2017

Behavior and production responses of pullets and laying hens to enriched housing and lighting

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Recommended Citation

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Behavior and production responses of pullets and laying hens to enriched housing and lighting

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Agricultural and Biosystems Engineering

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Ames, Iowa

2017

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ACKNOWLEDGMENTS

As I write down these words, I recall many fond memories from my Ph.D. study at Iowa State University. The path to earning a doctorate degree is not easy. I treasure all the experiences and adventures I have undergone on my journey to becoming a doctor. I am thankful to everyone who has encouraged, inspired, or helped me, and I wish to express my deepest gratitude for the support I have received during this process.

To my esteemed advisor - Dr. Hongwei Xin, I would like to express my sincerest gratitude for your unreserved guidance, continuous support, insightful comments, inspiring ideas, and uplifting encouragement throughout my Ph.D. study. You are not only a distinguished advisor for my research, but also an extraordinary mentor for my life.

To the rest of my committee members - Dr. Steven Hoff, Dr. Lie Tang, Dr. Suzanne Millman, and Dr. Zhengyuan Zhu, thank you for your valuable time and effort in helping me with my dissertation research projects. All your suggestions, comments, and insightful ideas, as well as those tough questions you posed, have inspired me to widen my research perspectives.

To a key number of individuals - Dr. Tong Wang, Dr. Petek Settar, Dr. Jasreen Sekhon, Lesa Vold, Maro Ibarburu, Kris Bell, my research group members (Tim Shepherd, Dr. Yang Zhao, Dr. Lilong Chai, Jofran Oliveira, Suzanne Leonard, and Dr. John Stinn), and my undergraduate research assistants (Haocheng Guo, Evan Anderson, John Remus, Dustin Kroening, Kyle Dresback, Jordan Keck, Jacyn Goebel, and Brad Richardson), thank you for all your efforts in helping me with my study, research, and life at Iowa State University.

To my dear friends - Guang Han, Tao Fei, Weijie Li, Qi Chen, Zhenping Liu, and Zhenhua Bai, thank you for your support and company. My friendships with you all really encouraged me when tackling these academic and life challenges. I know you guys will always have my back.

To my lovely family - my parents, grandparents, and girlfriend Yanxi Lu, thank you for your selfless love towards me and spiritual support throughout my Ph.D. study.

To the chickens that made my research possible - although I cannot remember each of your names, all your lives are respected. I hope you all feel proud of the contributions you have made.

ABSTRACT

The global demand for egg-source protein has been increasing rapidly along with the mounting public concerns over laying hen welfare. As a result, alternative hen housing has been emerging and adopted in different parts of the world, especially in developed countries. This dissertation had the overarching goal of generating the much-needed knowledge related to alternative laying hen housing design and management for improved laying hen welfare, efficiency of resource utilization, and production performance. Supporting this overarching goal were two primary research objectives that aimed to quantify behavioral and production performance responses of pullets and laying hens to perch design/configuration and light type/source. Toward that end, this dissertation covered five experiments that were conducted in controlled environment, aiming to supplement the existing knowledge base for the perches and lighting used in egg production systems. Each experiment aimed to fulfill a specific set of objectives, including: 1) examine perch-shape preference by laying hens and characterize temporal perching behavior of novice hens (no prior perching experience) after transfer from pullet-rearing cage to enriched colony setting (Chapter 2), 2) validate the suitability of the existing perch guideline on the minimum horizontal space requirement between parallel perches for laying hens (Chapter 3), 3) quantify the performance of a poultry-specific LED light *vs.* a warm-white fluorescent light with regards to their effects on pullet growing performance, activity levels, and welfare (Chapter 4), 4) investigate light preference of pullets and laying hens between a poultry-specific LED light *vs.* a warm-white fluorescent light, and evaluate the potential influence of prior lighting experience of birds on their subsequent preference for light (Chapter 5), and 5) evaluate the effect of light exposure of a poultry-specific LED light *vs.* a warm-white fluorescent light during rearing or laying phase on timing of sexual maturity, egg production performance, egg quality, and egg yolk cholesterol content of laying hens (Chapter 6).

The main findings from the experiments covered in this dissertation are as follows. The novice young hens showed increasing use of perches over time, taking them up to 5-6 weeks of perch exposure to approach stabilization of perching behaviors in the enrich colony setting; and the birds showed no preference for the perch shape of round or hexagon (Chapter 2). The horizontal distance of 25 cm between parallel perches was shown to be the lower threshold to accommodate the hen's perching behaviors (Chapter 3). The poultry-specific LED light and the fluorescent light yielded comparable growing performance, livability, and feather conditions of W-36 pullets during the rearing phase, but the poultry-specific LED light showed more stimulating effect on the pullet activity levels (Chapter 4). Pullets and laying hens exhibited a somewhat stronger choice for the fluorescent light as compared to the poultry-specific LED light, regardless of prior lighting experience; however, this tendency did not translate to differences in the proportion of feed use under each light type (Chapter 5). The poultry-specific LED lights yielded comparable production performance and egg quality of W-36 laying hens to the fluorescent lights (Chapter 6). Results from this dissertation research are expected to contribute to a) scientific information on laying hen perch design and placement and responses of novice birds to perch introduction, b) scientific evidence for setting or refining guidelines on horizontal distance of perches for laying hens in alternative hen-housing systems, and c) decision-making in selection of lighting type or source for efficient pullet rearing and egg production. The research also identified areas that may be considered in the future studies.

CHAPTER 1

GENERAL INTRODUCTION

Introduction

Egg production has undergone remarkable advancements over the past six decades. From 1960 to 2016, the annual egg supply in the U.S. has increased by approximately 60% (USDA, 2017). In the meantime, according to a life cycle assessment conducted by the Egg Industry Center, the total environmental footprints of the U.S. egg industry reduced drastically by over 50% over the period of 1960-2010 (Pelletier *et al.*, 2014). The advancements of the egg production were attributed to the improvements in poultry breeding and genetics, disease prevention and control, housing and environmental management, nutritional care and utilization efficiency in feed and other natural resources, as well as the increased crop yields (Xin and Liu, 2017). According to the "Chickens and Eggs 2016 Summary" from the National Agricultural Statistics Service (NASS), the U.S. annual average egg production on hand in 2016 was 279 eggs per layer (USDA, 2017). With an average of 365 million layers in stock during 2016, the U.S. annual total egg production reached 102 billion eggs (USDA, 2017). Though egg industry in the U.S. and many other countries has achieved an unprecedented production scale and efficiency, the global demand for egg-source protein has been increasing rapidly due to the growing population and rising income, particularly in developing countries. The world total population will reach 9.15 billion in 2050 according to the United Nations World Population Prospects-the 2008 revision (United Nations, 2008). Based on this assumption, the Food and Agriculture Organization (FAO) predicted that in order to satisfy the expected food and feed demand, global food production will be required to have a substantial increases of 70% by 2050, involving an additional quantity of approximately 40 million tons of egg production (FAO, 2009; Alexandratos and Bruinsma, 2012). Considering the scarcity of the natural resources that can be used for food and feed production, along with the increasing challenge to feed the world in the foreseeable future, further improvement in utilization efficiency of natural resources (e.g., feed, water, land, energy) in egg production is imperative.

