Evaluating the Effect of Daylength (24, 20, and 18 hours) during Brooding on Broiler Performance and Physiological Responses to Light Environment

By

Garret Gregory Ashabranner

(Under the Direction of Brian Fairchild)

ABSTRACT

Brooding broiler chicks under continuous or near-continuous light is common in poultry research and commercial poultry production. Despite its prevalence, there is a notable lack of research to support its use. The current research investigated the impact of various daylengths (24, 20, and 18 hours) during brooding on broiler performance and physiological responses to the light environment. Broiler performance and physiological responses were assessed in four experiments comprising of ten trials. Control birds reared under continuous light during brooding initially exhibited higher body weight during the first week. Following the introduction of the dark period for the control groups on Day 7, the treatment birds subjected to dark periods during brooding overtook the control group in body weight. From Days 28-42, no differences in performance were observed between the control and treatment groups. No significant differences were found in feed conversion between treatment and control groups were found during any experiment. Corticosterone and superoxide dismutase were consistently unaffected by either lighting program. The introduction of a dark period during brooding led to higher baseline and

nighttime elevation in melatonin levels in the treatment birds, persisting up to 35 days of age. Field trials conducted in commercial poultry houses further confirmed the research's findings, with no significant differences observed in performance or mortality when comparing control and treatment houses. This research illustrates that providing broiler chicks with a dark period from the day of placement does not yield detrimental effects on end-of-flock performance.

INDEX WORDS: photoperiod, light program, scotoperiod, dark period, melatonin

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Garret Gregory Ashabranner

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Garret Gregory Ashabranner

Major Professor:	Brian Fairchild
Committee:	Michael Czarick
	Adam Davis
	Prafulla Regmi
	Matthew Jones

Electronic Version Approved:

Ron Walcott
Vice Provost of Graduate Education and Dean of the Graduate School
The University of Georgia
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LITERATURE REVIEW

INTRODUCTION

The poultry industry has long debated whether to provide chicks with a dark period. Early publications 1960s suggested that broilers raised under continuous light performed better than those with a dark period (Skoglund et al., 1966; Shutze et al., 1960). Other sources suggested that broiler chicks should have at least 4 hours of darkness (Wilson et al., 1964). Even a publication from the 1940s argued that continuous lighting for chicks was not cost-effective at the time, advising that all farm animals, including chicks, required some period of darkness or dim light for rest (King and Chesnutt, 1948).

Broiler chicks are commonly subjected to continuous or near-continuous light for approximately the first week of their lives (Scanes and Christensen, 2020). Many broiler management guidelines support providing chicks with continuous or near-continuous light during brooding (Aviagen, 2018; Cobb, 2021). The underlying assumption is that continuous lighting allows chicks more time to consume feed during their rapid growth phase; however, this assumption has yet to be thoroughly studied with modern fast-growing broilers (Olanrewaju et al., 2006).

POULTRY LIGHT PERCEPTION

Physics of Light

What we humans and animals perceive as light is actually visible light, which encompasses a range of electromagnetic radiation wavelengths spanning roughly from infrared to ultraviolet radiation. Wavelength and frequency are the quantifiable characteristics of light. Wavelength is measured by the distance between two peaks or valleys within a wave, typically expressed in nanometers. On the other hand, frequency is the number of wave peaks passing through a point in one second. Wavelength and frequency are inversely proportional: longer wavelengths correspond to lower frequencies, as seen in red light, while shorter wavelengths, like those in blue light, are associated with higher frequencies (Waldman, 2002).

The color of visible light, also referred to as spectral color, is perceived by photoreceptors and determined by the dominant wavelength/frequency of the incoming light ray that enters the eye. An object's color arises from the wavelengths it reflects and emits, which subsequently enter the eye (Sweetnum, 2000). This phenomenon is species-specific and tied to individual eye physiology. For instance, certain reptiles can detect infrared wavelengths, allowing for modified thermal imaging (Williams, 2019). Birds, on the other hand, can see into ultraviolet wavelengths, providing them with a distinct perception of light and their surroundings. Their sensitivity to shorter ultraviolet wavelengths enables them to perceive vivid colors and visualize their environment similarly to how colors appear under black light (Morrison et al., 2018).

Light intensity pertains to the quantity of lumens falling on a specific surface area. Measurement units include lumens per square foot (footcandles) or lumens per square meter (lux). How humans perceive light intensity or brightness visually is tied to the amplitude of light

radiating from a source or reflecting off a surface and entering an organism's photoreceptors (Keller, 2014). Similar to visible wavelength and spectrum, light intensity perception varies across species and is influenced by ocular physiology. Humans have limited night vision, while nocturnal animals can perceive the "dim" light at night as visible. Birds, with a higher number of rods (the photoreceptive cells for dim light detection) compared to humans, can detect dim light more effectively (Lovette and Fitzpatrick, 2016).

Eye Physiology

Light has two physiological pathways to stimulate birds: retinal in the eye and extraretinal photoreceptors in the pineal gland and hypothalamus (Meyer, 1986). While the avian eye shares general characteristics with other vertebrate eyes, birds have evolved numerous adaptations that enhance their vision, making their eyes some of the most advanced in the animal kingdom. Some of these adaptations include oil droplet inclusions, glycogen deposits within their photoreceptors, well-developed areae and foveae within their retina, highly vascularized pecten within their vitreous body, and increased rod count. Vertebrate eyes consist of three layers: the internal retina or sensory tunic, the middle vascular tunic comprising the iris, ciliary body, and choroid, and the external fibrous tunic consisting of the cornea and sclera (Morrison et al., 2018; Meyer, 1986).

The process of vision transforms light energy into nerve impulses that the brain interprets as visual images. The dioptric media, including the cornea, aqueous humor, lens, and vitreous body, refract light rays before they reach the retina. The retina, protected within the eye, is the visual receptor and connects to the brain through the optic nerve. The cornea primarily

contributes to refraction, focusing light further into the eye onto the retinal receptors, rods, and cones (Kumar, 2015; Willis and Wilkie, 1999).

Suspending the eye's lens from the ciliary body are annular ligaments that separate the gel-like vitreous body from the aqueous chambers. The anterior chamber is situated between the cornea and iris, while the posterior chamber lies between the iris and lens. Fluid filling these chambers regulates intraocular pressure to maintain the eye's rigidity and shape. This fluid, secreted by the ciliary body into the posterior chamber, flows through the pupil and drains into the anterior chamber, eventually being discharged into the annular sinus venosus sclerae (canal of Schlemm) at the eye's ventral end (Egbuniwe and Ayo, 2016; Meyer, 1986).

The retina comprises two segments: an external cuboidal epithelium housing pigments and an internal neuroepithelium with various neuron and glial cell types. The retina's general structure includes a three-neuron chain spanning its thickness, forming the conduit for light-induced nerve impulses to reach the optic nerve. The first neurons in this chain are the rods and cones located on the outermost layer of the retina. The rods and cones invert and convert incoming images into electrical signals, which the retina and individual optic nerve fibers transmit. As light is absorbed, specific visual pigments in the rods and cones bleach, triggering impulses that travel through the optic nerve (Kumar, 2015; Willis and Wilkie, 1999). The avian retina showcases rods and cones, as well as their characteristic double cones. Rods function in low-light conditions, primarily operating in dim illumination. Avian rods feature cylindrical outer segments with discs containing the visual pigment rhodopsin. Cones, on the other hand, facilitate bright light and color vision (Lewis and Morris, 2006; Bowmaker and Knowles, 1977). Avian cones are characterized by conical outer segments containing colored oil droplets and the visual

pigment iodopsin. Birds possess single cones responsible for color perception with large circular oil droplets of varying colors, enabling them to perceive a wide range of hues.

The oil droplets filter light at different wavelengths into the visual pigments to better perceive color vision. The oxidation of the visual pigments by light triggers nerve impulses, aiding in color discrimination. The four visual pigments: short-wavelength (SWS) 1, SWS2, RH2, and long-wavelength sensitive (LWS) peak in sensitivity at wavelengths of 415, 455, 508, and 571 nm, respectively (Chemineau et al., 2007; Bowmaker and Knowles, 1977). Double cones consist of a tall, thin chief cone closely linked with a broad, short accessory cone, forming what is referred to as the double cone. These double cones are believed to detect colorless movement, although their exact function requires further study (Akyüz et al., 2018). Because of these distinctive characteristics, the avian eye can detect light wavelengths from 380 nm to 740 nm, encompassing the spectral range from the edge of infrared and into ultraviolet rays (Parvin et al., 2014). Birds are are most receptive to wavelengths in the range of 533-577 nanometers (nm) and exhibit the highest sensitivity to blue light at 480 nm and red light at 650 nm (Prescott and Wattes, 1999; Nicol, 2015).

The rods and cones possess light-sensitive dendritic endings and axons that synapse with the dendrites of the second neuron in the chain. Bipolar cells constitute the second neuron chain and further synapse with the third neuron chain known as ganglion cells (Meyer, 1986). Axons from ganglion cells travel along the retinal surface and exit the eye through the optic nerve. The retinal neuron chain also includes horizontal and amacrine cells, which regulate synaptic connections. Horizontal cells manage communication between rods/cones and bipolar cell dendrites, while amacrine cells facilitate communication between bipolar cell axons and ganglion cell dendrites (Jones et al., 2007; Prum, 1999). Visual impulses from the retina flow through the

optic nerve. The optic nerve branches three ways into the medial, lateral, and minor roots, where visual impulses travel into the forebrain for interpretation (Benzo, 1986).

Circadian Rhythms

Biological circadian rhythms are present in all organisms, from bacteria to humans (Vitaterna et al., 2001). Circadian rhythms can be defined as the physiological, physical, and behavioral cycles that follow a 24-hour period. These rhythms are regulated by internal biological clocks known as circadian oscillators. Organisms rely on environmental cues called Zeitgebers to determine their biological time. Among these Zeitgebers, light plays a prominent role in influencing biological, physiological, and behavioral rhythms within the 24-hour circadian cycle (Fleissner and Fleissner, 2002). The avian circadian rhythm relies on synchronization between the retina, pineal gland, and hypothalamus, and interactions among these components are responsible for circadian organization. Four photo-receptive areas in the brain include the pineal gland, lateral septal organ (LSO), preoptic area (POA), and Mediobasal hypothalamus (MBH). While photoreceptors in the retina are not necessary for circadian rhythms or reproductive cycles, these receptors in the retina and pineal gland play a role in detecting the dark period and stimulating melatonin production (Baxter et al., 2014). Elevated levels of melatonin signal the master clock of the hypothalamic suprachiasmatic nuclei, promoting rest and sleep (Calislar et al., 2018).

Circadian Disruption

Circadian disruption refers to the continuous disruption of the circadian system, which can be caused by various factors such as environmental conditions, social behavior, and seasonal changes (Vetter, 2020). The pineal gland plays a role in regulating the rhythmic patterns of melatonin production, which in turn influences the circadian behavior of poultry by activating and deactivating circadian oscillators (Calislar et al., 2018). A study on broilers reared under different lighting schedules (14L:10D, 17L:7D, 20L:4D, and 23L:1D) examined melatonin rhythms by measuring baseline melatonin levels on Day 21. The birds reared under 14L:10D, 17L:7D, and 20L:4D exhibited a nighttime melatonin increase above baseline levels. No significant change in baseline melatonin was observed in the 23L:1D group, suggesting that one hour of dark cannot elicit a physiological response (Schwean-Lardner et al., 2014). Another study found higher melatonin levels in birds exposed to extended dark periods (8 hours) compared to birds with near continuous or intermittent lighting schedules, with no changes in serotonin levels (Sun et al., 2017). An hour of light exposure during the dark period inhibits N-acetyltransferase activity and reduces melatonin production (Hamm and Menaker, 1980). When there is a change in the bird's environment, such as lighting, its circadian rhythm may be altered or disrupted. The rhythm can stabilize and adjust to the new conditions over a few days to weeks, depending on the bird's species, breed, and age (Norris and Carr, 2021). The potential consequences of circadian disruption caused by near-constant light extend beyond, negates the beneficial effects of a stable microbiome (Oliveira e Alvarenga et al., 2021).

Melatonin

Melatonin is a crucial factor in maintaining and regulating circadian rhythms in birds. Melatonin is rhythmically produced primarily by the pineal gland and, to a lesser extent, by the retina during the night or dark period (Pang et al., 1996; Brandstätter, 2002). The pineal gland is situated between the cerebral hemispheres and cerebellum and possesses functional photoreceptors and a circadian clock. Melatonin is released during the dark period when serotonin-N-acetyltransferase catalyzes serotonin to produce melatonin (Evered and Clark, 2009). The entry of light into the eyes stimulates retinal dopamine, inhibiting neural serotonin production. Norepinephrine produced during the light period is responsible for suppressing the catalyzation of melatonin by serotonin-N-acetyltransferase. The reduction of serotonin and serotonin-N-acetyltransferase is responsible for inhibiting melatonin during the light period (Appleby et al., 2004; Scanes and Dridi, 2021). Melatonin produced by the pineal gland is primarily released into the bloodstream. The retina and Harderian gland produce small rhythmic amounts of melatonin but do not contribute to plasma melatonin levels (Cogburn et al., 1987). Melatonin acts on three receptors: Mel1A, Mel1B, and Mel1C. All three receptors have a 7-transmembrane domain and a GTP-binding protein that inhibits adenylyl cyclase (Appleby et al., 2004; Scanes and Dridi, 2021). Melatonin is vital in regulating various physiological rhythms related to behavior, immune function, excretion, skeletal development, reproduction, thermoregulation, digestion, and the neuroendocrine system (Apeldoorn et al., 1999). Studies have shown that exposing chickens to constant light suppresses N-acetyltransferase activity (Tanabe et al., 1983). Disruption of circadian rhythms can occur when chickens have short dark periods, for example, broilers reared under a 12L:12D cycle exhibited rhythmic clock gene

activity, while those reared under a 23L:1D cycle showed no clock gene activity at 21 days of age (Hieke et al., 2019).

