed in room 2 but not transferred, and SS-T for chicks placed in room 2 and transferred to room 1. At 8 weeks, blood samples from all lines and treatment combinations were taken at 5 different time points (lights on, post lights on, and additional time points for room 2, 3 points during the gradual sunrise lighting).

On the day of placement, chicks received 24 hours of light. From day 2 to day 7, the lighting schedule was reduced to 23 hours of light, and 1 hour of dark. From day 8 on, lighting in both rooms was reduced to 18 hours of light, and 6 hours of dark. This lighting schedule remained in effect until sampling. At 8 weeks, birds, regardless of gender, from each experimental group were sampled be-

fore the lights turned on, every 20 minutes into SS lights turning on, and after the lights were fully on.

All quail were placed in fresh, pine shaving litter floor pens. Both rooms were in the same research house. Supplemental water was provided for the first week after placement, and water was provided ad libitum through a hanging nipple drinker water line throughout the experimental period. Feed was kept consistent for all treatments; a common commercial quail starter feed was provided ad libitum throughout the experiment period. Temperature and ventilation for both rooms were adjusted according to bird age.

A commercially available plasma corticosterone enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Inc.) and a microplate reader were used to determine plasma corticosterone concentrations. All samples were run in duplicate. Absorbance was measured at 405 nm (Corticosterone ELISA, n.d.).

To measure corticosterone concentrations, blood samples were collected into heparinized tubes and centrifuged to separate blood plasma. A single bird from each line, lighting program, and time of day was sampled. Due to low hatch numbers in two of the genetic lines, the sample size for this project was small and additional birds per time point could not be sampled. Blood samples were collected at 8 weeks of age once birds had been in produc-

tion for 2 weeks. For the sampling procedure, quail were euthanized by cervical dislocation, then decapitated, and several milliliters of blood were collected into a heparinized tube. The blood was then centrifuged at $1000 \times G$ for 10 minutes, and plasma separated from the red blood cells and white blood cells. Plasma samples were frozen for evaluation of corticosterone later.

As a result of low hatch numbers in quail lines, multiple samples per time point were not possible. Therefore, statistical analysis could not be conducted to determine interactions between lines and treatments; however, means with standard errors are presented for sampling times within lighting treatment and for birds that remained in their original lighting treatment versus those transferred between lighting treatments.

Results and Discussion

Rooms varied based on the methods of how lights were turned on and off. Results of this study indicated that birds subjected to sudden on/off lighting had greater corticosterone concentrations overall than birds subjected to sunrise/sunset lighting (Fig. 1). Sunrise/sunset lighting better mimics the lighting conditions a quail would receive in the wild. Although these quail lines have been continuously raised and selected in a situation where lights were sud-