Along with the increasing demand for animal-source protein over the past six decades is the mounting public concerns over animal welfare, which continually calls for the industries and legislations to improve animal welfare during production. The mounting pressure for the egg industry has led to development and adoption of alternative egg production systems (e.g., enriched colony, cage-free aviary, free-range housing) that aim to better accommodate natural behaviors of birds (e.g., perching, nesting, dustbathing, foraging), thereby yielding plausibly improved animal welfare (Xin and Liu, 2017). Work on alternative egg production systems started in the 1970s and was most active in the 1980s, and primarily aimed at reducing welfare problems during egg production by replacing conventional cages (Appleby, 2003). One of the most important milestones of the egg industry is the passing of the European Union Council Directive 1999/74/EC, a legislation that established the minimum standards for protection of laying hens, including the ban on conventional cages in EU from 2012 (Council Directive 1999/74/EC, 1999). Because of the EU’s ban on conventional cages, the alternative housing systems have been finding increasing adoption in egg production worldwide. As most laying hens are still housed in conventional cages in the United States (approximately 85%) and many other major egg-producing countries (e.g., China, Mexico, Japan, Indian, and Brazil), a substantial increase in adoption of the alternative housing systems is likely to happen in the foreseeable future (e.g., more than 100 retailers, grocers, restaurant chains and entertainment companies in the U.S. have pledged to source only cage-free eggs by 2025 or 2030, amounting to more than 72% of the current U.S. national layer inventory) (Xin and Liu, 2017). However, the so-called welfare-friendly alternative housing systems also have their own disadvantages regarding the laying hen welfare, such as piling, pecking, keel bone deformation, and mechanical injuries that lead to elevated mortality or morbidity. To fulfil the increasing demand for ameliorating laying hen welfare, research toward eliminating the negative impacts of the alternative housing systems on laying hens is urgently needed.

Based on the information described above, research described in this dissertation had the overarching goal of generating the much-needed knowledge related to alternative laying hen housing design and management for improved laying hen welfare, efficiency of resource utilization, and production performance. Supporting the overarching goal were two primary research objectives that aimed to quantify behavioral and production performance responses of pullets and laying hens to perch design/configuration and light type/source. Perch and lighting are two crucial external factors in egg production systems that impact bird behavior, development, production performance, health, and welfare. The importance of perch and lighting has made them research hotspots in the scientific and industry communities for several decades. The following sections describe perches and lighting used in egg production systems.

Perches and Lighting Used in Egg Production Systems

Perches in Egg Production Systems

Modern breeds of laying hens originated from red junglefowl (*Gallus gallus*) in that red junglefowl was first domesticated in Asia at least five thousand years ago. Perching is a natural behavior of red junglefowl (Fig. 1). Under natural conditions, red junglefowl usually perch on tree branches or bushes to roost at night to keep themselves away from potential dangers from the ground (e.g., night-hunting ground predators) (Struelens and Tuyttens, 2009). Despite the long-term domestication, perching behavior has not been lost in domestic laying hens (Fig. 1). Indeed, laying hens are highly motivated to roost on elevated perches at night in modern egg production systems when elevated perches are provided (Weeks and Nicol, 2006; Hester, 2014). Research found that hens were prepared to work by pushing open weighted doors for access to perches for nighttime roosting, and displayed signs of unrest when roosting was thwarted (Olsson and Keeling, 2000; Olsson and Keeling, 2002). A summary of scientific studies regarding perch use and perching behaviors of laying hens is listed in Table 1. Typically, when perch space is sufficient, most of laying hens (about 80-100% of the total hens) will roost on elevated perches throughout the nighttime. In contrast, the use of perches is considerably less during the daytime as compared to nighttime. During the daytime, laying hens jump on and off perches frequently and spend about 25-50% of time roosting on perches. According to the scientific evidence about hen motivation to perch, perching behavior has been considered a high behavioral priority of laying hens.

Figure 1. Red junglefowl roosting on tree branches (left[1]) and laying hens roosting on perches (right[2]).

With the scientific knowledge indicating that perching is a high behavioral priority of laying hens, requirements or legislations for providing appropriate perches to laying hens appeared. Switzerland first established legislation to improve welfare of laying hens in that conventional cages were banned in 1992 and all housing systems must provide at least 14 cm of elevated perches per hen (HÄne *et al.*, 2000; Käppeli *et al.*, 2011). Thereafter, the EU Directive set forth the minimum standards, which states that perch must have no sharp edges and perch space must be at least 15 cm per hen in alternative hen housing systems. In addition, horizontal distance between perches and between perch and wall should be at least 30 and 20 cm, respectively (Council Directive 1999/74/EC, 1999). As a result, perch became one of the most essential enrichments in alternative housing systems. However, ambiguities and debates existed due to unclear statement in perch design (e.g., material, color, height, shape, and size) and lack of substantive scientific information at that time. Some researchers criticized that this directive was more about satisfying public opinion than to meet laying hen's actual need (Savory, 2004). In the U.S., there is no specific legislation regarding the

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[1] Source:https://www.cacklehatchery.com/media/catalog/product/cache/1/image/9df78eab33525d08d6e5fb8d27136e95/s/h/shutterstock_160677413.jpg

[2] Source:http://media.npr.org/assets/img/2014/12/29/enriched-cage_custom-bdef4c96a151db26825b3bc07edeae34c13a5072-s900-c85.jpg

use of perches in egg production systems so far. However, due to the increasing adoption of enriched colony and cage-free systems, there are several certification programs (e.g., UEP Standard, American Humane Certified Standard, and HFAC Standard) that set standards for providing laying hen perches in alternative housing systems. For illustration, a summary of legislations or standards for providing perches in egg production systems is listed in Table 2.