The hypothalamus is located deep within the preoptic area of the forebrain. Light with long wavelengths and high intensity is required to penetrate the skull and brain tissue to stimulate the hypothalamus. Light stimulation of the hypothalamus directly controls the release of gonadotropin-releasing hormone (Chowdhury et al., 2010). Monochromatic light sources with longer wavelengths, such as red light, have been shown to be more effective in penetrating the skull and stimulating the reproductive axis in birds than white light, which contains all colors (Lewis and Morris, 2000; Baxter et al., 2014).

Rest and sleep have roles in poultry's expected performance, behavior, and physiological function. It is suggested that most vertebrates need at least four hours of undisturbed sleep to exhibit a complete sleep cycle (Blokhuis, 1983; Blokhuis, 1984). This suggestion was supported by Schwean-Lardner et al. (2014), who demonstrated a significant peak in melatonin levels during the four-hour dark period when comparing a lighting schedule of 23 hours of light and 1 hour of dark (23L:1D) to a schedule of 20 hours of light and 4 hours of dark (20L:4D). No notable change in baseline melatonin was observed in the 23L:1D schedule, indicating that one hour of dark is insufficient to elicit a physiological response. Broilers reared under lighting schedules with over four hours of uninterrupted dark exhibited significant elevation in melatonin levels above baseline during their respective dark periods (Schwean-Lardner et al., 2014).

Melatonin modulates antioxidant enzyme activities by interacting with calmodulin and inhibiting the inactivation of nuclear Retinoid-related orphan receptor α (RORα) melatonin receptors (Tomas-Zapico and Coto-Montes, 2005). This nuclear transcription factor subsequently decreases the expression of NF-jB-induced antioxidant enzymes (Tomas-Zapico and Coto-

Montes, 2005). Therefore, melatonin's effect on antioxidant enzyme activities is mediated by inhibiting the RORα pathway. Melatonin not only directly scavenges free radicals but has associations with enzymes like superoxide dismutase involved in the metabolism of free radicals, contributing to its important actions in oxidative defense (Tomas-Zapico and Coto-Montes, 2005).

Dark periods have been shown to improve immune response by increasing melatonin levels (Moore and Siopes, 2000). Japanese quail exposed to 6-8 hour dark periods exhibited higher innate cellular and humoral immune responses than quail reared under constant light. These quail raised under constant light also had a higher heterophil/lymphocyte ratio (H/L ratio). Supplementing melatonin to Japanese quail reared under constant light resulted in dose-dependent improvements in immune response and a better H/L ratio, indicating reduced stress (Moore and Siopes, 2000).

In ovo, injection of melatonin into turkey eggs led to increased lymphoproliferative cell-mediated immune response. This response in the treated birds approached mature levels by Day seven post-hatch, while control embryos did not reach mature levels until Day 21 post-hatch (Moore and Siopes, 2005). Similar results were observed in antibody responses when poults were exposed to chukar red blood cells (Moore and Siopes, 2005). Another study demonstrated that broilers supplemented with melatonin in their feed exhibited lymphoid hyperplasia in the liver, spleen, and bursa, which may be responsible for increased red blood cell count, hemoglobin, packed cell volume, and leukocytic count (Ahmad et al., 2011b). Other research has shown that melatonin administration reduced blood glucose levels (Bermudez et al., 1983) and increased white blood cell counts in broilers (Brennan et al., 2002).

Corticosterone

Birds exhibit a daily pattern of corticosterone, with peak levels occurring during the dawn and dusk phases. These cycles in corticosterone are natural and do not necessarily indicate stress. In chickens, this rhythmic increase in corticosterone stimulates foraging and feeding behavior, so broilers show more feeding activity before the lights are turned off and after they are turned back on corresponding to greater corticosterone levels (Hess, 2002; Norris and Carr, 2021). In a commercial broiler house, corticosterone levels range from 340 pg/mL to 1,070 pg/mL, with a mean of 624 pg/mL, (Thaxton et al., 2005). Corticosterone levels are higher during the light period, lower during the dark period, and peak at the beginning of the light period (Smoak and Birrenkott, 1986). The diurnal rhythm of corticosterone varies depending on age, rearing conditions, sampling technique, and photoperiod (Smoak and Birrenkott, 1986; Webb and Mashaly, 1985). Circadian rhythms of corticosterone do not develop until later in life. Research by Webb and Mashaly indicated that laying hens exhibit developed circadian corticosterone patterns at 11 weeks old. Chicks have higher circulating corticosterone levels than adults, which is believed to drive their search for food when young (Webb and Mashaly, 1985).

There are conflicting results in the literature regarding the effect of photoperiod length on corticosterone levels. Some studies suggest that day length does not significantly impact corticosterone production (Renden et al., 1994), while Buckland et al. found that broilers reared under intermittent (1 hour L: 3 hour D) light had lower corticosterone levels compared to birds exposed to near continuous light (Buckland et al., 1976). Under optimal conditions, the length of the photoperiod may not induce a stress response, and the photoperiod itself may not directly affect corticosterone production. Indirectly, the feeding behavior, the hunger response, and their

correlation to corticosterone may be the driving factors for corticosterone rhythms (Smoak and Birrenkott, 1984; Renden et al., 1994).

Photoperiod Effect on Poultry Microflora

After hatching, the chick microbiome is undeveloped and undergoes colonization and development within the first three days of life via inoculation from foraging through their environment (Ding et al., 2017). The photoperiod, which affects the overall physiology of birds, is believed to influence the gut microflora. A study by Wang (2018) discovered that chickens reared under 12.5L:11.5D had higher colonization of opportunistic pathogenic Aeriscardovia and Delftia, in their ceca. In contrast, birds exposed to 8L:16D of light had a greater relative abundance of Lactococcus, a beneficial bacterium, in their ceca (Wang et al., 2018). It is suggested that disruptions in the circadian rhythm hinder the establishment of stable microbial communities in the intestines (Hieke et al., 2019). An extended dark period promotes greater diversity in the gut microflora. Birds reared under a 12L:12D light-dark cycle exhibited significantly higher diversity in their intestinal microbiome compared to those reared under a 23L:1D cycle. Furthermore, this study demonstrated improved microbial acquisition and oscillation in the ceca of birds raised under a 12L:12D cycle (Hieke et al., 2019). The role of the photoperiod on poultry microbiome is a new area of research, and the mechanisms involved in altering microbial activity need to be further researched.

Light During Incubation

Studies have shown that rhythmic melatonin secretion in chicken embryos during incubation depends on the occurrence of environmental stimuli (Csernus et al., 2007; Csernus et al., 1998). Chicken embryos can start developing circadian rhythms around 18 days of incubation, and this effect can be enhanced by providing a light/dark cycle during incubation. Exposure to a light/dark cycle during incubation can stimulate arylalkylamine N-acetyltransferase, the enzyme responsible for melatonin production, as early as ten days of incubation (Zeman et al., 1999; Zeman and Illnerová, 1990; Zeman et al., 2004). Furthermore, N-acetyltransferase activity in the pineal gland is higher during dark periods; in embryos exposed to a light/dark cycle, it can be twice as high during the dark period (Binkley and Geller, 1975). By 18 days of incubation, chicken embryos exposed to light/dark cycles can exhibit rhythmic melatonin production (Zeman et al., 1999; Binkley and Mosher, 1984). On Day 19 of incubation, chicken embryos incubated under (12L:12D) showed higher melatonin levels compared to those incubated under constant dark (0L:24D) or (1L:23D), though no differences were observed at five weeks after hatching (Archer and Mench, 2014). Providing a light schedule during egg incubation reduces fear responses in chicks (Archer and Mench, 2017). Chicks incubated with a light/dark schedule exhibited shorter tonic immobility times through latencies to right themselves and lower inversion intensity at 42 days of age than those without a light/dark schedule (Archer and Mench, 2017). These results did not indicate that a specific duration of light or dark was superior (Archer and Mench, 2017).

PHOTOPERIOD

Performance

The photoperiod is an important factor influencing poultry performance, welfare, and health. While broilers were historically raised under continuous or near continuous light, recent decades have seen a growing recognition of the benefits a dark period offers (Classen et al., 1991). As a result, incorporating a dark period into broiler lighting programs has become commonplace (Scanes and Christensen, 2019). A "dark period," also called a scotophase, signifies the dark duration within a 24-hour period. It is important to highlight that in many studies, the treatment lighting period is initiated when birds are 7 or 8 days old. Consequently, further investigation is required to address the early lighting program research gap.

It is generally accepted that broiler body weight and feed consumption increase with increased light period (Classen, 2004a). Dark periods starting between Days 2 and 7 decrease early feed consumption and body weight (Özkan et al., 2006; Classen, 2004a). Mortality increases linearly with day length, and feed efficiency is consistently better with birds given a dark period (Ingram and Hatten, 2000; Schwean-Lardner et al., 2012b). Though an initial decrease in body weight and feed consumption is expected when comparing birds with and without a dark period, research has shown that broilers exhibit compensatory growth. The drop in feed consumption and body weight can be expected for the first two weeks after the dark period is initiated; however, by the end of the flock, broilers have repeatedly shown to catch up and show no differences in performance compared to birds reared with continuous or near continuous light (Classen et al., 2004a; Lien et al., 2007). In recent years, it has been considered that day length does not significantly affect performance under optimal conditions by the end of

the flock (Olanrewaju et al., 2018). More current research comparing dark periods starting at the day of placement found no differences in body weight, body weight gain, feed consumption, feed conversion ratio (FCR), or crop fill for 14 days comparing 20L:4D and 23L:1D (Magee et al., 2022; Olanrewaju et al., 2018).

Step-up lighting programs

It was eventually accepted that the leg health issues and metabolic disorders associated with giving birds constant light throughout growout negated any performance benefits; researchers began experimenting with novel lighting programs in the 1990s (Classen et al., 1991). One of these programs was the step-up lighting program, where birds were subjected to near-continuous light during brooding. Afterward, birds received extended dark periods, that slowly decreased as they matured. These gradually increasing light period schemes have been shown to decrease body weight initially; however, at the end of the flock, they show no difference or higher body weights and reduce mortality compared to birds under continuous/near continuous light (Riddell et al., 1992; Rozenboim et al., 1999a; Charles et al., 1992).

Step-down lighting programs

Step-down lighting programs were another non-continuous lighting program researched heavily in the 1990s and early 2000s. This lighting program involves brooding chicks with continuous to near continuous light and gradually increasing the dark period throughout growout. Step-down lighting programs initially decreased bird body weight and feed consumption compared to continuous light; however, they have consistently shown no differences by the end of the flock (Classen et al., 1991; Downs et al., 2006; Lien et al., 2009). These lighting programs

decrease mortality and skeletal diseases in broilers drastically; because of this, they have been heavily implemented in modern commercial production (Scanes and Christensen, 2019).

Dawn/Dusk Simulation

A dawn/dusk simulation refers to a lighting program that, at a set time point, gradually decreases the light intensity until the dark period and gradually increases the light intensity as the lights turn on. Broiler lighting systems typically turn off and on as the dark period starts and stops. Broilers learn to adapt and expect these controlled lighting programs. Research on dawn/dusk cycles gradually increases or decreases the light intensity as the lights turn off and on, specifically in broilers, is limited compared to studies on laying hens. Exploring this area further could improve broilers' productivity and welfare (Tanaka and Hurnik, 1991; James et al., 2020). Research has shown that longer dawn/dusk cycles in intermittent lighting systems, equal to or exceeding 30 minutes, exhibit better welfare characteristics with lower corticosterone and H/L ratio levels and higher feed consumption. Short dawn/dusk cycles in intermittent lighting systems exhibit higher body weight, corticosterone, and H/L ratio levels in broilers (Nelson et al., 2020; Lewis et al., 2010). Birds also exhibit increased activity during a step-up (dawn) lighting function compared to a step-down (dusk) function (Kristensen et al., 2006b).

Physiology, and Behavior

Dark periods have been found to significantly reduce mortality related to sudden death syndrome in poultry (Schwean-Lardner et al., 2014). Dark periods can also result in lower leg abnormalities and gait scores, indicating better bone health (Renden et al., 1996; Schwean-Lardner et al., 2014). Long-lived layers, turkeys, and broiler breeders develop eye abnormalities

such as the loss of corneal convexity, corneal flattening, buphthalmos, hyperopia, and blindness when reared under continuous or near continuous light (Li et al., 1995; Ashton et al., 1973; Cummings et al., 1986). The present hypothesis for explaining the benefits of a dark period is the role of melatonin. A daily dark period is required for the rhythmic secretion of melatonin, adding to the development of the circadian rhythm and resting/activity cycles (Tanabe and Nakamura, 1983; Schwean-Lardner et al., 2014).