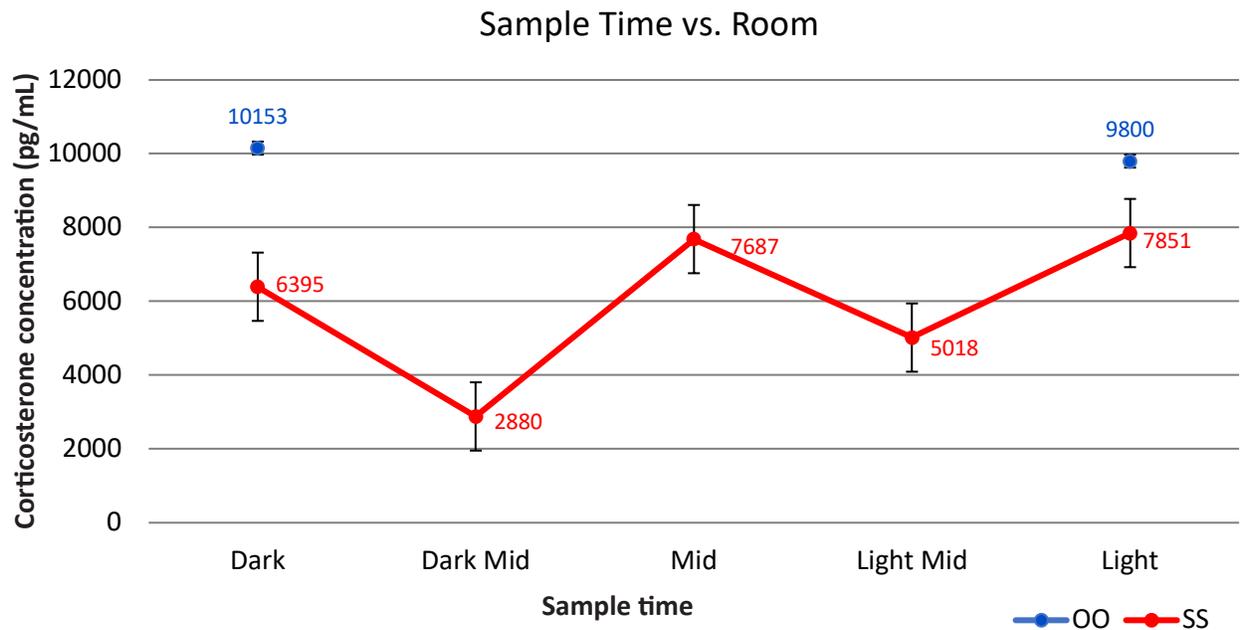


Fig. 1. Corticosterone concentrations (pg/mL) mean for each sampling time. OO—standard on/off lighting, SS—sunrise/sunset lighting. Dark—before lights on at 8:00, dark mid—20 min into lights on, mid—40 minutes into lights on, light mid—60 minutes into lights on, light—lights on at full intensity after 9:00. $n = 51$, samples analyzed in duplicate.

den on and sudden off, having an environment that better mimics natural lighting that a quail would be subjected to in the wild appears to lower stress and has the potential to improve animal welfare conditions. These results are similar to the findings of Ryu and colleagues' fish study and dimming lights (2019).

Quail transferred between rooms at 4 weeks, regardless of line or treatment, had greater corticosterone concentrations than quail that remained in the same treatment throughout the study (Fig. 2). Transfer between rooms represents an additional stress. Even though quail were allowed to acclimate to the new rooms for 4 weeks, the period may have been too short to overcome the negative impacts of the transfer stress.

As light intensity increased, so did corticosterone concentrations for lines R, L, and A. Line A and R had corticosterone concentrations greater than H and L before the lights turned on, then settled between H and L while the lights were turning on, then were the lowest of all bloodlines once the lights were at full intensity (Fig. 3). The H and L lines were originally selected for their corticosterone levels after a brief mechanical stress was applied to them (Satterlee and Johnson, 1978). It is possible the H line has a lower basal level of corticosterone as observed by the low corticosterone level exhibited by the H line quail during the dark period; however, when a stress or change in environment is applied to the quail, a sudden

but drastic increase in circulating corticosterone levels can be observed.

Maintaining low levels of corticosterone could be key to improving production, performance, welfare, growth rate, and egg production. Many environmental factors have been researched thoroughly to find out what it takes to help birds grow faster, but the main research in lighting has been focused on light intensity (Raccoursier et al., 2019) and day length. What little research that has gone into light transitions has been conducted in fish (Ryu et al., 2019).