Effects of providing perches to laying hens and laying hen perching behaviors have drawn extensive attention of researchers and egg producers over the past four decades. Many studies have been conducted to investigate perch design (e.g., type, shape, size, texture, and material) and spatial perch arrangement (e.g., height, angle, and relative location). These studies mainly focused on the effects of perch provision on production performance (e.g., body weight, egg production, egg quality, feed usage, and feed efficiency), health and welfare (e.g., skeletal and feet health, feather condition, and physiological stress), and perching behaviors (e.g., perch use and preference) of laying hens (Struelens and Tuyttens, 2009; Hester, 2014; Panel and Ahaw, 2015). Results of studies from both laboratory and commercial settings have shown benefits as well as detriments of providing perches to laying hens. For example, use of perches can stimulate leg muscle deposition and bone mineralization (Enneking *et al.*, 2012; Hester *et al.*, 2013a), increase certain bone volume and strength (Hughes *et al.*, 1993; Appleby and Hughes, 1990; Barnett *et al.*, 2009), reduce abdominal fat deposition (Jiang *et al.*, 2014), and reduce fearfulness and aggression (Donaldson and O'Connell, 2012). However, keel bone deformities, foot disorders (e.g., bumble foot) and bone fractures have also been reported to be associated with perches (Appleby *et al.*, 1993; Tauson and Abrahamsson, 1994; Donaldson *et al.*, 2012). Moreover, controversies occur when contradictory results are derived from different experiments. For instance, some studies showed beneficial impacts of perches on feather condition or mortality of laying hens (Duncan et al., 1992; Glatz and Barnett, 1996; Wechsler and Huber-Eicher, 1998), whereas others showed detrimental impacts (Tauson, 1984; Moinard *et al.*, 1998; Hester *et al.*, 2013b). Recently, European Food Safety Authority (EFSA) Panel on Animal Health and Animal welfare (AHAW) conducted systematic and extensive literature reviews to assess the appropriate height and position of perches, as well as perch design features (e.g., material, color, temperature, shape, width, and length), and found that relevant features of perches are often confounded with others with regards to their impacts on laying hens (Panel and Ahaw, 2015). In addition to perch characteristics mentioned above, the management of pullets and laying hens (e.g., timing of perch introduction to birds) will also have an impact on laying hen perching behaviors and performance. Research found that rearing pullets without early access to perches, in some ways, would impair the spatial cognitive skills of hens (Gunnarsson *et al.*, 2000), thus may be detrimental to their subsequent perching ability and long-term welfare. Similarly, studies showed that early assess to perches had positive effects on musculoskeletal health of pullets as well as subsequent long-term health of hens (Hester *et al.*, 2013a; Yan *et al.*, 2014; Habinski *et al.*, 2016).

Table 1. Summary of studies regarding perch, perch use, and perching behaviors of laying hens

Breed	Age (wk)	Perch			Perch Utilization		Reference
		Space (cm/bird)	Type	Height (cm)	Daytime (%)	Night (%)	
White Leghorn	22-82	12	round wood (d = 33 mm)	7.5	20-50	80-100	Tauson (1984)
White Leghorn	16-56	16	round wood (d = 33 mm)	7.5	25		(Braastad (1990)
ISA Brown	18-71	11.25 15	rectangular (50 × 25 mm)	7.5	25	76-85	Appleby *et al.* (1992)
ISA Brown	20-72	11.25 15 22.5	round softwood (d = 35 mm)	7.5	41-47	60-72 72-78 99	Duncan *et al.* (1992)
ISA Brown	18-72	15	rectangular softwood (50 × 25 mm)	9	25	90-94	Appleby *et al.* (1993)
White Leghorn	19-80	12	round hardwood (d = 36 mm)	7	25	90	Abrahamsson and Tauson (1993)
White Leghorn	20-80	12 16	round softwood (d = 36 mm)	7.5	20-26	93-99	Tauson and Abrahamsson (1994)
White Leghorn	20-80		plastic mushroom (48 × 68), round softwood (d = 36)		23-25	88-94	
ISA Brown	20-44	15	rectangular softwood (50 × 25 mm)	9	32-37	92-98	Appleby and Hughes (1995)
ISA Brown	18-72	12 13 14 15	rectangular softwood (50 × 25 mm)	9	30-36	81-95	Appleby (1995)
White Leghorn	19-30	15		45 70	31-35		Wechsler and Huber-Eicher (1998)
White Leghorn	36	90	rectangular hardwood (45 × 45 mm)	23 43 63		97-99	Olsson and Keeling (2000)
ISA Brown	43-52	15		17.5 35 70	24	18	Cordiner and Savory (2001)
White Leghorn	3-18	10 20	softwood rails with beveled edges (30 × 30 mm)	20 40 60	38		Newberry *et al.* (2001)
Lohmann Brown, Lohmann White, Hy-Line White, Hy-Line Brown	20-80	12 15				65-88	Wall and Tauson (2007)
White Leghorn	16-42	17	rectangular wood (23 × 30 mm)		28	65-70	Valkonen *et al.* (2009)
Hy-Line Brown	29-67	15	oval wood (36 × 30 mm)	9	21-37	30-66	Barnett *et al.* (2009)
Bovans Goldline	18-24		rectangular wood (13, 30, 45, 60, 75, 90, 105 × 15 mm)	12	47-51		Struelens *et al.* (2009)
White Leghorn	18-27	20	round wood, steel, and rubber cover (d = 27, 34, 45 mm)	40		97.5	Pickel *et al.* (2010)
White Leghorn	18	20	round metal (d = 34 mm)	40		93	Pickel *et al.* (2011)

Table 2. Legislations or standards for providing perches to laying hens in egg production systems

Standard/Legislation	Housing Type	Requirements
EU Directive (Council Directive 1999/74/EC, 1999)	non-cage systems	▪ at least 15 cm per hen ▪ at least 30 cm horizontal distance between perches ▪ at least 20 cm horizontal distance between the perch and the wall ▪ no sharp edges ▪ must not be mounted above the litter
	enriched cages	▪ at last 15 cm per hen
UPE Standard (UEP, 2017)	cage-free	▪ at least 15 cm per hen ▪ at least 30 cm horizontal distance between perches ▪ at least 30 cm horizontal distance between the perch and the wall ▪ at least 20% of the perch elevated to a minimum of 40 cm above the adjacent floor ▪ at least 20 cm from the top of the perch to the ceiling or other structures
American Humane Certified Standard (Amercian Humane, 2017)	enriched colony	▪ at least 15 cm per hen ▪ at least 24 cm of clear head height above (20 cm for perches over internal feed troughs) ▪ 25-45 mm in width at the top ▪ a gap of no less than 13 mm on either side of any perch ▪ no sharp edges
American Humane Certified Standard (Amercian Humane, 2016)	cage-free	▪ at least 15 cm per hen ▪ at least 30 cm horizontal distance between perches ▪ at least 30 cm horizontal distance between the perch and the wall ▪ at least 20% of the perch elevated to 40-100 cm above the adjacent floor ▪ at least 24 cm of clear height above perches (20 cm of clear height over internal feed troughs) ▪ 25-45 mm in diameter
HFAC Standard (HFAC, 2017)	all systems	▪ at least 15 cm per hen ▪ at least 30 cm horizontal distance between perches ▪ at least 20 cm distance from any wall or ceiling ▪ at least 20% of the perch elevated 40-100 cm above the adjacent floor ▪ a gap of no less than 13 mm on either side of any perch ▪ at least 2.54 cm wide at the top (rounded perches must have a diameter of not less than 2.54 cm and not greater than 7.6 cm) ▪ no sharp edges ▪ replacement pullets must have access to perches starting before 4 weeks of age

Lighting in Egg Production Systems

Artificial light sources have been used in egg production systems for many decades (Fig. 2). As light is a crucial environmental factor that affects behavior, development, production performance, health, and well-being of poultry (Lewis and Morris, 1998; Parvin *et al.*, 2014), lighting in egg production systems has drawn much attention from both scientific and industrial communities. In general, lighting used in egg production systems has various characteristics that can greatly impact birds, mainly including photoperiod, light intensity, and light wavelength or color.