Light/dark cycles affect many physiological responses in poultry. Constant exposure to light can disrupt circadian rhythms and damage photoreceptors (Aschoff, 1989). Studies involving layers, broilers, and Japanese quail exhibit higher stress responses to shorter dark periods through higher corticosterone, H/L ratios, longer tonic immobility durations (a fear response test correlated to longer immobility indicating more stress/fear), adrenal weights, and plasma-free amino acids (Campo et al., 2007; Zulkifi et al., 1998; Freeman et al., 1981; Moore and Siopes, 2000). In more recent studies, no differences were observed in corticosterone levels comparing lighting treatments, suggesting lighting programs may have little stress response to birds raised under optimal conditions (Magee et al., 2023; Olanrewaju et al., 2018). Birds exposed to dark periods have improved immune response via higher antibody titers, nonspecific cellular immune responses, and humoral immune responses in chickens and Japanese quail (Moore and Siopes, 2000). While the quail groups reared under constant light exhibited immunosuppression (Moore and Siopes, 2000; Kirby and Froman, 1991). Broiler chicks raised under a lighting program with over four hours of dark exhibited higher melatonin during the dark period and less fluctuating baseline melatonin levels compared to chicks reared under continuous or near continuous light (Magee et al., 2023; Özkan et al., 2006; Schwean-Lardner et al., 2014).

Continuous (24-hour) light throughout the flock has demonstrated significant health and welfare implications. Sudden death syndrome, a growth/metabolic-related condition, is more prevalent in birds exposed to continuous light schedules (Classen et al., 2004b; Riddell and Classen, 1992). The length of the day is correlated to instances of mortality and leg issues, as indicated by gait scores (Schwean-Lardner et al., 2013). Providing broilers with a dark period has consistently been shown to reduce gait scores, leg abnormalities, and tibial dyschondroplasia (Blair et al., 1993; Sanotra et al., 2002; Sorensen et al., 1999; Karaarslan and Nazlıgül, 2018). Dark periods have also been shown to reduce culling due to leg issues.

In some cases, bone ash content is also increased in broilers given a dark period, indicating higher bone density (Brickett et al., 2007). It is theorized that providing broilers with dark periods stimulates activity patterns. Increased bird activity is associated with better skeletal health and promotes bone development and repair (Riddell and Classen, 1992; Brickett et al., 2007).

Behavioral patterns in birds are closely tied to the light/dark cycle, with sleep and rest primarily occurring during the dark period (Appleby et al., 2004). While birds can sleep during light periods, the quality and duration of their sleep are typically inadequate (Ayala-Guerrero et al., 2003; Bonnet, 2005). Light plays a significant role in regulating bird activity (Kristensen et al., 2006a). Constant light programs negatively impact the birds' circadian rhythms, modifying behaviors such as standing, pecking, scratching, and vertical wing shaking (Sanotra et al., 2001). Birds reared with longer dark periods exhibit more behaviors like preening, wing shaking, litter picking, and dustbathing, which are generally considered comfort behaviors, as well as shorter latencies to lay. Longer dark periods tend to also make birds more active during the light (Sanotra et al., 2002). Longer day lengths are associated with decreased overall bird activity,

which is linked to reduced bone health and behaviors associated with bird comfort (Bayram et al., 2010; Schwean-Lardner et al., 2012a). Laying hens reared under continuous light tend to have higher fear responses during tonic immobility tests than birds reared under dark periods (Campo et al., 2007; Campo and Davila, 2002). Research suggests longer daylengths reduce behaviors such as preening, stretching, dustbathing, and foraging in broilers and turkeys (Schwean-Lardner et al., 2016). Prolonged day length disrupts the diurnal patterns of poultry (Manser, 1996), including eating and drinking patterns. Birds tend to exhibit increased feeding behavior when the lights come on after fasting at night and before the dark period in anticipation of another fasting period (May and Lott, 1994). Broilers raised under constant light do not establish a strong feeding rhythm (Ferrante et al., 2006), but they can adjust their feeding patterns in response to changing photoperiods (Duve et al., 2011). The most feeding activity in birds typically occurs within the first hour after the lights turn on (Aldridge et al., 2021a). In broilers, a longer photoperiod is associated with a lower percentage of time spent standing, walking, feeding, and drinking at 27 and 42 days of age (Schwean-Lardner et al., 2012a). As the lights come on and in anticipation of their upcoming dark period, broilers exhibit increased activity in terms of walking, standing, feeding, and drinking. Furthermore, longer dark periods correspond to higher levels of activity. Birds exposed to near continuous light (<4 hours) show significantly lower daytime activity compared to those with longer dark periods (Schwean-Lardner et al., 2014).

INTERMITTENT LIGHTING

Performance

Intermittent lighting programs, which involve a 24-hour photo cycle with multiple dark periods, have been extensively studied. These programs have been reported to enhance broiler performance and reduce the occurrence of leg abnormalities and sudden death syndrome (Rahimi et al., 2005; Ohtani and Leeson, 2000). Intermittent lighting programs yield similar results to step-down lighting schemes. The concept behind intermittent lighting is that broilers can systematically eat and drink during the light period while resting and conserving energy during the dark period (Robbins et al., 1984; Simons and Haye, 1985). Broilers reared under intermittent light have had mortality from sudden death syndrome decreased by 37% compared to continuous lighting (Ononiwu et al., 1979). Intermittent lighting programs have been shown to depress broiler growth initially but show no differences by the end of the flock (Ohtani and Leeson, 2000). Various intermittent lighting schemes have a significant improvement in feed conversion when compared to near-continuous light and programs with dark periods over eight hours; however, no differences in feed consumption or body weight were observed (Abbas et al., 2008; Apeldoorn et al., 1999; Rahimi et al., 2005; Buys et al., 1998). Studies have reported increased body weight and feed consumption with birds reared under intermittent lighting schedules with no differences in feed conversion (Onbasilar et al., 2007; Petek et al., 2005; Duve et al., 2011).

On the other hand, other studies found no differences between intermittent and continuous light with decreased performance compared to a step-down lighting program (Renden et al., 1996; Newberry et al., 1985). Intermittent lighting studies often do not report other environmental conditions that may affect performance, such as temperature and relative

humidity. Some studies do not compare lighting schemes with equal light and dark hours; this may contribute to variability in performance results between studies. Many of these studies do not test treatments against a continuous dark period, so the benefits of intermittent light compared to simply having dark periods have yet to be researched extensively.

Physiological responses

Several studies have investigated the effects of intermittent lighting on broiler chickens. Plasma corticosterone levels have been reported to be lowered in broilers raised under intermittent lighting (Buckland et al., 1976; Abbas et al., 2008). Lower overall heat production has been observed in birds with intermittent light (1 hour L: 3 hours D). Birds' heat production follows the light/dark cycles of the lighting scheme. The heat production of birds under intermittent light continually fluctuates, increases as the lights turn on, and decreases as the lights turn off. Heat production stays relatively consistent in birds reared under continuous light (Buyse et al., 1994). Intermittent light is suggested to influence the blood chemistry of the bird. Some studies have found higher white blood cell counts, plasma T3 levels, serum melatonin, serum superoxide dismutase, and T-lymphocyte proliferation compared to broilers reared under continuous light (Zheng et al., 2013; Abbas et al., 2008; Kiger et al., 2000; Kuhn et al., 1996).

Intermittent light has been reported to reduce leg abnormalities. Reduced gait scores and tibial dyschondroplasia have been observed in birds reared under intermittent light compared to continuous light. An increase in bird activity is normally observed in intermittent lighting treatment, which is thought to contribute to better leg health (Wilson et al., 1984; Buckland et al., 1976; Wong-Valle et al., 1993). Alternatively, some research has found no differences in tibial dyschondroplasia, carcass characteristics, or relative asymmetry (Onbasilar et al., 2007). The

studies that found a difference in skeletal health also reported better performance, whereas the study that reported no differences in leg health also had no differences in performance. The rate of growth may have a greater contribution to the occurrence of leg abnormalities than the lighting program.

Broilers develop a modified diurnal and circadian rhythm when reared on intermittent light exhibited by their feeding behavior (Classen, 2004a; Classen et al., 2004b). Broilers will adjust their feeding patterns to match the photoperiod. Birds with uninterrupted light periods exhibited more feeding activity throughout the day, while the intermittent lighting group had higher feeding activity 20 minutes before and after each dark period (Duve et al., 2011). Lower broiler fear response has been observed in birds reared under intermittent lighting exhibited by shorter tonic immobility test times (Onbasilar et al., 2007).

INTENSITY

Performance

Light intensity plays a significant role in shaping bird behavior and is commonly used as a management tool. Increasing light intensity stimulates bird activity, such as walking, standing, feather pecking, cannibalism, and fighting (Alvino et al., 2009b; Newberry et al., 1986). In commercial broiler production, it is common practice to start with a high light intensity and gradually decrease it as the flock progresses; this allows newly hatched chicks to explore their environment and find food and water (Bell et al., 2002). Reducing light intensity as birds age promotes greater energy utilization for weight gain and decreases activity (Deaton et al., 1976). Light intensities exceeding 20 lux are associated with decreased body weight due to increased

energy expenditure on activity, whereas light intensities below 20 lux are linked to increased body weight and are theorized to be caused by decreased activity (Buyse et al., 1996; Charles et al., 1992; Ahmad et al., 2011). Extremely low light intensities below 1 lux can lead to metabolic disorders, skeletal disorders, retinal degradation, blindness, and reduced feed intake in broilers and turkeys (Ashton et al., 1973; Siopes et al., 1984). Some studies have compared different light intensities' effects on lean carcass weight, breasts, tenders, wings, legs, and fat deposition and found they were heavier in light intensities less than 2 lux; however, these differences are relative to body weight and may not be directly related to the light intensity (Lien et al., 2008; Deaton et al., 1988). Other studies have shown no differences in body weight, body weight gain, feed conversion, corticosterone, or mortality when comparing light intensity. These same studies did show that light intensities between 5 to 20 lux had better carcass yield (Olanrewaju et al., 2011; Deep et al., 2010). Research conducted on brooding light intensity found that mortality decreased while body weight, feed consumption, and feed efficiency increased with increasing light intensity for the first 14 days. The paper suggests a minimum of 5 lux should reduce early chick mortality (Deep et al., 2013). Recent research comparing preference lighting programs suggests that feed efficiency is better in 5 and 10-lux groups and broilers given choice lux groups than broilers reared at 20 lux (Aldridge et al., 2022). Having a uniform light intensity distribution in a commercial broiler house is difficult. Often, sunlight will come into the chicken house as the ventilation fans turn on and off. A study tested birds exposed to variable light intensity through fans. It showed no differences in body weight or BWG compared to birds with constant light intensity, except the variable light group exhibited higher FCR (Purswell and Olanrewaju, 2017).

Physiological responses

Studies in broilers and turkeys have consistently found birds reared with low light intensities under 1 lux compared to those exposed to intensities above 5 lux potentially leading to myopia (nearsightedness) (Siopes et al., 1984; Blatchford et al., 2009; Deep et al., 2013; Rault et al., 2017). Corticosterone levels increase with light intensity (Kang et al., 2020). Light intensities do not affect melatonin concentrations if the photoperiod length is the same (Deep et al., 2012). Varying light intensities have shown to influence blood chemistry such as pH, Na+, K+, Cl+, pCO2, Hb, and Hct in broiler chickens. Studies by the same authors concluded that changes in performance may have a more significant effect than light intensity and photoperiod length (Olanrewaju et al., 2012; Olanrewaju et al., 2013). Leg abnormalities might be observed more in higher light intensities. These leg issues are most likely correlated to body weight rather than light intensity (Kristensen et al., 2006a; Rault et al., 2017).

Light intensity has a major effect on bird activity. Foraging, pecking, and aggressive behavior, as well as overall bird movement, increases with increasing light intensity (Newberry et al., 1986; Blatchford et al., 2009; Deep et al., 2012; Alvino et al., 2009a; Kristensen et al., 2007). The greater the difference in light intensity between the light and dark period, the more active the birds were during the day and the more they rested during the dark period. Alternatively, lowering the light intensity will decrease light-period activity and increase dark-period activity. The contrast in light intensity between the light and dark periods has a greater influence on bird behavior than the length of the dark/light period (Alvino et al., 2009b; Blatchford et al., 2012). Traditionally, broiler house lights are positioned to provide uniform light intensity across the house, aiming to eliminate dark or shadow areas (Bell et al., 2002). Recent research has explored the placement of strategic light intensities and their effects on bird

behavior. Chickens prefer to eat in higher light intensities and rest in lower intensities. Studies have looked at placing supplementary lights over the feeders and drinkers, resulting in lower light intensities in non-feeder and drinker areas and higher intensities in the feeder/drinker area. The findings suggest that broilers prefer and consume more feed in areas with higher light intensities than the feeders and drinkers in low light intensity (Aldridge et al., 2021a; Raccoursier et al., 2019).

SPECTRUM

Performance

A significant amount of research has been conducted to investigate the effects of different responses of light wavelengths on chickens' performance and physiological function, considering their wide spectral range of visible light. Various studies have shown that light at different spectra has varying impacts on performance and behavioral stimuli (Lewis and Morris, 2000). Specifically, monochromatic lighting studies have revealed that different wavelengths or colors affect bird performance differently (Parvin et al., 2014). Red lights have been found to increase aggression, cannibalism, and feather pecking, while blue lights are associated with a calming effect on broilers (Lewis and Morris, 1998; Prayitno et al., 1997a). Chicken reproduction is stimulated by longer orange/red wavelengths that can easily penetrate the skull (Lewis and Morris, 2006). These longer wavelengths are not commonly used in broilers as they are typically slaughtered before sexual maturation (Hassan et al., 2013).