While short-term quantification of corticosterone can be done by sampling blood, long-term corticosterone status can be determined by measuring corticosterone stored in the feather (Bortolotti et al., 2008). In Bortolotti and colleagues' study, corticosterone concentrations in molted feathers of partridges were measured with a methanol-based extraction technique and correlated with egg production recorded over time. Although the lines of quail were not able to be kept separate to measure egg production because of inadequate facilities, several anecdotal observations were noted for each room. Quail subjected to traditional lighting (OO) came into production quicker than SS. While quail in SS took longer to come into lay and reach peak production, the hens in SS consistently laid 2 to 3 times more eggs per day than OO. If the increased corticosterone concentrations caused decreased egg production, it could also cause problems with hatchability

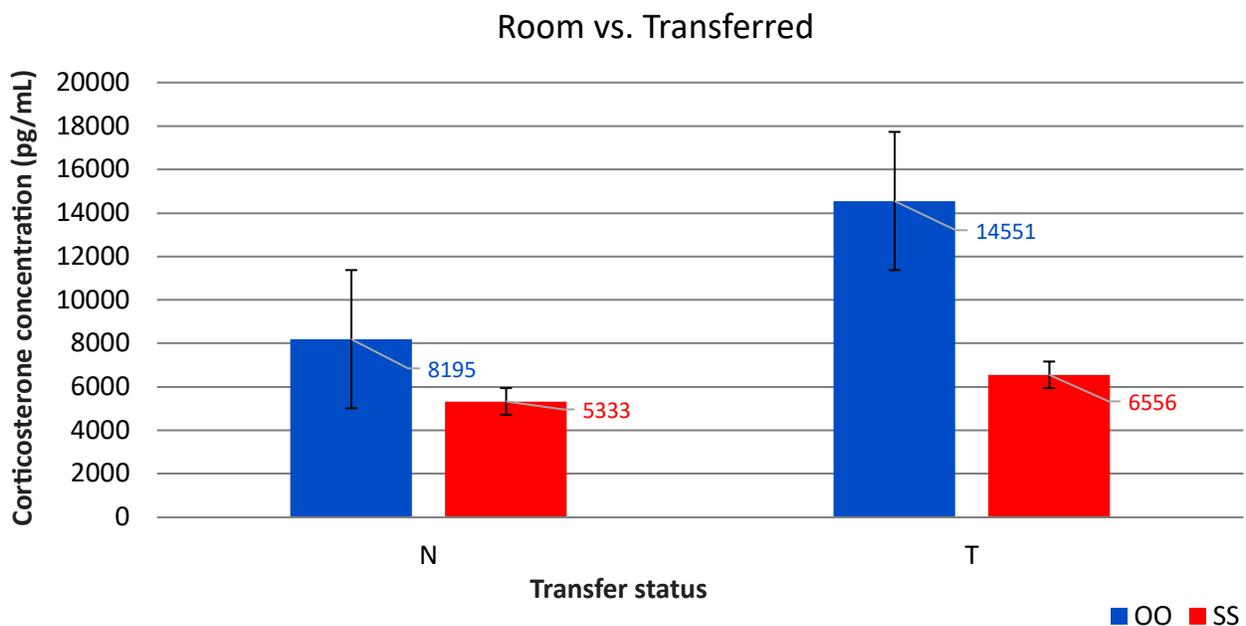


Fig. 2. Fig. 2. Corticosterone concentrations (pg/mL) by room and transfer status. OO—standard on/off lighting, SS—sunrise/sunset lighting. N = not transferred, T = transferred. n = 51, samples analyzed in duplicate.

and growth. Corticosterone has also been shown to impact embryonic survival and offspring growth (Peixoto et al., 2020).

Not only could the transferred quail have been impacted by the light systems changing, but also by the handling and move. Handling stress response could be avoided with a remote blood sampling system like one created for turkeys (McMurtry and Brocht, 1984), but quail are small and this might not be the best option. Corticosterone has a half-life of 22 minutes in male broilers (Birrenkott and Wiggins, 1984) and 9.8 minutes in Coturnix quail (Kovács and Péczely, 1983), so it would not remain in the body for that long, but the stress induced during transport from room to room could have affected the birds' growth. Hull and colleagues (2007) found that young Coturnix quail dosed with corticosterone had lower weights of the spleen, thymus, bursa, muscle, testes, and oviduct. While stressors and egg production tracked with egg charts is beyond the scope of this study, it merits further research.