Research on poultry lighting dates back to the early 1930s. Since then, extensive research has led to a broad understanding of lighting effects on poultry. The early studies mainly focused on the impacts of photoperiod and light intensity on behavior, development, production, and reproductive traits of poultry. For example, studies found that sexual development and maturity of pullets were associated with changes in photoperiod, while activity levels of birds were positively correlated to light intensity. All those early studies have led to the establishments of general lighting guidelines on photoperiod and light intensity for improved animal performance and energy efficiency (e.g., ASABE EP344.4 - Lighting systems for agricultural facilities, Hy-Line Commercial Layers Management Guideline).

In more recent decades, the emphasis of poultry lighting has been placed on various light colors (e.g., blue, green, red, and white) and lighting sources (e.g., incandescent, fluorescent, and LED lights) (Lewis and Morris, 2000; Parvin *et al.*, 2014). A list of studies concerning these aspects is summarized in Table 3. The transformation of research emphasis to light colors and lighting sources was mainly caused the increasing understanding on poultry physiology (e.g., poultry vision) and the advancement of lighting technology (e.g., the emerging LED lights). Research has shown that poultry and humans have different light spectral sensitivities (Fig. 3) (Prescott *et al.*, 2003; Saunders *et al.*, 2008). When humans have three types of retinal cone photoreceptors, poultry have five that are sensitive to ultraviolet, short-, medium-, and long-wavelength lights (Osorio and Vorobyev, 2008). Compared to humans, poultry can perceive light not only through their retinal cone photoreceptors in the eyes, but via extra retinal photoreceptors in the brain (e.g., pineal and hypothalamic glands) (Mobarkey *et al.*, 2010). Retinal cone photoreceptors produce the perception of light colors by receiving lights at the peak sensitivities of approximately 415, 450, 550, and 700 nm (Lewis and Morris, 2000). In contrast, the extra retinal photoreceptors can only be activated by long-wavelength lights (e.g., red) that can penetrate the skull and deep tissue of poultry head (Lewis and Morris, 2000). With the knowledge of the spectral sensitivity of poultry, considerable efforts have been made to understand poultry responses to light stimulus and to impact poultry (e.g., growth, reproduction, and behavior) by manipulating light stimulations to their retinal and extra-retinal photoreceptors.

Research has demonstrated that red lights have an accelerating effect on sexual development and maturity of poultry, and can facilitate egg production as compared to short-wavelength lights (e.g., green and blue lights) (Woodard *et al.*, 1969; Harrison *et al.*, 1969; Pyrzak *et al.*, 1987; Gongruttananun, 2011; Min *et al.*, 2012; Huber-Eicher *et al.*, 2013; Baxter and Joseph, 2014; Wang *et al.*, 2015; Yang *et al.*, 2016). In contrast, some studies found that exposure to short-wavelength lights (e.g., green and blue lights) led to improved egg quality (e.g., increased egg weight, shell thickness, or shell strength) as compared to exposure to long-wavelength lights (e.g., red light) (Pyrzak *et al.*, 1987; Er *et al.*, 2007; Min *et al.*, 2012; Hassan *et al.*, 2014; Li *et al.*, 2014). In addition, blue lights are found to be more associated with improving growth, calming the birds, and enhancing the immune response (Prayitno *et al.*,1997; Rozenboim *et al.*, 2004; Cao *et al.*, 2008; Xie *et al.*, 2008; Sultana *et al.*, 2013). Based on these earlier research findings, many lighting manufacturers have designed LED lights specifically for poultry production by integrating some light traits that have been shown to be beneficial to certain poultry production aspect (e.g., growth, reproduction, or well-being). Figure 4 illustrates the spectral characteristics of some emerging poultry-specific LED lights by comparing with the traditional incandescent and fluorescent lights. It is well known that the LED lights have advantages over the traditional incandescent and fluorescent lights on their operational characteristics (e.g., more energy-efficient, durable, and dimmable). As the emerging poultry-specific LED lights are increasingly finding applications in egg production systems, the increasing adoption of the emerging LED lights may contribute to the further improvement of egg production.

Figure 2. Examples of artificial light sources used in laying hen housing systems[3].

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[3] Source:https://www.hato.lighting/sites/default/files/HATO%20CORAX%20lighting%20layer%20stable%20600x400_0.jpg

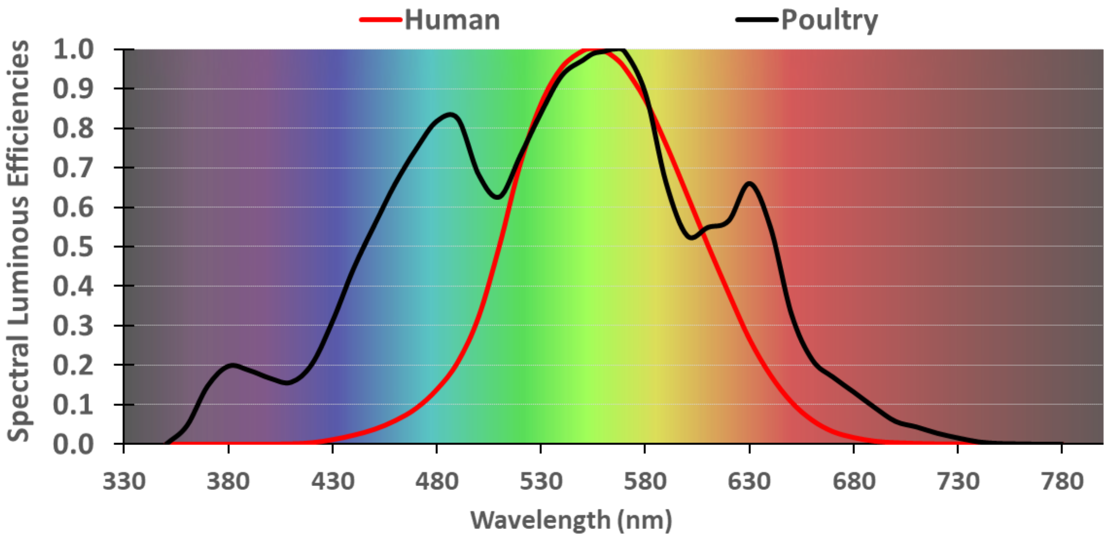

Figure 3. Spectral sensitivities of humans and poultry at various wavelengths[4].