On the other hand, shorter blue/green wavelengths have been reported to stimulate growth (Lewis and Morris, 2006). Specifically, research findings indicate that broilers reared

under green and blue monochromatic lights tend to have higher body weights compared to birds reared under red and white lights. Birds exposed to green light perform better, displaying lower feed to gain rates and higher meat yield (Rozenboim et al., 1999b; Cao et al., 2008; Halevy et al., 1998; Cao et al., 2012). On the other hand, other studies indicate there are no significant differences in performance when comparing birds reared under red, white, green, or blue monochromatic light (Rozenboim et al., 1999b; Senaratna et al., 2008; Franco et al., 2022a). Some researchers have reported that brooding broilers under green light and transitioning them to blue light by days 10-20 will accelerate growth compared to staying on one specific monochromatic light (Cao et al., 2012; Rozenboim et al., 2004; Oke et al., 2021). There is a trend in significantly higher body weights by the end of growout in birds reared under full-spectrum light compared to monochromatic light (Riber, 2015). More recent research has been conducted to provide broilers with a light spectrum that includes ultraviolet. There is a trend in better performance in birds exposed to ultraviolet, though these differences in performance are not significant (House et al., 2020; James et al., 2020; Olanrewaju et al., 2016). Overall, the performance differences when comparing birds reared under different light spectra are small; it is suggested that economics should be the driving factor when choosing which wavelength to rear birds under (Purswell et al., 2018; Lewis and Morris, 2000). Many spectrum studies presented did not indicate that light intensity was corrected for the specific monochromatic wavelength associated with the visible light spectrum of chickens. This variation in light intensity may be a factor in the inconsistent results in these studies.

Physiological responses

Light spectrum has been shown to influence many physiological aspects in poultry. Birds reared under green and blue lights have exhibited higher testosterone, satellite cell counts, and myofiber growth than those reared under white and red lights (Cao et al., 2008). Researchers theorized that the increased body weight is due to these physiological responses (Halevy et al., 1998; Cao et al., 2008; Rozenboim et al., 1999a). Blue light has also been found to decrease corticosterone levels and increase melatonin production, potentially reducing oxidative damage (Abdel-Azeem and Borham, 2018; Abdo et al., 2017). On the other hand, monochromatic green light increases clocking genes expression associated with melatonin production and arylalkylamine N-acetyltransferase expression. Specifically, monochromatic green light has been observed to inhibit gonadotropin-releasing hormone (GNRH-I) expression, leading to increased melatonin secretion and stimulation of the melatonin receptor pathway in the chick brain (Jiang et al., 2016; Cao et al., 2017). While monochromatic red light has been found to suppress melatonin synthesis (Liu et al., 2019; Zhang et al., 2017; Jin et al., 2011). Studies in broilers and turkeys reared under blue light have shown lower H/L ratios and reduced fear response compared to white and green lights (Franco et al., 2022b; Scott and Siopes, 1994). Birds reared under blue light have higher T-lymphocytes and increased humoral immune response to Newcastle disease vaccine than birds reared under red light; however, the mechanism for this is unclear (Xie et al., 2008). The overall differences in the physiological responses to different spectra are questionable. Like performance, many of these studies may not have corrected for the specific monochromatic wavelength associated with the visible light spectrum of chickens. Again, it is suggested that the driving force behind light selection should be economical (Olanrewaju et al., 2019; Lewis and Morris, 2000).

A study comparing red and blue monochromatic light showed that red light increased behaviors such as standing, walking, drinking, wing stretching, and aggression while decreasing dozing, sleeping, and pecking, as compared to blue light. (Prayitno, 1997b). Alternatively, another study examined birds' preferences in different colored-lighted areas and found that birds tended to stay longer and consume more in green-lighted areas. (Khosravinia, 2007; Mendes et al., 2013).

Summary

Incorporating dark periods into broiler lighting programs has proven beneficial for performance and well-being. Maintaining continuous 24-hour lighting can lead to various health issues in broilers, including leg disorders and sudden death syndrome, which dark periods can help alleviate. The secretion of melatonin, regulating circadian rhythms, and the modulation of immune responses in poultry are all influenced by the cycling of light and darkness. Light intensity plays a crucial role in shaping poultry behavior: higher light intensities tend to increase activity levels, while lower intensities promote rest and feeding. Studies exploring light wavelengths have revealed a range of effects on poultry performance and behavior. It is worth mentioning that the differences in performance and physiological responses to different spectra are often subtle, with economic considerations often guiding the selection of light sources. In conclusion, it is evident that the photoperiod, light intensity, and light wavelength significantly impact poultry performance, wellbeing, and physiology. Implementing optimal lighting practices can not only enhance productivity but also ensure the well-being of broilers.

CHAPTER 2

STATEMENT OF PURPOSE

This literature review offers a comprehensive overview of how light factors such as photoperiod, intensity, and spectrum influence poultry. With the exception of one case study conducted in 1966 (Skoglund., 1966), virtually no research on photoperiod during the first week of growout has been conducted. The topic this dissertation concentrates on the influence of photoperiod during the initial week of life on broilers. Previous research has demonstrated that lighting programs can impact bird performance and regulate physiological factors like corticosterone, melatonin, and superoxide dismutase. These programs have been linked to positive effects on broiler leg health, particularly in terms of gait scores and tibial breaking strength in older birds. The current study aims to explore the impact of photoperiod during the first week of age on these aspects in broilers.

Providing continuous or near continuous light during brooding is based on the premise that it will enhance performance and reduce early chick mortality by allowing chicks more time for feeding. Even though this is a common practice, minimal research supports these practices. Existing research on lighting programs primarily focuses on photoperiod, light intensity, and light spectrum initiated post-brooding. The well-established body of knowledge of lighting programs consistently shows the benefits of dark periods on broilers after brooding. This contrasts the perception that providing chicks with continuous light is beneficial. With all the

research supporting the use of dark periods in older birds, it can be argued that providing chicks with a dark period could be advantageous.

Many welfare guidelines require at least four hours of darkness after the brooding period. This requirement stems from the understanding that a minimum of four hours of uninterrupted darkness is necessary for chickens to complete a full sleep and melatonin cycle, an aspect of their physiological well-being. Given our evolving understanding of circadian rhythm development and disruption in poultry, exposing chicks to continuous light during brooding may raise welfare concerns in the future. It is conceivable that future welfare guidelines will require the inclusion of a dark period during the brooding phase.

Integrators are unlikely to adopt a four or six-hour dark period during brooding without proper research as to its effects on broiler weight gain, feed consumption, feed efficiency, leg health, and physiological function. This research aims to investigate how various photoperiods during brooding influence broiler performance and physiological responses.

CHAPTER 3

MATERIALS AND METHODS

Three experiments were conducted at the University of Georgia Poultry Research Center. The University of Georgia Institutional Animal Care and Use Committee (IACUC # A2022 05-001-Y1-A0) approved all procedures in this study. A total of six rooms were utilized in each experiment. Three rooms served as controls with no dark period (24L:0D) from Day 0 to Day 7 while the birds in the other three rooms were given a dark period from Day 0 to Day 7. The fourth experiment was conducted on a commercial broiler farm. A total of four houses were utilized in this experiment. Two houses served as controls with 24L:0D from placement to Day 5, then 20L:4D until Day 7, and then set to 18L:6D from Day 8 until the end of each trial on Day 42. The other two houses served as treatments with 18L:6D from placement until the end of each trial on Day 42.

The light treatments for each Experiment were:

- Experiment 1. A 14-day experiment analyzing 24L:0D vs. 20L:4D
- Experiment 2. A 21-day experiment analyzing 24L:0D vs. 18L:6D
- Experiment 3. A 42-day experiment analyzing 24L:0D vs. 18L:6D
- Experiment 4. A 42-day experiment analyzing 24L:0D vs. 18L:6D in commercial broiler houses

Birds in the control rooms were provided the same dark period as the treatment birds starting on Day 8. All rooms (Experiments 1, 2, and 3) had a 30-minute dawn/dusk cycle before and after each dark period.

Facilities

Each trial utilized six 6.1 m X 7.3 m rooms with four 1.5 m X 1.2 m pens resulting in a total of 24 pens. Each room was equipped with two exhaust fans 22.86 cm AT09Z2CP (1,070 cfm @ 0.254 cm) and 45.72 cm AT18ZCP (4,120 cfm @ 0.245), (Munters Corporation, Lansing, MI, United States), a 100,000 Btu/hr forced air furnace (LB White - Guardian) and two continuously operating 45.72 cm circulation fans (AT18 4,360 cfm @ 0.254 cm) (Munters Corporation, Lansing, MI, United States). Light was provided by six dimmable LED bulbs (General Electric relax LED Comfortable Soft White Light A19 Medium Base, 2700K, 800 lumens) controlled by an automatic digital light dimmer (PLS-2400 MR4, Precision Lighting Systems, Hot Springs, AR, United States of America). Light intensity was set to 40 Lux at floor level in each pen throughout the trial. Water was provided by a one meter long Ziggity drinker line equipped with three nipples (Ziggity Systems INC. Middlebury, IN, United States of America) and fed utilizing Chore-Time Konavi feeders (Chore-Time Milford, IN, United States of America). Each pen had approximately 8 cm of fresh pine shavings covering the concrete floor. The litter was cleaned out and fresh bedding was applied in between each trial.

Bird Management

On day of hatch, Cobb byproduct male broiler chicks were weighed and separated into nine weight categories: <32, 32-34, 35-37, 38-40, 41-43, 44-46, 47-49, 49-51, and >51 grams. Chicks were then evenly selected from each weight category and placed into pens to minimize differences in initial pen weights. The environmental controllers in all rooms were programmed with the same temperature curve (Table 1). Throughout the experiments, a starter (0-21D), grower (22-35D), and finisher (36-42D) feed (Table 2) was offered ad libitum. Body weights were determined by weighing birds on Days 0, 3, 7, 10, and weekly thereafter (depending on the

specific experiment). Feeders were weighed every time bird weights were measured and were used to calculate feed consumption and feed to gain ratio. Mortality and culled birds were necropsied and recorded daily.

Blood Sampling Procedure

Weekly blood samples were taken from one random bird in each pen during the middle of the dark period (~3am) and the middle of the light period (~3pm). A red headlamp was used to collect samples in the dark period to keep the birds calm. Blood was drawn via aortic cardiac puncture and stored in sodium heparin tubes; birds were euthanized by cervical dislocation after the sample was taken. Each blood sample was taken within a minute of handling the bird as to not confound any sample due to stress. Blood was then centrifuged at 1,000 rpm and 4°C. The blood plasma was then collected off and stored in labeled microcentrifuge tubes at -20°C for later analysis. Plasma samples were thawed and analyzed for corticosterone, superoxide dismutase, and melatonin using a microplate spectrophotometer (Victor Nivo Multimode Microplate Reader, PerkinElmer. Waltham, MA, United States of America).

Table 1. Room temperature setpoints used to calculate curves guidelines.

Bird Age (Day)	**Experiment 1 Room Temperature (˚C)**	**Experiment 2 Room Temperature (˚C)**	**Experiment 3 Room Temperature (˚C)**
0	33.9	33.9	33.9
7	30.0	30.0	30.0
14	27.8	27.8	27.8
21	-	26.1	26.1
28	-	-	23.3
35	-	-	21.1

Table 2. Starter, grower, and finisher diet formulations for Experiments 1, 2, and 3.

Ingredient	Starter diet*	Grower diet	Finisher
	%		
Corn	55.241	61.333	64.353
Soybean meal	38.178	32.137	29.287
Soybean oil	2.073	2.289	2.496
Dicalcium phosphate (18.5%)	1.621	1.506	1.317
Limestone	1.341	1.271	1.169
Salt	0.428	0.430	0.402
DL-Methionine	0.419	0.401	0.379
L-Lysine HCL	0.263	0.239	0.223
Choline chloride (60%)	0.247	0.216	0.193
L-Threonine	0.008	-	-
Vitamin premix[1]	0.100	0.100	0.100
Trace mineral premix[2]	0.080	0.080	0.080
Calculated analysis			
Crude protein (%)	22.091	19.784	18.716
M.E. (Kcal/Kg)	2975.000	3050.000	3100.000
Calcium (%)	0.900	0.840	0.760
Phosphorus (%)	0.690	0.646	0.600
Available Phosphorus (%)	0.450	0.420	0.381
Choline (mg/kg)	1700.000	1600.000	1499.998
Sodium (%)	0.200	0.200	0.180
Digestible - Met + Cys (%)	0.910	0.850	0.830
Digestible - Lysine (%)	1.220	1.050	0.970
Digestible - Threonine (%)	0.830	0.724	0.676
Digestible - Isoleucine (%)	0.834	0.738	0.694
Digestible - Valine (%)	0.891	0.800	0.757
Digestible - Arginine (%)	1.336	1.176	1.101

*Studies 1 and 2 the birds were only fed starter feed

[1]Supplied per kilogram of diet: vitamin A, 5,511 IU; vitamin D3, 1,102 ICU; Vitamin E, 11.02 IU; vitamin B12, 0.01 mg; Biotin, 0.11 mg; Menadione, 1.1 mg; Thiamine, 2.21 mg; Riboflavin, 4.41 mg; d-Pantothenic Acid, 11.02 mg; Vitamin B6, 2.21 mg; Niacin, 44.09 mg; Folic Acid, 0.55 mg; Choline, 191.36 mg.

[2] Supplied per kilogram of diet: Mn, 107.2 mg; Zn, 85.6 mg; Mg, 21.44 mg; Fe, 21.04; Cu, 3.2 mg; I, 0.8 mg; Se, 0.32 mg.

Experiment 1

A 14-day experiment analyzing 24L:0D vs. 20L:4D Day 0 – Day 7**.** In the first experiment, 22 chicks were placed in each pen (four per replicate room) resulting in an initial stocking density of 0.09 m^2/bird, with a final stocking density of 0.12 m^2/bird after euthanizing birds for blood sampling each week. Three rooms were assigned as control with 24L:0D from placement to Day 7 and then set to 20L:4D from Day 8 until the end of each trial on Day 14. The other three rooms were assigned as treatment with 20L:4D from placement until the end of each trial on Day 14. Pen weights were taken on Days 0, 3, 7, 10, and 14. Birds were individually weighed on Days 0, 7, and 14 to calculate uniformity. Corticosterone and melatonin were assayed using ELISA kits (Cayman Chemicals Corticosterone ELISA Cat#501320) and (Genway Biotech Melatonin ELISA Kit GWB-7A8704) according to the manufacturer's instructions. Day 7 plasma samples analyzed for melatonin were diluted by a factor 1:2 to be within the range of the ELISA kit. This experiment consisted of three replicate trials and the light treatments were alternated by room to account for possible room effects.