Bird welfare continues to be at the forefront of the poultry industry, but it is usually measured by broken bones at the processing plant. Not only does corticosterone provide a good measure of welfare, but it can also provide insight into how to improve production and production practices. Since corticosterone can be traced both long-term and short-term, this could help producers fine-tune their lighting practices and track changes within weeks.

Conclusions

Initial findings from this study suggest that sunrise/sunset lighting may be better for bird health and welfare than traditional on/off lighting, as indicated by corticosterone response. This research is the first step in determining new techniques regarding lighting that producers can utilize to help improve the health and welfare of their production flocks. Future research that expands upon this data and looks at a larger sample size of quail is imperative moving forward. Although it does appear that the stress response of lighting was not line specific, focusing on one single line of quail subjected to either on/off lighting or sunrise/sunset lighting is necessary in the future.

Acknowledgments

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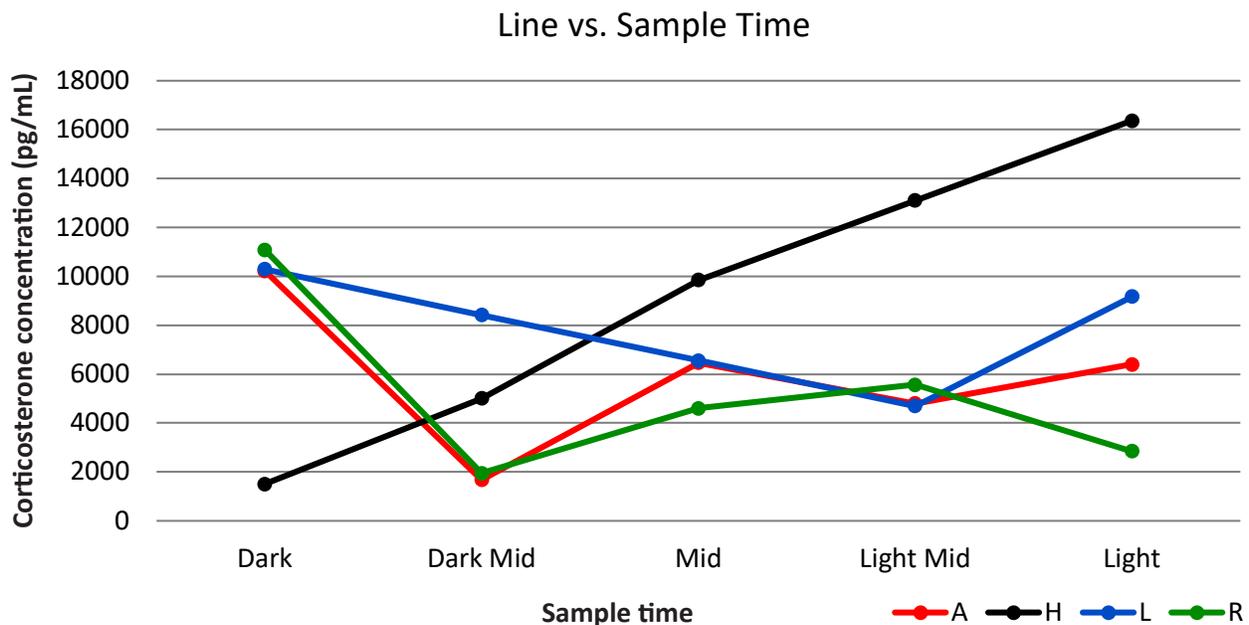


Fig. 3. Corticosterone concentrations (pg/mL) by line and sample time. A = Arkansas Random, H = High Stress, L = Low Stress, R = Random. Dark—before lights on, dark mid—20 min into lights on, mid—40 minutes into lights on, light mid—60 minutes into lights on, light—lights on at full intensity. n = 51, samples analyzed in duplicate.

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