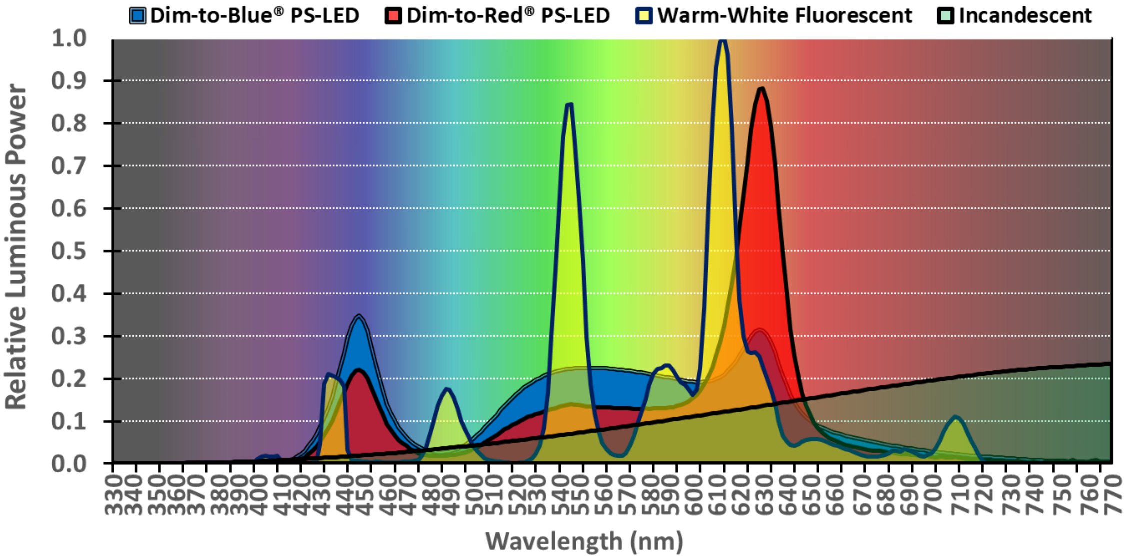

Figure 4. Spectral characteristics of the incandescent light, fluorescent light (warm-white), and poultry-specific LED lights (Dim-to-Blue® PS-LED and Dim-to-Red® PS-LED, PS-LED = poultry-specific LED light)[5].

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[4] Data from book: Poultry lighting – the theory and practice. Peter Lewis (2006)

[5] Figure from paper: Choice between fluorescent and poultry-specific LED lights by pullets and laying hens. Liu *et al.* (2017)

Table 3. Summary of studies regarding light colors or lighting sources in egg production systems

Experimental Light	Test Parameters	Reference
incandescent, cool-white, soft-white fluorescent, green, gold, blue, red	mortality, age at sexual maturity, egg production	Carson *et al.* (1958)
red, green, white fluorescent	cannibalism, body weight, mortality, egg production	Schumaier *et al.* (1968)
blue, green, red, clear	sexual maturity, egg production, egg weight	Harrison *et al.* (1969)
incandescent, blue, greed, red	egg production	Harrison (1972)
incandescent, fluorescent	body weight, feed intake, egg production, fertility and hatchability of eggs	Sipoes (1984)
blue, green, red, cool-white, sunlight-simulating fluorescent, incandescent	sexual maturity, body weight, abdominal fat	Pyrzak *et al.* (1986)
blue, green, red, cool-white, simulated-sunlight fluorescent, incandescent	egg production, egg quality	Pyrzak *et al.* (1987)
incandescent, compact fluorescent	preference	Widowski *et al.* (1992)
incandescent, fluorescent	physical activity, energy expenditure	Boshouwers and Nicaise (1993)
high-frequency, low-frequency compact fluorescent	preference	Widowski and Duncan (1996)
mini-fluorescent, green, red, infrared LED	egg production, feed consumption, egg quality	Rozenboim *et al.* (1998)
high-pressure sodium, incandescent	preference	Vandenbert and Widowski (2000)
blue, green, red LED	egg weight, egg quality	Er *et al.* (2007)
white, green	body weight, feed intake, sexual maturity, egg production, egg quality	Lewis *et al.* (2007)
red, orange, yellow, green, blue, violet	mortality, sexual maturity, egg production, feed consumption, egg quality	Kavtarashvili *et al.* (2007)
fluorescent, red LED	body weight, feed consumption, mortality, sexual maturity, egg production, egg quality, eye morphology	Gongruttananun (2011)
incandescent, white, blue, red LED	sexual maturity, egg production, egg quality, feed intake, feed conversion, ovary weight,	Min *et al.* (2012)
white, green, red LED	behavior, body weight, feed consumption, sexual maturity, egg production	Huber-Eicher *et al.* (2013)
incandescent, blue, yellow, green, red, white LED	egg production, egg weight, feed intake, egg quality	Borille *et al.* (2013)
red, green, blue, white	egg production, egg weight, egg quality, feed intake, feed conversion, sexual maturity, reproductive hormones	Hassan *et al.* (2013)
green, white, red	sexual maturity, egg production, body weight, stress	Baxter *et al.* (2014)
white, green, red, blue	behavior, egg production, egg weight, feed intake, feed conversion, egg quality	Hassan *et al.* (2014)
blue, green, red, white	body weight, sexual maturity, egg production, egg quality, fertility and hatchability, hormone	Li *et al.* (2014)
incandescent, fluorescent, LED	body weight, sexual maturity, egg production, egg quality, feed intake, feed conversion,	Kamanli *et al.* (2015)
blue, green, red, white	egg production, melatonin receptors	Li *et al.* (2015)
red, white, blue, yellow, green	egg production, egg weight, feed conversion, egg quality,	Borille *et al.* (2015)
blue, green, red, yellow	egg production, egg weight, mortality, bacterial strain	Svobodová *et al.* (2016)
fluorescent, LED	light operational traits, egg production, egg quality, mortality, feed intake, feed conversion, stress, welfare	Long *et al.* (2016a) Long *et al.* (2016b)

Existing Issues and Research Needs

With regards to the perch used in egg production systems, although extensive research has been conducted to investigate the effects of perch provision on perching behaviors, production performance, health, and welfare of laying hens, neither the egg industry nor the scientific community has designed a perfect perching system so far. As described earlier, the provision of perches in hen housing systems could still lead to many detrimental effects (e.g., keel bone deformities, foot disorders, and bone fractures) that would negatively impact production and welfare of the birds. Therefore, to enhance production efficiency and welfare of laying hens, considerable efforts are still needed towards optimizing perch design (e.g., shape, size, texture, material, and temperature), spatial arrangement (e.g., height, angle, and relative position), and management (e.g., timing of bird's introduction to perches).