Experiment 2

A 21-day experiment analyzing 24L:0D vs. 18L:6D Day 0 – Day 7. In the second experiment 23 chicks were placed in each pen (four per replicate room) resulting in an initial stocking density of 0.08 m^2/bird, with a final stocking density of 0.11 m^2/bird after euthanizing birds for blood sampling each week. Three rooms were assigned as control with 24L:0D from placement to Day 7 and then set to 18L:6D from Day 8 until the end of each trial on Day 21. The other three rooms were assigned as treatment with 18L:6D from placement until the end of each trial on Day 21. Birds were individually weighed on Days 0, 7, 14, and 21 to calculate uniformity. On Days 3 and 10 birds were weighed by total pen weight. The same corticosterone

ELISA kit from Experiment 1 was used. A different melatonin ELISA kit was used in this experiment because it was found to be more accurate and economical; this ELISA was validated by the authors (MP Biomedicals Direct Melatonin EIA Kit CAT# 07P534A). The enzymatic antioxidant associated, superoxide dismutase, was added to this experiment (Cayman Chemicals SOD Assay Kit CAT #706002). Days 7 and 14 plasma samples analyzed for melatonin were diluted by a factor of 1:2.5 to be within the range of the ELISA kit. All plasma assay kits were used according to the manufacturer's instructions. This experiment consisted of two replicate trials and the light treatments were alternated by room to account for possible room effects.

Experiment 3

A 42 day experiment analyzing 24L:0D vs. 18L:6D Day 0 – Day 7. In the third experiment 28 chicks were placed in each pen (four per replicate room) resulting in an initial stocking density of 0.07 m^2/bird, with a final stocking density of 0.12 m^2/bird after euthanizing birds blood sampling each week. Three rooms were assigned as control with 24L:0D from placement to Day 7 and then set to 18L:6D from Day 8 until the end of each trial on Day 42. The other three rooms were assigned as treatment with 18L:6D from placement until the end of each trial on Day 42. Birds were individually weighed on Days 0, 7, 14, 21, and 42 to calculate uniformity. On Days 3, 10, 28, and 35, birds were weighed by total pen weight. The same blood plasma assays conducted in this experiment were repeated from Experiment 2.

On Day 21, two birds that showed no visual sign of leg problems were selected from each pen and banded. This was done to observe any development of leg issues after three weeks of age. On Day 42, before the completion of each trial, the two banded birds from each pen (total = 24) were observed for gait scores according to a Three-Point Gait-Scoring System (Webster et al., 2008). After gait scoring, each bird was cervically dislocated, tibias were collected, and all

tissue was removed from the bones. One tibia from each bird was assayed for bone-breaking strength via a servo hydraulic testing machine (Stable Micro Systems' TA.HDplusC Contact Texture Analyzer, Godalming Surrey GU7 1YL United Kingdom), as described in (Regmi et al., 2015). This experiment was replicated two times and the light treatments were alternated by room to account for room effects.

Experiment 4

A 42-day experiment analyzing 24L:0D vs. 18L:6D Day 0 – Day 5. This experiment was conducted across three consecutive flocks in four commercial broiler houses, each measuring 15.24 meters in width and 152.4 meters in length. Before the first trial the grower cleaned out and applied approximately three inches of pine shavings on the packed dirt floor of the broiler houses. Before the second and third trial the grower decaked the houses and broilers were reared on used litter. Chicks were placed with an initial stocking density of 0.08 m2/bird, 0.09 m2/bird, and 0.08 m2/bird for the first, second, and third flocks, respectively. Two houses were assigned as control with 24L:0D from placement to Day 5, then 20L:4D until Day 7, and then set to 18L:6D from Day 8 until the end of each trial on Day 42. The other two houses were assigned as treatment with 18L:6D from placement until the end of each trial on Day 42. Light treatments were alternated by house to account for house effects across the three trials (Table 3).

Birds were individually weighed 200 birds per house (100 birds in the front half and 100 birds in the back half) on Days 1, 3, 7, 14, 21, 28, 35, and 42 to calculate uniformity and average bird weight. In addition, the farmer's mortality and cull sheets were recorded weekly. A transect sampling method was used on Day 42 for the second and third flocks. This method was adapted from previous studies (BenSassi et al., 2019; Marchewka et al., 2013). Trained observers followed designated paths within each house, as indicated by the black dashed lines in Figure 1.

During these observations, the observers tallied the number of birds that could not walk visually observed along each transect. Finally, upon the completion of each flock, the integrator provided data on final body weight, livability, and feed conversion ratio (FCR).

During the first and second trial, broilers were caught and processed at 47 days of age where on the third trial broilers were caught and processed on 45 days of age. The integrator provided total house wight of the broilers, total feed consumption, and feed conversion ratio separated by house on the day the birds were sent to processing.

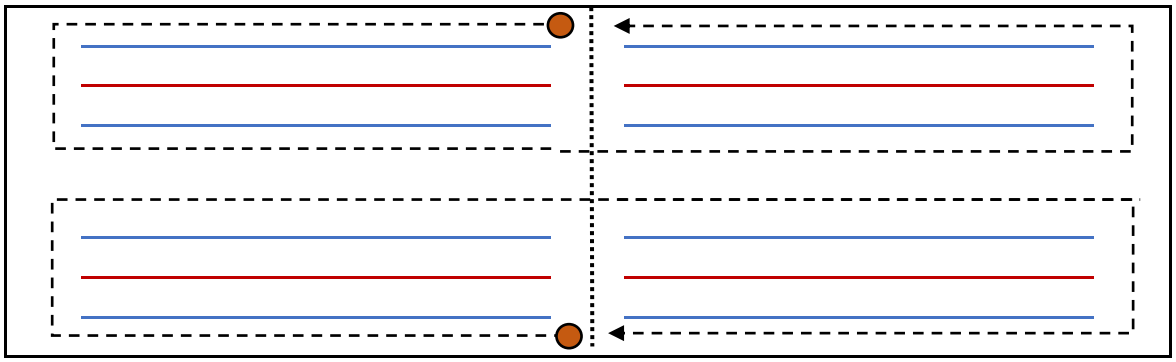

Figure 1. Transect sampling method path within the chicken house. Black dashed line indicates the path walked by observers. The red indicates feed line and the blue indicates the water lines of the house

Table 3. Photoperiod and light intensities regime for Experiment 4

	Control		Treatment	
Day	Light/Dark hours	Intensity	Light/Dark hours	Intensity
0	24L:0D	35.0 lux	18L:6D	35.0 lux
5	20L:4D	35.0 lux	18L:6D	35.0 lux
7	18L:6D	35.0 lux	18L:6D	35.0 lux
10	18L:6D	17.5 lux	18L:6D	17.5 lux
14-42	18L:6D	12.5 lux	18L:6D	12.5 lux

Data analysis

Data from these four experiments were analyzed with room (pen trials experiments 1, 2, and 3) and house (field trial experiment 4) as the experimental units. Because there were no significant differences between trials within an experiment, data were combined using trial as a blocking factor. The arcsine transformation normalized proportional data (uniformity, mortality, gait scores, lames observations in experiment 4) before analysis and back transformed means are presented. Performance and plasma assay data were analyzed using the ANOVA procedure (JMP Pro, ver. 15). The means of the variables found to be significantly different were separated by Tukey's method. Variables were considered statistically significant at $P \leq 0.05$.

CHAPTER 4

Results and Discussion – Pen Trials

Room temperatures for all trials were maintained within ± 0.8°C of the specified temperature curve. Relative humidity within the rooms was maintained between 35-65% for all trials. No significant differences were observed in temperature and RH between rooms and trials.

No significant differences in mortality were observed across all trials between control and treatment. Due to the absence of trial interactions, data from Trials 1, 2, and 3 (14 day – 24L vs. 20L:4D) were combined for Experiment 1 (P=0.10244), data from Trials 4 and 5 (21 day – 24L vs. 18L:6D) were combined for Experiment 2 (P=0.11156), and data from Trials 6 and 7 (42 day – 24L vs. 18L:6D) were combined for Experiment 3 (P=0.17707).

Experiment 1 (14 days – 24L vs. 20L:4D)

Performance

The treatment group exhibited significantly (P≤0.05) lower BW (82 vs. 85g) and BWG (39 vs. 42g) on Day 3. This trend continued into Day 7, where BW (182 vs. 188g), and BWG (139 vs. 145g) were significantly lower compared to the control group (Table 1). No differences in 0-14 ADG were seen between control and treatment. The pattern of higher body weight in the control birds changed after the birds were exposed to their first 4-hour dark period of Day 7. By Day 10, the treatment birds displayed significantly higher BW (306 vs. 299g) than the control birds. On Day 14, the treatment group continued to outperform the control birds with

significantly higher BWG (355 vs. 337g) and ADG (51 vs. 48g), compared to the control group. No significant differences were observed in feed to gain throughout the experiment.

The current results support previous findings that suggest birds adjust their feed intake and compensate growth in response to photoperiod. Classen et al. (1991) found that broilers reared under a step-down lighting program consumed similar amounts of feed by the end of the flock as broilers raised under 23L:1D for the entire flock. The current results differ from the findings of Magee et al. (2022), who found no performance differences between birds subjected to different light schedules (23L:1D vs. 20L:4D) on either Day 7 or 14. This difference in conclusions may be due to the one-hour dark period that Magee et al. (2022). The room setup with only one pen and sample size may not have given Magee et al. (2022) the statistical power needed to observe a significant difference. The differences in the use of both males and female, diet, and melatonin ELISA in Magee et al. (2022) may also have contributed to the differences in melatonin compared to the current research.

Blood Plasma Characteristics

Mean intra- and inter-assay coefficient of variations (CV) for the current studies blood plasma data were 11.81 and 4.61, respectively. Table 6 shows Intra- and inter-assay (CV) by experiment. Of the four blood plasma sample collection periods, significant differences ($P \leq 0.05$) in corticosterone were only observed in the treatment group during the dark period of Day 6 (3.4 vs. 2.2ng/mL) (Table 5). The corticosterone levels were within normal physiological ranges for broilers (Korte et al., 1997). The results of the current study concur with Magee et al. (2023), who also reported no corticosterone differences at Day 14 in birds subjected to different light schedules (23L:1D vs. 20L:4D).

Treatment birds exhibited elevated plasma melatonin levels on Day 6 during both the dark (366 vs. 155 pg/mL) and light periods (251 vs. 59 pg/mL), as well as during the dark and light periods on Day 13 (275 vs. 128 pg/mL) and (113 vs. 35 pg/mL) respectively (Table 5). The current data are similar to Schwean-Lardner et al. (2014), who found a 20L:4D cycle increased baseline melatonin and dark period elevations compared to birds reared under 23L:1D at 21 days of age. The current data differs from the melatonin levels observed in Magee et al. (2023), where birds exposed to different light schedules (23L:1D vs. 20L:4D) showed no melatonin increase during the dark. Though the experimental design of Magee et al. (2023) is comparable to this research, their research found no melatonin elevation during the dark period of either control or treatment, differs from the research in previous studies on melatonin production in poultry (Calislar et al., 2018).