In terms of the lighting used in egg production systems, more energy-efficient, readily-dimmable, long-lasting, and more affordable LED lights are increasingly finding applications in egg production operations. Just as CFL lamps have been replacing incandescent lamps, LED lights will replace CFL lamps and become the predominant lighting source in the foreseeable future. However, the existing lighting guidelines or recommendations (e.g., Hy-Line Commercial Layers Management Guideline) were mainly established based on the traditional incandescent or CFL lights, which may not accurately reflect the operational characteristics and impact of the LED lights on birds. In addition, despite anecdotal claims about advantages of some commercial poultry-specific LED lights over traditional incandescent or fluorescent lights on poultry performance and behavior, data from controlled comparative studies are lacking. Therefore, there is a need for more research regarding the impact of poultry-specific LED lights on poultry and the corresponding lighting strategy for sustainable egg production.

Objectives and Outline of the Dissertation

This dissertation includes seven chapters. Besides the current chapter (Chapter 1), each of the following five chapters (Chapters 2-6) represents an experiment conducted in an environment-controlled laboratory that supplements the existing knowledge base on behavior and production responses of pullets and laying hens to the enriched housing (with perches) and lighting (poultry-specific LED light *vs.* fluorescent light). All the experiments are summarized in the final chapter (Chapter 7), along with a general discussion on the practical implications and future research needs. The experiments in this dissertation address the following specific objectives:

1) Advance the understanding of perch-shape preference by laying hens and characterize temporal perching behavior of novice hens after transferred from pullet-rearing cage into enriched colony setting, achieved by continuously quantifying perch utilization and perching behaviors of hens using a sensor-based automated perching monitoring system (Chapter 2);
2) Validate the suitability of the existing perch guideline on the minimum horizontal space requirement between parallel perches for laying hens, achieved by assessing the behavior responses of laying hens to a range of horizontal distances between parallel perches (Chapter 3);
3) Assess the performance of a commercial poultry-specific LED light *vs.* a warm-white fluorescent light with regards to their effects on pullet growing performance, activity levels, and welfare conditions, achieved by measuring physiological conditions of

individual birds and quantifying flock movement index using computer vision analysis (Chapter 4);

4) Explore light preference of pullets and laying hens between a commercial poultry-specific LED light *vs.* a warm-white fluorescent light, and evaluate the potential influence of prior lighting experience of birds on their subsequent preference for light, achieved by comparing their free-choice behaviors in preference test compartments (Chapter 5); and
5) Evaluate the effect of light exposure of a poultry-specific LED light *vs.* a warm-white fluorescent light during rearing or laying phase on timing of sexual maturity, egg production, egg quality, and egg yolk cholesterol content of laying hens (Chapter 6).

Key Experimental Setups and Methods Used in the Dissertation Research

Sensor-Based Automated Perching Monitoring

A real-time, sensor-based perching monitoring system was built by incorporating six pairs of load-cell sensors (Model 642C, Revere Transducers Inc., Tustin, CA, USA) supporting six metal perches, coupled with a LabVIEW-based data acquisition system (version 7.1, National Instrument Corporation, Austin, TX, USA). This monitoring system consisted of a compact FieldPoint controller (NI cFP-2020, National Instrument Corporation) and two 8-channel thermocouple input modules (NI cFP-TC-120, National Instrument Corporation), collecting data at 1 Hz sampling rate. In each of the experimental pens (Fig. 5), a pair of load-cell sensors was fitted with the adjustable brackets and coupled to a metal perch, forming the weighing perch (Fig. 6a). The data acquisition system automatically read analog voltage outputs of the weighing perches and converted the electronic signals to load weight using pre-defined calibration equations (Fig. 6b), thereby providing real-time measurement of load weight on the perches (Fig. 6c). The load weight of perching birds on each perch was then converted to the number of perching birds on the corresponding perch (Fig. 6d) by using a series of determined weight thresholds. With using this system, perching behaviors of the experimental birds were continuously monitored throughout the test period.

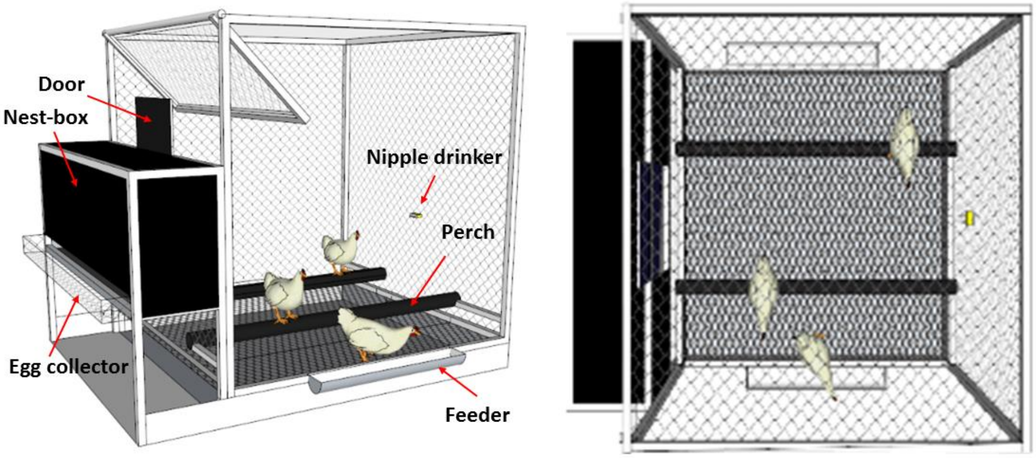

Figure 5. A schematic representation of the experimental pen[6].

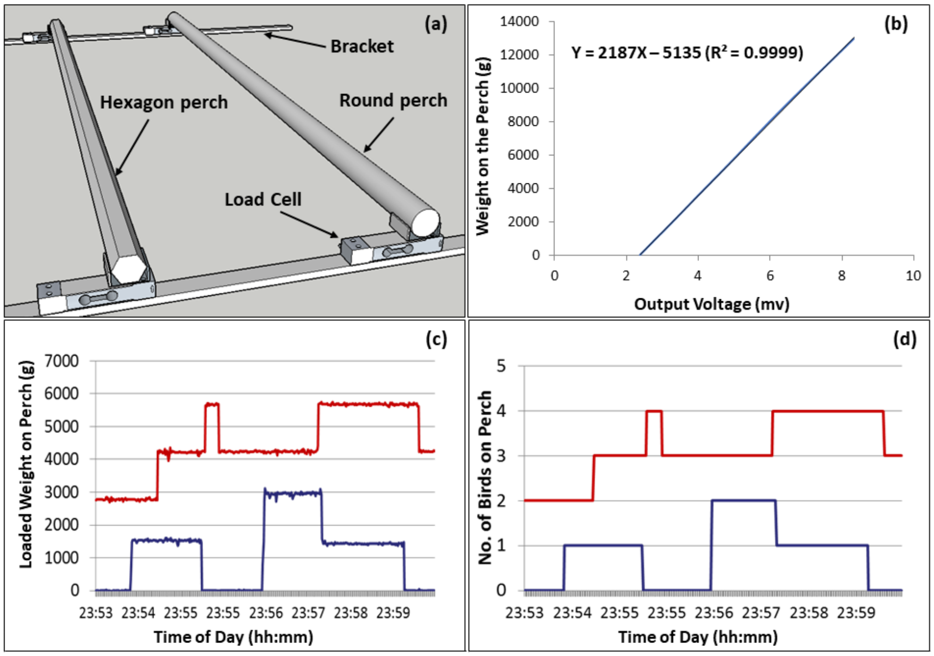

Figure 6. An automated perching monitoring system. (a) weighing perches, (b) linear response of loadcell scale output to load weight, (c) load weight of perching hens on each perch, (d) number of perching birds on each perch.

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[6] Figure from paper: Effects of horizontal distance between perches on perching behaviors of Lohmann hens. Liu and Xin (2017)

Computer Vision-Based Locomotion Quantification

Locomotion behaviors of pullets were recorded using four cameras (720P HD, night vision, Backstreet Surveillance Inc., UT, USA) per room (Fig. 7) at 5 frames per second (FPS). Video analysis was done using automated image processing programs developed in MATLAB (MATLAB R2014b, The MathWorks, Inc., Natick, MA, USA), mainly including image stitch, subtraction, conversion and binarization.

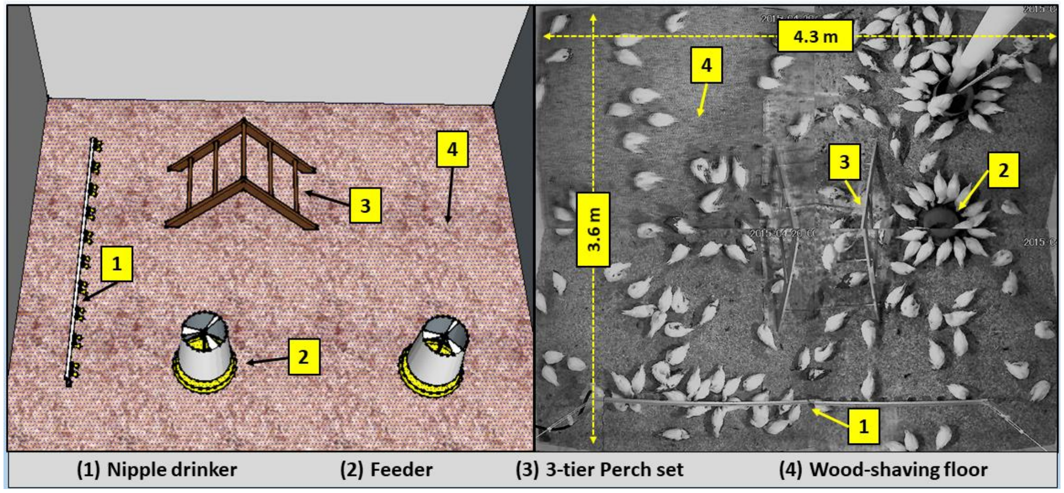

Figure 7. Schematic (left) and top photographic view (right) of the pullet-rearing room[7].

Movement index (**MI**) was used as the behavioral parameter for quantifying locomotion of the pullets, defined as the ratio of cumulative displacement area caused by moving pullets to the entire floor area at 1-s intervals. To calculate MI, image processing procedures were applied to the captured time-series video frames (5 FPS) according to the following equations.

$$P_m(x, y, f) = P(x, y, f) - P(x, y, f-1) \qquad [1]$$

$$P_m'(x, y, f) = 0.2989 \times P_m(x, y, f)_R + 0.5870 \times P_m(x, y, f)_G + 0.1140 \times P_m(x, y, f)_B \qquad [2]$$

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[7] Figure from paper: Effects of light-emitting diode light v. fluorescent on growing performance, activity levels and well-being of non-beak-trimmed W-36 pullets. Liu *et al.* (2017)

$$P_m''(x, y, f) = \begin{cases} 1, & P_m'(x, y, f) > \tau \\ 0, & otherwise \end{cases} \quad [3]$$

$$MP(f) = 100 \times \frac{\sum_{(x,y)\in I(f)} P_m''(x, y, f)}{\sum_{(x,y)\in I(f)} 1} \quad [4]$$

Where $P_m(x, y, f)$ is the difference of the RGB values of the pixels at coordinate (x, y) between two successive image frames f and $f-1$; $P(x, y, f)$ is RGB value of the pixel at coordinate (x, y) of the image fame f; $P_m'(x, y, f)$ is the difference of the intensity values of the pixels at coordinate (x, y) between two successive image frames f and $f-1$; $P_m(x, y, f)_R$, $P_m(x, y, f)_G$, $P_m(x, y, f)_B$ represents red, green and blue color value of $P_m(x, y, f)$, respectively; $P_m''(x, y, f)$ is the binary value of $P_m'(x, y, f)$, 1 or 0, representing pixel with or without movement, respectively; τ is the threshold for detecting movement; $MP(f)$ is the ratio of movement pixels between two successive image frames (f and $f-1$) to the entire image frame pixels of frame f; $I(f)$ is image frame f (Fig. 8). MI over 1-s interval at time t, MI(t), was calculated as

$$MI(t) = \left(\sum_{f=1}^{r} MP(f)\right)_t \quad [5]$$

where r represents frame rate, r = 5 FPS. To minimize the noises and random errors derived from video recording procedures, mean movement index (**MMI**) over 1-minute interval at minute i, $MMI(i)$, was calculated, of the following form,

$$MMI(i) = \left(\frac{\sum_{t=1}^{60}(MI(t))}{60}\right)_i \quad [6]$$

The resultant time-series MMI values were used to elucidate the pullet activity levels.