Table 4. Performance parameters between broilers with exposure to continuous light (control) or 4 hours of darkness (treatment) during the first week post-hatch – Experiment 1

			Day				
Parameter	**Treatment**	**N**	**0**	**3**	**7**	**10**	**14**
Body weight (g)	Control	9	43 ± 0.5	85 ± 0.8*	188 ± 3.2*	299 ± 6.2*	524 ± 6.9
	Treatment	9	43 ± 0.6	82 ± 1.0	182 ± 2.7	306 ± 4.7	537 ± 7.9
	P-value		1.0000	**0.0003**	**0.0061**	**0.0190**	0.0520
Weekly BWG (g)	Control	9	-	-	145 ± 3.1*	-	337 ± 5*
	Treatment	9	-	-	139 ± 2.8	-	355 ± 6
	P-value		-	-	**0.0047**	-	**0.0050**
Weekly ADG (g)	Control	9	-	-	21 ± 0.4	-	48 ± 0.8*
	Treatment	9	-	-	20 ± 0.4	-	51 ± 0.8
	P-value		-	-	0.1114	-	**0.0069**
Overall BWG (g)	Control	9	-	42 ± 1.2*	145 ± 3.1*	279 ± 3.4	501 ± 4.8
	Treatment	9	-	39 ± 1.5	139 ± 2.8	285 ± 2.6	514 ± 3.8
	P-value		-	**0.0014**	**0.0047**	0.0624	0.0509
Overall ADG (g)	Control	9	-	14 ± 0.5	21 ± 0.4	28 ± 0.4	36 ± 0.4
	Treatment	9	-	13 ± 0.5	20 ± 0.4	29 ± 0.3	37 ± 0.3
	P-value		-	0.1517	0.1141	0.0881	0.1006
Feed to Gain (g:g)	Control	9	-	0.85 ± 0.037	1.05 ± 0.012	1.11 ± 0.010	1.19 ± 0.008
	Treatment	9	-	0.88 ± 0.027	1.06 ± 0.005	1.10 ± 0.011	1.20 ± 0.009
	P-value		-	0.1429	0.4366	0.2667	0.4243
Uniformity (% CV)	Control	9	-	-	10.24 ± 0.52	-	10.47 ± 1.13
	Treatment	9	-	-	9.68 ± 0.55	-	9.98 ± 0.86
	P-value		-	-	0.4754	-	0.6449

Values are mean ± SEM of 3 rooms across 3 trials to see n = 9

All birds were on the same photoperiod (20L:4D) from Days 7-14

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 5. Plasma corticosterone and melatonin comparing broilers with exposure to continuous light (control) or 4 hours of darkness (treatment) during the first week post-hatch – Experiment 1

			Day			
			6		13	
Parameter	**Treatment**	**N**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**
Corticosterone (ng/mL)	Control	9	2.2 ± 0.22*	2.3 ± 0.27	2.6 ± 0.41	1.4 ± 0.23
	Treatment	9	3.4 ± 0.51	2.8 ± 0.33	3.1 ± 0.57	1.7 ± 0.32
	P-value		**0.0350**	0.2018	0.4765	0.5281
Melatonin (pg/mL)	Control	9	155 ± 15*	59 ± 20*	128 ± 8*	35 ± 6*
	Treatment	9	366 ± 35	251 ± 74	275 ± 38	113 ± 15
	P-value		**<0.0001**	**0.0253**	**0.0014**	**0.0003**

Values are mean ± SEM of 3 rooms across 3 trials to see n = 9

All birds were set to the same photoperiod (18L:6D) from Days 7-14

Lights came on at 5:00am and turned off at 1:00am from 0-14 days in the treatment birds

Lights came on at 5:00am and turned off at 1:00am from 7-14 days in the control birds

[1] Samples were collected during the middle of the dark period (~3am)

[2] Samples were collected during the middle of the light period (~3pm)

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 6. Intra- and inter-assay coefficient of variations (CV) for the current studies blood plasma data

Assay	Experiment	Average	
		Inter-assay variability (CV)	**Intra-assay variability (CV)**
Corticosterone	1	12.77	5.58
	2	11.50	5.80
	3	13.40	6.30
Melatonin	1	10.20	2.67
	2	13.43	4.39
	3	12.83	4.11
Superoxide dismutase	2	7.16	4.03
	3	13.15	3.96

Standards and controls were run in triplicate
All samples were run in duplicate

Experiment 2 (21 days – 24L vs. 18L:6D)

Performance

As observed in Experiment 1, treatment birds in Experiment 2 displayed significantly ($P \leq 0.05$) lower BW (79 vs. 84g), BWG (36 vs. 42g), and ADG (12 vs. 14g) on Day 3. The trend continued into Day 7, with the control birds outperforming the treatment birds BW (182 vs.166g), BWG (139 vs.123g), and ADG (20 vs.18g) (Table 7). The control birds began to underperform relative to the treatment birds while adjusting to the six-hour dark period beginning on Day 7. Unlike in Experiment 1, there were no significant differences in Day 10 performance (BW, 0-10 BWG, or FI) between the two groups in this experiment. During Week 2, the treatment pens exhibited higher weekly BWG (318 vs. 295g) and weekly ADG (45 vs. 42g). By Day 21, no differences in performance were observed comparing control and treatment.

The difference between the Day 10 data of Experiments 1 and 2 is most likely due to the difference in photoperiod between the two experiments. Because the treatment birds in Experiment 2 received a six-hour dark period, their growth curve changed and did not show higher growth until Day 14. No differences in feed to gain were observed throughout the experiment. The treatment group was significantly more uniform on Day 7 (9 vs. 11%), with no differences in uniformity observed on Days 14 and 21.

Blood Plasma Characteristics

No differences in plasma corticosterone or superoxide dismutase levels were found when comparing control and treatment groups throughout the experiment (Table 8). Treatment birds exhibited significantly ($P \leq 0.05$) higher plasma melatonin levels during the dark periods of Day 6 (462 vs. 135pg/mL), 13 (368 vs. 227pg/mL), and 20 (297 vs. 107pg/mL) (Table 8). The plasma

melatonin was also significantly higher during light periods of Day 6 (208 vs. 63pg/mL), 13 (70 vs. 26pg/mL), and 20 (31 vs. 12pg/mL).

These findings are similar to those of Schwean-Lardner et al. (2014), who reported that a 17L:7D light cycle increased baseline melatonin as well as dark period elevations compared to birds reared under 23L:1D at 21 days of age. Özkan et al. (2006) found comparable melatonin concentrations on Day 21 (165 and 295pg/mL) when comparing birds reared with and without dark periods (24L vs. 16L:8D) from Day 2 to 49. Although the initiation of the dark period and the photoperiod length of Schwean-Lardner et al. (2014) and Özkan et al. (2006) differs from this research, the pattern in melatonin peak during the night and elevated daytime concentrations is comparable with this research.

Table 7. Performance parameters between broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch – Experiment 2

			Day					
Parameter	**Treatment**	**N**	**0**	**3**	**7**	**10**	**14**	**21**
Body weight (g)	Control	6	43 ± 0.4	84 ± 0.6*	182 ± 1.7*	287 ± 3.2	477 ± 6.3	1,036 ± 18.3
	Treatment	6	43 ± 0.4	79 ± 0.7	166 ± 2.7	292 ± 2.9	484 ± 7.5	1,057 ± 15.1
	P-value		1.0000	**0.0003**	**0.0004**	0.2168	0.2742	0.0785
Weekly BWG (g)	Control	6	-	-	139 ± 1.8*	-	295 ± 5.8*	559 ± 14.2
	Treatment	6	-	-	123 ± 3.1	-	318 ± 5.1	573 ± 9.1
	P-value		-	-	**0.0003**	-	**0.0010**	0.2360
Weekly ADG (g)	Control	6	-	-	20 ± 0.3*	-	42 ± 0.9*	80 ± 2.0
	Treatment	6	-	-	18 ± 0.4	-	45 ± 0.8	82 ± 1.3
	P-value		-	-	**0.0003**	-	**0.0028**	0.2251
Overall BWG (g)	Control	6	-	42 ± 0.7*	139 ± 1.8*	244 ± 3.0	434 ± 6.7	993 ± 18.8
	Treatment	6	-	36 ± 0.8	123 ± 3.1	250 ± 2.9	441 ± 7.9	1,014 ± 15.4
	P-value		-	**0.0002**	**0.0003**	0.7184	0.2657	0.0807
Overall ADG (g)	Control	6	-	14 ± 0.3*	20 ± 0.3*	25 ± 0.3	31 ± 0.5	47 ± 0.8
	Treatment	6	-	12 ± 0.2	18 ± 0.4	25 ± 0.3	32 ± 0.6	48 ± 0.7
	P-value		-	**<0.0001**	**0.0003**	0.7184	0.2019	0.1114
Feed to Gain (g:g)	Control	6	-	0.88 ± 0.015	1.03 ± 0.011	1.10 ± 0.006	1.19 ± 0.006	1.25 ± 0.013
	Treatment	6	-	0.90 ± 0.018	1.04 ± 0.005	1.10 ± 0.010	1.19 ± 0.012	1.23 ± 0.007
	P-value		-	0.4000	0.2458	0.7794	0.6887	0.0991
Uniformity (% CV)	Control	6	-	-	11.02 ± 0.86*	-	10.86 ± 1.08	8.34 ± 0.42
	Treatment	6	-	-	8.51 ± 0.50	-	8.34 ± 0.42	7.75 ± 0.22
	P-value		-	-	**0.0303**	-	0.0564	0.1114

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were on the same photoperiod (18L:6D) from Days 7-21

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 8. Plasma corticosterone, melatonin, and superoxide dismutase comparing broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch – Experiment 2

			Day					
			6		13		20	
Parameter	**Treatment**	**N**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**
Corticosterone (ng/mL)	Control	6	4.4 ± 0.56	3.6 ± 0.56	5.3 ± 0.65	3.2 ± 0.82	2.1 ± 0.27	2.0 ± 0.33
	Treatment	6	4.7 ± 0.91	3.9 ± 0.68	4.0 ± 0.15	3.5 ± 1.20	2.9 ± 0.72	1.6 ± 0.37
	P-value		0.7799	0.7990	0.1694	0.8549	0.8549	0.4840
Melatonin (pg/mL)	Control	6	135 ± 28*	63 ± 10*	227 ± 35*	26 ± 5*	107 ± 11*	12 ± 2*
	Treatment	6	462 ± 21	208 ± 26	368 ± 38	70 ± 13	295 ± 51	31 ± 6
	P-value		**<0.0001**	**<0.0001**	**0.0156**	**0.0046**	**0.0023**	**0.0074**
SOD (units/mL)	Control	6	135 ± 8	143 ± 7	130 ± 11	141 ± 7	131 ± 8	126 ± 13
	Treatment	6	143 ± 5	133 ± 4	141 ± 5	131 ± 9	140 ±9	136 ± 7
	P-value		0.3819	0.2968	0.3600	0.4366	0.5158	0.4509

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were set to the same photoperiod (18L:6D) from Days 7-21

Lights came on at 6am and turned off at 12am from 0-21 days in the treatment birds

Lights came on at 6am and turned off at 12am from 7-21 days in the control birds

[1] Samples were collected during the middle of the dark period (~3am)

[2] Samples were collected during the middle of the light period (~3pm)

* Indicates a significant difference (P≤0.05) comparing control and treatment

Experiment 3 (42 days – 24L vs. 18L:6D)

Performance

Similar to the previous experiments, treatment birds on Day 3 displayed significantly ($P \leq 0.05$) lower BW (87 vs. 81g), BWG (37 vs. 43g), and ADG (12 vs. 14g). The trend continued to Day 7 with the control birds outperforming the treatment birds BW (190 vs.180g), BWG (140 vs.130g), and ADG (20 vs.19g) (Table 9). By Day 10, however, performance differences between control and treatment groups were no longer observed. By Day 14, the same trend seen in Experiments 1 and 2 was observed, with the treatment group growth surpassing the control birds BW (514 vs. 500), BWG (470 vs. 456g), Weekly BWG (334 vs. 310g), and Weekly ADG (48 vs. 44g).

Unlike the findings in Experiment 2, in this experiment the treatment group continued to outperform the control group BW (1,045 vs. 1,021g) to Day 21, possibly due to more uniform birds. There were higher cases of omphalitis in the second experiment, which may be why the birds varied in weight, leading to higher standard error, which may be a factor leading to no significant difference in Day 21 BW in Experiment 2.

From Days 28 to 42, no differences in performance were observed between the control and treatment groups (Table 10). Consistent with the previous experiments, no feed to gain differences were observed throughout the trial.

The treatment group showed better uniformity on Days 7 (10 vs. 12%) and Day 21 (7 vs. 11%). On Days 14 and 42, no significant differences in uniformity were observed between the control and treatment birds. Gait scores were collected and no significant differences between treatment and control were found since no birds with scores above 0 in either trial were

observed. There were no differences in tibial breaking strength between treatment and control groups (Table 11).

Blood Plasma Characteristics

No differences were observed in corticosterone levels, and consistently fell within normal physiological ranges (Table 12-13). Similar trends in melatonin were observed in Experiment 3 as in Experiments 1 and 2. During the dark periods treatment birds exhibited significantly ($P \leq 0.05$) higher plasma melatonin levels on Days 6 (468 vs. 192pg/mL), 13 (346 vs. 120pg/mL), 20 (300 vs. 89pg/mL), 27 (280 vs. 65pg/mL), and 35 (245 vs. 83pg/mL) (Figure 3). During the light periods, treatment birds exhibited significantly higher plasma melatonin levels on Days 6 (73 vs. 24pg/mL), 13 (109 vs. 30pg/mL), 20 (68 vs. 16pg/mL) and 27 (55 vs. 17pg/mL). These treatment differences were no longer in the light period of Day 35 and the light/dark periods of Day 42. The findings from this research are similar to Özkan et al. (2006), who reported similar melatonin concentrations on Day 49 to this research in broilers reared under different lighting schedules (24L vs. 16L:8D) from Day 2 to 49.

No significant differences were seen in SOD levels between the two groups. This observation aligns with the findings by Ayo et al. (2018) and Mosleh et al. (2016), which found no differences in plasma SOD between birds reared under different photoperiods.

Table 9. Performance parameters between broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch (0-21 days) – Experiment 3

			Day					
Parameter	**Treatment**	**N**	**0**	**3**	**7**	**10**	**14**	**21**
Body weight (g)	Control	6	45 ± 0.2	87 ± 1.5*	190 ± 3.9*	305 ± 4.1	500 ± 8.9*	1,021 ± 10.8*
	Treatment	6	45 ± 0.2	81 ± 0.2	180 ± 2.3	306 ± 2.8	514 ± 7.6	1,045 ± 7.8
	P-value		1.0000	**<0.0001**	**0.0006**	0.8693	**0.0391**	**0.0401**
Weekly BWG (g)	Control	6	-	-	140 ± 3.8*	-	310 ± 5.0*	477 ± 3.6
	Treatment	6	-	-	130 ± 2.0	-	334 ± 5.6	487 ± 6.6
	P-value		-	-	**0.0007**	-	**0.0004**	0.2389
Weekly ADG (g)	Control	6	-	-	20 ± 0.3*	-	44 ± 0.7*	68 ± 0.6
	Treatment	6	-	-	19 ± 0.2	-	48 ± 0.8	70 ± 1.0
	P-value		-	-	**0.0008**	-	**0.0003**	0.3018
Overall BWG (g)	Control	6	-	43 ± 1.1*	140 ± 3.8*	274 ± 3.3	456 ± 8.7*	977 ± 10.6*
	Treatment	6	-	37 ± 0.9	130 ± 2.0	275 ± 2.7	470 ± 7.4	1,001 ± 7.8
	P-value		-	**<0.0001**	**0.0007**	0.9409	**0.0390**	**0.0412**
Overall ADG (g)	Control	6	-	14 ± 0.3*	20 ± 0.3*	27 ± 0.3	33 ± 0.7	47 ± 0.4*
	Treatment	6	-	12 ± 0.3	19 ± 0.2	27 ± 0.2	34 ± 0.5	48 ± 0.3
	P-value		-	**<0.0001**	**0.0008**	0.9999	0.1299	**0.0213**
Feed to Gain (g:g)	Control	6	-	0.83 ± 0.020	1.00 ± 0.013	1.08 ± 0.007	1.16 ± 0.004	1.24 ± 0.012
	Treatment	6	-	0.84 ± 0.015	1.01 ± 0.005	1.07 ± 0.003	1.15 ± 0.002	1.25 ± 0.015
	P-value		-	0.4463	0.2960	0.3176	0.1305	0.4974
Uniformity (% CV)	Control	6	-	-	11.69 ± 0.49*	-	10.01 ± 0.73	10.86 ± 0.95*
	Treatment	6	-	-	9.51 ± 0.43	-	7.34 ± 1.28	7.01 ± 1.29
	P-value		-	-	**0.0078**	-	0.1006	**0.0375**

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were on the same photoperiod (18L:6D) from Days 7-42

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 10. Performance parameters comparing broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch (28-42 days) – Experiment 3

			Day		
Parameter	**Treatment**	**N**	**28**	**35**	**42**
Body weight (g)	Control	6	1,798 ± 22.3	2,680 ± 19.0	3,413 ± 48.7
	Treatment	6	1,810 ± 15.7	2,689 ± 29.4	3,433 ± 52.2
	P-value		0.5085	0.8179	0.7307
Weekly BWG (g)	Control	6	733 ± 13.4	838 ± 16.1	733 ± 33.7
	Treatment	6	721 ± 14.9	835 ± 21.0	744 ± 33.0
	P-value		0.5088	0.8931	0.6759
Weekly ADG (g)	Control	6	105 ± 1.9	120 ± 2.3	105 ± 4.8
	Treatment	6	103 ± 2.1	119 ± 2.9	106 ± 4.7
	P-value		0.5531	0.8488	0.6740
Overall BWG (g)	Control	6	1,754 ± 22.2	2,636 ± 18.9	3,369 ± 48.7
	Treatment	6	1,766 ± 15.4	2,645 ± 29.4	3388 ± 52.0
	P-value		0.5207	0.8145	0.7287
Overall ADG (g)	Control	6	63 ± 0.8	75 ± 0.5	80 ± 1.2
	Treatment	6	63 ± 0.6	76 ± 0.9	81 ± 1.3
	P-value		0.6424	0.7551	0.8126
Feed to Gain (g:g)	Control	6	1.31 ± 0.021	1.45 ± 0.021	1.54 ± 0.017
	Treatment	6	1.31 ± 0.018	1.46 ± 0.026	1.53 ± 0.025
	P-value		0.9277	0.5236	0.8546
Uniformity (% CV)	Control	6	-	-	9.01 ± 0.45
	Treatment	6	-	-	9.34 ± 0.67
	P-value		-	-	0.6862

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were on the same photoperiod (18L:6D) from Days 7-42

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 11. Tibia breaking strength comparing 42 day-old broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch

Treatment	**N**	**Breaking Strength (N)***
Control	6	434 ± 21
Treatment	6	443 ± 28
P-value		0.7991

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were on the same photoperiod (18L:6D) from Days 7-42

*Breaking strength expressed in Newtons of force used to fracture the bone completely

Table 12. Plasma corticosterone, melatonin, and superoxide dismutase comparing broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch (Days 6-20) – Experiment 3

			Day					
			6		13		20	
Parameter	**Treatment**	**N**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**
Corticosterone (ng/mL)	Control	6	2.2 ± 0.64	3.0 ± 0.66	1.8 ± 0.36	1.4 ± 0.17	1.6 ± 0.18	1.3 ± 0.25
	Treatment	6	2.6 ± 0.51	2.6 ± 0.81	1.7 ± 0.38	1.2 ± 0.45	1.7 ± 0.11	0.9 ± 0.24
	P-value		0.6476	0.7452	0.9586	0.7201	0.8804	0.4163
Melatonin (pg/mL)	Control	6	192 ± 36*	24 ± 3*	120 ± 29*	30 ± 11*	89 ± 19*	16 ± 4*
	Treatment	6	468 ± 33	109 ± 26	346 ± 16	73 ± 13	300 ± 43	68 ± 48
	P-value		**0.0002**	**0.0208**	**<0.0001**	**0.0055**	**0.0012**	**0.0036**
SOD (units/mL)	Control	6	123 ± 5	123 ± 6	81 ± 23	97 ± 23	116 ± 10	121 ± 8
	Treatment	6	126 ± 8	126 ± 8	107 ± 22	102 ± 22	124 ± 9	124 ± 5
	P-value		0.7781	0.7414	0.4504	0.8822	0.5452	0.7453

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were set to the same photoperiod (18L:6D) from Days 7-21

Lights came on at 6am and turned off at 12am from 0-21 days in the treatment birds

Lights came on at 6am and turned off at 12am from 7-21 days in the control birds

[1] Samples were collected during the middle of the dark period (~3am)

[2] Samples were collected during the middle of the light period (~3pm)

* Indicates a significant difference (P≤0.05) comparing control and treatment

Table 13. Plasma corticosterone, melatonin, and superoxide dismutase comparing broilers with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first week post-hatch (Days 27-41) – Experiment 3

			Day					
			27		34		41	
Parameter	**Treatment**	**N**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**	**Dark[1]**	**Light[2]**
Corticosterone (ng/mL)	Control	6	1.1 ± 0.17	1.1 ± 0.18	0.6 ± 0.26	0.7 ± 0.20	0.8 ± 0.27	0.5 ± 0.07
	Treatment	6	1.2 ± 0.20	1.2 ± 0.33	0.9 ± 0.25	0.8 ± 0.13	0.5 ± 0.12	0.7 ± 0.12
	P-value		0.7680	0.8624	0.4034	0.7621	0.3266	0.0905
Melatonin (pg/mL)	Control	6	65 ± 33*	17 ± 4*	83 ± 8*	35 ± 11	92 ± 30	9 ± 3
	Treatment	6	280 ± 48	55 ± 9	245 ± 49	39 ± 11	131 ± 50	13 ± 3
	P-value		**0.0045**	**0.0041**	**0.0091**	0.8418	0.5226	0.3363
SOD (units/mL)	Control	6	141 ± 11	136 ± 13	140 ± 15	119 ± 15	149 ± 5	116 ± 24
	Treatment	6	148 ± 8	135 ± 11	143 ± 15	139 ± 13	139 ± 11	138 ± 12
	P-value		0.6457	0.9478	0.8973	0.3144	0.4011	0.4304

Values are mean ± SEM of 3 rooms across 2 trials to see n = 6

All birds were set to the same photoperiod (18L:6D) from Days 7-21

Lights came on at 6am and turned off at 12am from 7-21 days in the control birds

Lights came on at 6am and turned off at 12am from 0-21 days in the treatment birds

[1] Samples were collected during the middle of the dark period (~3am)

[2] Samples were collected during the middle of the light period (~3pm)

* Indicates a significant difference (P≤0.05) comparing control and treatment

Pen Trial Discussion Experiments 1, 2, and 3

Performance

The performance patterns observed in these experiments align with previous research indicating that any growth depression induced by early dark periods tends to not affect bird weight at market age, resulting in no overall performance differences (Özkan et al., 2006; Olanrewaju et al., 2018; Classen et al., 2004; Lien et al., 2007). This suggests that birds are able to adjust their feed intake in response to photoperiod changes by the end of the flock, ultimately leading to comparable final performance under optimal conditions (Classen et al., 2004). Classen et al. (1991) suggested that androgenic hormone production may be responsible for this compensatory growth observed in birds subjected to early dark periods. Later, these same researchers found birds reared with early dark periods that increased over time had higher androstenedione, testosterone, and body weight than birds raised under 23L:1D for seven weeks. Charles et al. (1992) suggest that the higher body weight may be associated with the birds preparing for sexual maturity stimulated by adequate nutrition and light which may have increased the androstenedione and testosterone. This does not explain the compensatory growth of the birds in the current study as they were far too young to be photostimulated into sexual maturity. Compensatory growth in broilers is not well understood, however, it has been repeatedly observed in research. It is theorized that birds that are feed restricted from nutritional or lighting programs will metabolically compensate in response to the restriction (Zhan et al., 2007).

It is a common theory among poultry producers that Day 7 weights are correlated with the final flock performance (Aviagen, 2018; Cobb, 2021). The current research suggests Day 7 body weights are relative to the lighting program, and it may not be applicable to compare

broiler performance under different lighting programs at Day 7. Day 14 performance may better indicate broiler performance when comparing lighting programs. The current research also suggests that early dark periods do not affect performance; rather, they modify the growth curve of birds, ending with similar final body weights as birds reared under constant light.

The treatment birds caught up to the control birds in body weight after the control birds were given their first dark period. This phenomenon may be related to the biological concept of Eskin's knee, which refers to the curve changes in behavior and/or growth an animal must undergo to adjust to an environmental change (Menaker et al., 1978).

Corticosterone

All plasma corticosterone levels between the treatment and control groups in the three experiments remained within normal physiological ranges (Korte et al., 1997). The notable difference in corticosterone in the first experiment during the dark period of Day 6 is likely attributed to human error during blood sampling. It was the first time some researchers sampled blood via cardiac puncture. Because of this, some bleeding time took longer than 60 seconds and may have affected the results. These findings align with previous research suggesting photoperiod has minimal effects on bird stress when reared under optimal conditions (Smoak and Birrenkott, 1984; Renden et al., 1994). The dimming function used to simulate a dawn/dusk may have imprinted birds to anticipate the dark period. This time may have allowed the birds to bed down or get ready for the dark period instead of being surprised by a sudden dark period.

Superoxide dismutase

No differences in SOD were observed in these experiments. In hindsight, conducting a comprehensive oxidative panel to capture a broader range of oxidative defense mechanisms

might have been beneficial. Research by Baykalir et al. (2020) reported that a photoperiod of 16L:8D, in combination with a stressor, led to higher SOD levels compared to broilers reared under continuous or intermittent lighting programs. During the current experiments, birds were meticulously reared under optimal conditions.

Birds reared under optimal conditions are expected to have similar SOD levels. SOD will be decreased as it is depleted, scavenging free radicals in the body, thus indicating oxidative stress. Birds subjected to oxidative stress will have lower SOD compared to birds with no exposure to this stress (Fridovich, 1975). Introducing a stressor capable of oxidative damage may be necessary to observe differences in SOD when comparing birds reared under different photoperiods.

Gait scores and Tibial Breaking Strength

Leg abnormalities have been linked to photoperiod as it correlates with rest at night and increased activity during the day contributing to stronger bones (Lewis and Morris, 2006), and melatonin has been shown to regulate bone growth by increasing osteoblast differentiation and mineralization of the bone matrix (Roth et al., 1999). The combination of metabolic and hormonal changes during sleep and the activity during the light period has a greater influence on leg health than resting at night (Classen et al., 1991). This study did not find significant differences in ultimate force required to fracture the bone, possibly due to the control and treatment groups experiencing different lighting programs only during the first week before leg issues typically develop (Schwean-Lardner et al., 2013; Sherlock et al., 2010). Dark periods have a greater effect on gait scores later in the bird's life when the bird is carrying more weight and not as active (Classen et al., 1991).

The birds in this study were reared under optimal conditions in a controlled pen trial setting. The optimal environmental conditions, litter moisture, bird activity, and nutrition may have contributed to the absence of significant differences in gait scores and tibial breaking strength. Broilers challenged at early ages with suboptimal nutrition, high litter moisture, high bird densities, or even bacterial chondronecrosis that could initiate lameness might benefit from the early dark periods.

Melatonin

Based on the current research, dark periods from the day of placement can increase melatonin concentration in chicks. The current results are comparable to research by Archer and Mench (2014) and Zeman et al. (1999), which found that melatonin production can be stimulated as early as Day 16 of incubation by introducing a light/dark cycle in the incubator. This research also supports the findings of Magee et al. (2023), who found higher melatonin in birds brooded under 20L:4D, although the pattern in melatonin differed from these results.

By Day 21 in Experiments 2 and 3, control birds still did not catch up with the melatonin production of the treatment birds despite being on the same lighting schedule for 14 days. The higher melatonin observed in the treatment birds on Day 7 is a physiological response from the photoperiod. The absence of light stimulation during the dark period results in a series of biochemical/enzymatic reactions that stimulates the pineal gland to synthesize melatonin (Binkley, 1990).

The feedback loop between the suprachiasmatic nuclei (SCN) and the pineal gland directly influences circadian clocking mechanisms. Melatonin stimulated by the light/dark cycle and rhythmically produced acts upon the SCN, directly affecting clocking mechanisms. The

SCN, in turn, regulates core circadian clocking genes that provide positive and negative feedback loops across the body. As clocking genes are expressed and develop circadian rhythmicity, the SCN dictates melatonin secretion by way of neural signals which parallel the light/dark cycle. In turn, the pineal gland is stimulated by both the SCN and the dark cycle to maintain circadian function (Binkley, 1990). This is why melatonin will still increase during the expected dark period for a few days when providing birds 24 hours of light after developing a rhythm. Even though the pineal gland is not stimulated by a dark period, the circadian pacemaker in the SCN is anticipating a dark period. The pineal gland stops producing extra melatonin after not being stimulated by a light/dark cycle; this will cause the circadian rhythm not to be maintained, and the SCN will slowly stop signaling melatonin to be synthesized (Scanes and Dridi, 2022). The current research observed no differences in the two-to-nine-fold increase in melatonin from dark to light periods in control and treatment groups. This indicates that there are most likely no differences in the broiler's circadian rhythms of melatonin. Simply the treatment birds' dark period stimulated a higher melatonin setpoint compared to the control birds via the early feedback loop development between the SCN and pineal gland of the treatment birds.

In Experiment 3, it was only during the light period of Day 35 that the melatonin concentrations normalized between control and treatment birds. As chickens age, their pineal gland becomes less photosensitive. It is theorized that after the bird's circadian rhythm develop, it is less sensitive to react to minor environmental changes (Doi et al., 1995). This may be why the treatment birds developed a higher melatonin setpoint, why it took longer for control birds to reach the same levels as the treatment, and why the overall melatonin concentration decreased over the course of each flock. Stimulating higher melatonin on the day of placement might set

the SCN circadian pacemaker, while the pineal gland is most photoreceptive, leading to higher levels of melatonin (Binkley, 1990).

By setting the photoperiod (18L:6D), the plasma melatonin levels were persistently higher compared to birds brooded under 24 hours of light. The sleep and rest induced by the early dark period are easily observable natural behaviors that do not affect bird performance. Melatonin has been shown to improve immune function, mediate stress responses, and promote growth and development (Calislar et al., 2018). This suggests that birds reared under an extended early dark period during brooding may be better equipped to handle stressors and disease challenges. Melatonin has a strong relationship to the oxidative defense of the body (Tomas-Zapico and Coto-Montes, 2005). The current study did not find any differences in mortality or observable bird health. Some of the beneficial aspects of higher melatonin may have yet to be observed due to these birds being reared under optimal conditions. Future studies should delve deeper into the effects of an early dark period on birds exposed to various challenges, such as the oxidative stress related to heat stress and mycotoxins, as well as varying disease states. Conducting challenge studies to evaluate whether the heightened melatonin levels can mitigate oxidative stress in birds raised under suboptimal conditions could lead to novel lighting programs that could potentially benefit bird health.

Additional research needs to investigate the combined effects of incubating broiler eggs under the same photoperiod as they will experience post-placement; this may further stimulate and synchronize the bird's internal clock. Synchronizing chicks to the photoperiod during incubation might establish long-lasting behavioral and circadian rhythms.

Another area of research is to investigate broiler chicks' hormonal and circadian development by subjecting them to varying lighting schedules during the brooding phase. This

includes examining melatonin production patterns during brooding and assessing any indications of circadian disruption in chicks exposed to continuous light. Gaining insights into how the photoperiod during brooding can enhance performance, and physiological function has the potential to optimize and refine poultry farming practices in the future.

Sleep is a natural behavior exhibited by all vertebrates. Many welfare guidelines require a dark period in older birds. For example, the National Chicken Council Welfare Guidelines requires at least four hours of darkness every 24 hours except for the first week of age (NCC, 2017). The results of this study contribute to the understanding of the impact of photoperiod on broiler performance and melatonin levels, suggesting that implementing an early dark period may align with the UK Farm Animal Welfare Council's "Five Freedoms" of animal welfare, specifically the freedom to express natural behavior, i.e., sleep, without compromising overall bird performance (FAWC, 2010). This research has indicated that early dark periods have no detriment to flock performance; with all the known benefits of dark periods in older birds, it is reasonable to speculate that chicks might benefit from being provided a dark period every day of the flock.

Results and Discussion – Field Trials

Experiment 4

Temperatures in the four study houses were kept within 3.2°C of the integrator-provided temperature curve. Relative humidity within the houses was maintained between 45-65% for all trials. No significant differences were observed in temperature and RH between houses and trials across this experiment.

Chicks were placed in all four study houses within four hours of one another in all three trials. Breeder flock age varied within each trial and between all three trials. In Trial 1, all four houses were placed with chicks from 30-week-old breeder flocks. In Trial 2, one control and one treatment house were placed from 25-week-old breeder flocks, and the other control and treatment houses were placed from 28-week-old breeder flocks. In Trial 3, one control and one treatment house were placed from 28-week-old breeder flocks, and the other control and treatment houses were placed from 32-week-old breeder flocks.

Inclusion body hepatitis (IBH) which is caused by an adenoviruses causes liver lesions and can lead to elevated mortality upwards of 20% (Noormohammadi, 2022), was observed in all four houses across the three trials. Broilers tested positive for (IBH) during the third week of each trial. This factor may have influenced the outcomes compared to the results from the pen trials. The field data from Trials 8, 9, and 10 conducted in commercial broiler houses were combined for analysis due to the absence of trial interactions.

Performance

No statistically significant differences between treatment and control houses in BW, BWG, and uniformity were observed. Although the control houses showed a numerical increase in percent mortality, this difference did not differ statistically (Table 14). Similarly, the percentage of lame birds on Day 42 were numerically higher in the control houses but did not differ statistically (Table 15).

Performance data on the day the birds were caught and processed Day 47 (Trials 1 and 2) and Day 45 (Trial 3) provided by the broiler company did not reveal any significant differences in BW, livability, and feed conversion ratio (FCR). It is worth noting that the company data

indicated a 50-gram weight difference between the treatment and control houses, contrasting with the weights collected by the researchers. This discrepancy could be attributed to logistical inaccuracies, such as keeping coops of birds from different houses on separate trucks (Table 16). Even in the presence of IBH, the lighting program did not appear to have a detrimental effect on the health of the birds.

Table 14. Performance parameters between broilers reared in commercial houses with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first five days post-hatch

			Day							
Parameter	**Treatment**	**N**	**1**	**3**	**7**	**14**	**21**	**28**	**35**	**42**
Body weight (g)	Control	6	54 ± 2	81 ± 5	153 ± 8	388 ± 29	796 ± 54	1,333 ± 82	2,027 ± 95	2,703 ± 111
	Treatment	6	56 ± 2	84 ± 5	154 ± 8	395 ± 26	817 ± 46	1,366 ± 81	2,043 ± 109	2,722 ± 109
	P-value		0.5007	0.4454	0.8342	0.7288	0.5186	0.5415	0.8493	0.8062
Uniformity (% CV)	Control	6	10 ± 1	15 ± 2	16 ± 1	16 ± 1	17 ± 2	15 ± 2	14 ± 2	15 ± 2
	Treatment	6	12 ± 2	13 ± 0.5	14 ± 1	16 ± 1	16 ± 1	16 ± 2	14 ± 2	14 ± 1
	P-value		0.6033	0.3528	0.4006	0.4933	0.4554	0.4022	0.6349	0.2442
Weekly BWG (g)	Control	6	-	-	-	235 ± 22	408 ± 27	537 ± 36	695 ± 44	676 ± 48
	Treatment	6	-	-	-	240 ± 19	422 ± 21	549 ± 37	677 ± 55	679 ± 57
	P-value		-	-	-	0.8663	0.6850	0.8190	0.8080	0.9650
Overall BWG (g)	Control	6	-	27 ± 4.8	99 ± 7.5	334 ± 29.0	742 ± 53.9	1,279 ± 82.3	1,974 ± 95.3	2,650 ± 111.5
	Treatment	6	-	28 ± 4.5	98 ± 7.2	338 ± 25.8	760 ± 45.6	1,309 ± 80.8	1,987 ± 109.3	2,666 ± 108.5
	P-value		-	0.9214	0.9254	0.9200	0.8057	0.7990	0.9303	0.9193
Dead (%)	Control	6	-	0.17 ± 0.01	0.48 ± 0.08	0.73 ± 0.09	1.08 ± 0.12	5.14 ± 1.88	6.43 ± 2.30	7.34 ± 2.47
	Treatment	6	-	0.19 ± 0.02	0.5 ± 0.06	0.76 ± 0.06	1.03 ± 0.05	4.47 ± 2.40	5.33 ± 2.58	5.85 ± 2.56
	P-value		-	0.3914	0.8609	0.7710	0.6179	0.7808	0.6580	0.5510
Culls (%)	Control	6	-	0.18 ± 0.03	0.33 ± 0.02	0.58 ± 0.03	0.84 ± 0.03	1.35 ± 0.13	1.68 ± 0.18	1.97 ± 0.20
	Treatment	6	-	0.18 ± 0.02	0.35 ± 0.03	0.60 ± 0.04	0.85 ± 0.04	1.31 ± 0.13	1.62 ± 0.16	1.87 ± 0.16
	P-value		-	0.9118	0.4400	0.5157	0.7554	0.7186	0.5928	0.4190
Mortality (%)	Control	6	-	0.34 ± 0.03	0.81 ± 0.10	1.31 ± 0.10	1.92 ± 0.12	6.48 ± 1.98	8.12 ± 2.44	9.31 ± 2.63
	Treatment	6	-	0.36 ± 0.03	0.84 ± 0.06	1.36 ± 0.06	1.88 ± 0.04	5.78 ± 2.53	6.95 ± 2.73	7.71 ± 2.71
	P-value		-	0.5641	0.7598	0.6926	0.7202	0.7766	0.6530	0.5408

Values are mean ± SEM of 2 houses across 3 trials to see n = 6

Control birds were given a photoperiod of 24L:0D from 0-5 days, 20L:4D from 6-7 days, and 18L:6D from 7-42 days.

Treatment birds were given a photoperiod of 18L:6D from 7-42 days

Table 15. Percentage of broilers that could not walk observed through transect walk between broilers reared in commercial houses with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first five days post-hatch

Treatment	N	Average count	Culls observed %
Control	4	119	0.49 ± 0.29
Treatment	4	86	0.34 ± 0.12
P-value			0.1651

Values are mean ± SEM of 2 houses across 2 trials to see n = 4
Control birds were given a photoperiod of 24L:0D from 0-5 days, 20L:4D from 6-7 days, and 18L:6D from 7-42 days.
Treatment birds were given a photoperiod of 18L:6D from 7-42 days

Table 16. Day of harvest performance parameters provided by the integrator between broilers reared in commercial houses with exposure to continuous light (control) or 6 hours of darkness (treatment) during the first five days post-hatch

Treatment	N	Body Wt. (g)	Livability (%)	Feed to Gain (g:g)
Control	6	2,911 ± 63	89 ± 4	1.74 ± 0.05
Treatment	6	2,861 ± 37	92 ± 3	1.74 ± 0.03
P-Value		0.3622	0.3414	0.8241

Values are mean ± SEM of 2 houses across 3 trials to see n = 6
Control birds were given a photoperiod of 24L:0D from 0-5 days, 20L:4D from 6-7 days, and 18L:6D from 7-42 days.
Treatment birds were given a photoperiod of 18L:6D from 7-42 days
Days

Discussion Experiment 4

This research found no differences in performance comparing control and treatment houses throughout the flock. The sample size, conditions in the field, as well as the IBH may explain the differences in performance results as compared to the pen trials.

The mortality rate for the last three weeks of the flocks was not significantly different but was numerically higher in the control houses. This may be associated with the early dark period. More controlled challenge studies may be needed to better understand how the physiological responses caused by dark periods during brooding affect disease-challenged birds.

A higher sample size (houses/treatment) would improve the evaluation of early dark periods in commercial settings. A systematic approach should be used where an entire complex is used to compare continuous light versus dark periods during brooding. The combination of a larger sample size and the grower's varying management styles may show significant effects in performance when utilizing dark periods during brooding.

This study's findings challenge the prevailing theory that providing broiler chicks with continuous or near-continuous light yields performance benefits, indicating that such lighting practices do not contribute to improved end-of-flock performance. From a more applied standpoint, providing a dark period from the beginning of the flock does not have appreciable negative impacts on broiler performance as previously thought by many poultry producers.

CHAPTER 5

Conclusions

The results of this study provide several key insights into the effects of providing a dark period through the entire flock on broiler performance and physiological responses.

- Both the four and six-hour dark periods during brooding significantly reduce body weight during the first week of age compared to broilers brooded under continuous light.
- The birds brooded with dark periods exhibited compensatory growth with significantly higher body weight, and weight gain than broilers brooded with continuous light from Days 10 to 21.
- Feed efficiency and end-of-flock performance are unaffected by brooding broilers under dark periods.
- No differences in plasma corticosterone or superoxide dismutase were noted when broilers were provided a dark period in the first week of grow-out.
- Birds provided with a dark period during brooding exhibit significantly higher melatonin levels during the light and dark periods than broilers brooded under continuous light.
- Brooding broilers with dark periods initiated elevated melatonin setpoint levels during the light and dark periods until five weeks of age.
- Performance of birds in a commercial broiler house setting is unaffected by brooding broilers with dark periods.

Early dark periods reduced weight at the end of the first week but no differences in performance were observed by the end of the flock. The melatonin production in the broiler chicks brooded with a dark period suggests early stimulation of the pineal gland, leading to higher melatonin levels throughout the flock.

In the modern poultry industry, there is a pressing need to balance the demands of feeding a growing global population, addressing consumer-driven animal welfare concerns, and providing straightforward recommendations for poultry growers to implement. This research offers a framework for a simple lighting program that does not negatively impact bird performance and is compatible with broiler welfare guidelines. Lighting programs like this can be seamlessly integrated into every chicken house equipped with a lighting system, and it comes at no additional cost to the grower. While minimal, one could argue that this light program would lower grower expenses by utilizing less energy from lights in the first week. This approach is one way to address the key challenges of performance and animal welfare compatibility while ensuring practicality and affordability for poultry producers.

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