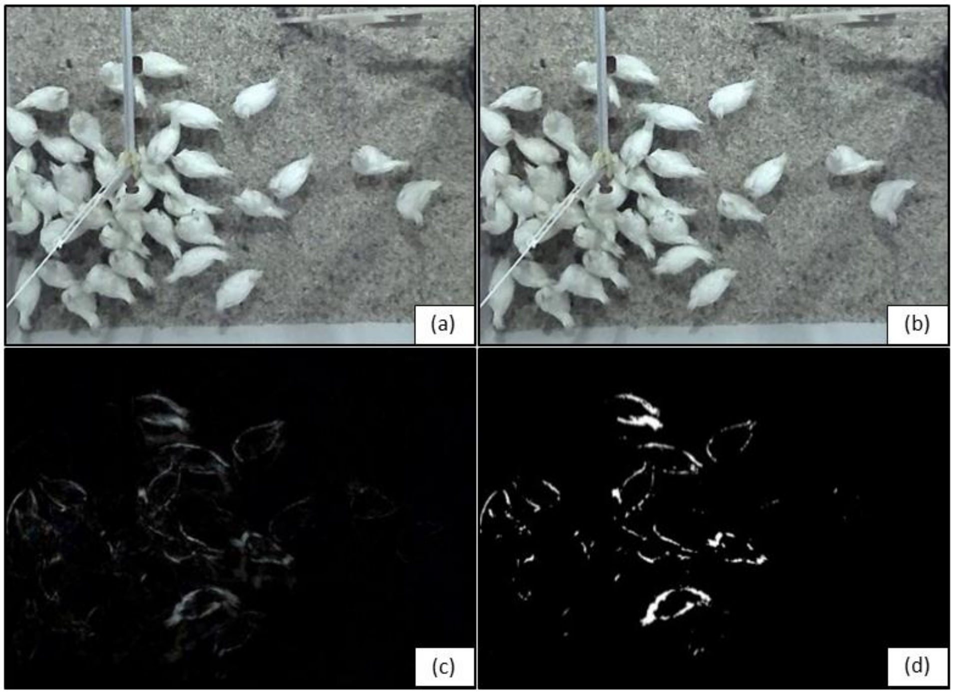

Figure 8. Image processing for determining movement index[8]. (a) Current image frame *I(f)*, (b) previous image frame *I(f-1)*, (c) grey-scale differential between *I(f)* and *I(f-1)*, (d) binary differential.

Computer Vision and Sensor-Based Preference Assessment

A real-time sensor-based feeding monitoring system was built by incorporating four load-cell scales (RL1040-N5, Rice Lake Weighing Systems, Rice Lake, WI, USA) with a LabVIEW-based data acquisition system (version 7.1, National Instrument Corporation). The system consisted of a compact FieldPoint controller (NI cFP-2020, National Instrument Corporation) and multiple thermocouple input modules (NI cFP-TC-120, National Instrument Corporation). The data were collected at 1-s intervals. Feeder weight was used for determining daily feed use by calculating the feeder weight difference between the beginning and the end of the day.

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[8] Figure from paper: Effects of light-emitting diode light v. fluorescent on growing performance, activity levels and well-being of non-beak-trimmed W-36 pullets. Liu *et al.* (2017)

A real-time vision system was built and used by incorporting four infrared video cameras (GS831SM/B, Gadspot Inc. Corp., Tainan city, Taiwan, China) and a PC-based video capture card (GV-600B-16-X, Geovision Inc., Taipei, Taiwan, China) with a surveillance system software (Version 8.5, GeoVision Inc.). One camera was installed atop each cage and recording top-view images. This vision system could record images from all four cameras simultaneously at 1 FPS. Distribution of the birds in the light preference test compartments (**LPTC**) (Fig. 9) was analyzed using an automated image processing program in MATLAB (R2014b, MathWorks Inc.) and VBA programs in Excel (Microsoft Office 2016, Redmond, WA, USA).

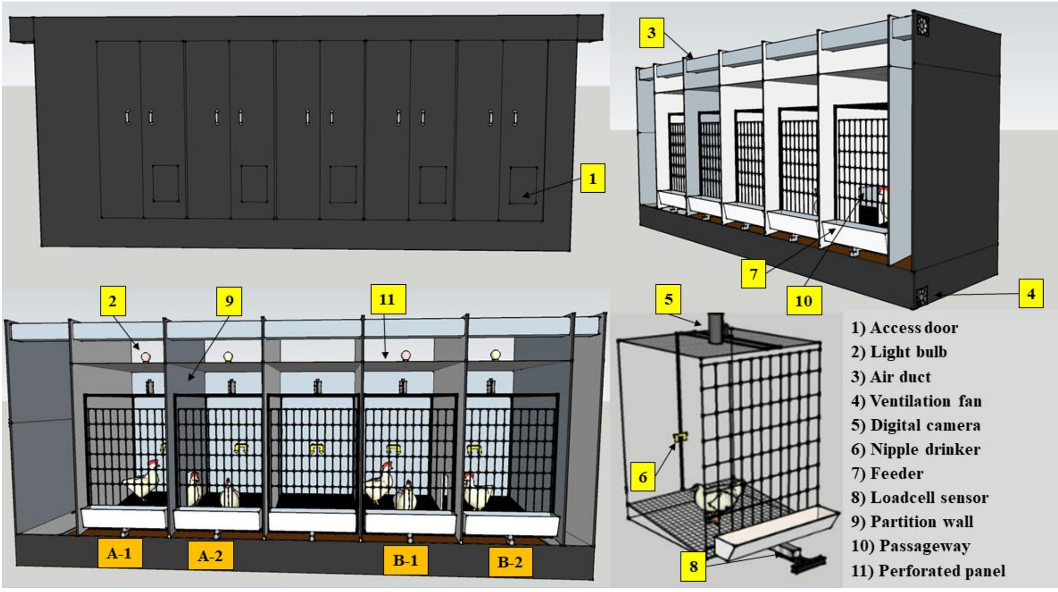

Figure 9. A schematic representation of the light preference test system[9].

The algorithm for determining the dristribution of the birds in the LPTCs consisted of four main procedures: 1) extracting pixels representing the birds in each image (Fig. 10a-e), 2) counting number of bird blobs detected in each image (Fig. 10e), 3) determining area of each

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[9] Figure from paper: Choice between fluorescent and poultry-specific LED lights by pullets and laying hens. Liu *et al.* (2017)

blob (Fig. 10f), and 4) determining the number of birds in each cage (Fig. 11). The two simultaneous images from each pair of LPTC were analyzed separately for each cage. As such, if a bird is passing through or staying at the passageway, one bird would be detected as two blobs, one per image (Fig. 11). A blob could also be a single bird, or multiple contacting birds. Contacting birds were not individually segmented during the image processing. With only three birds in LPTC, there were a maximum of four total detected blobs and 10 possible scenarios for distributions of the birds (Fig. 11). With the knowledge of number of blobs in each cage and area of each blob, the number of birds in each cage was determined using an automated VBA program in Excel.

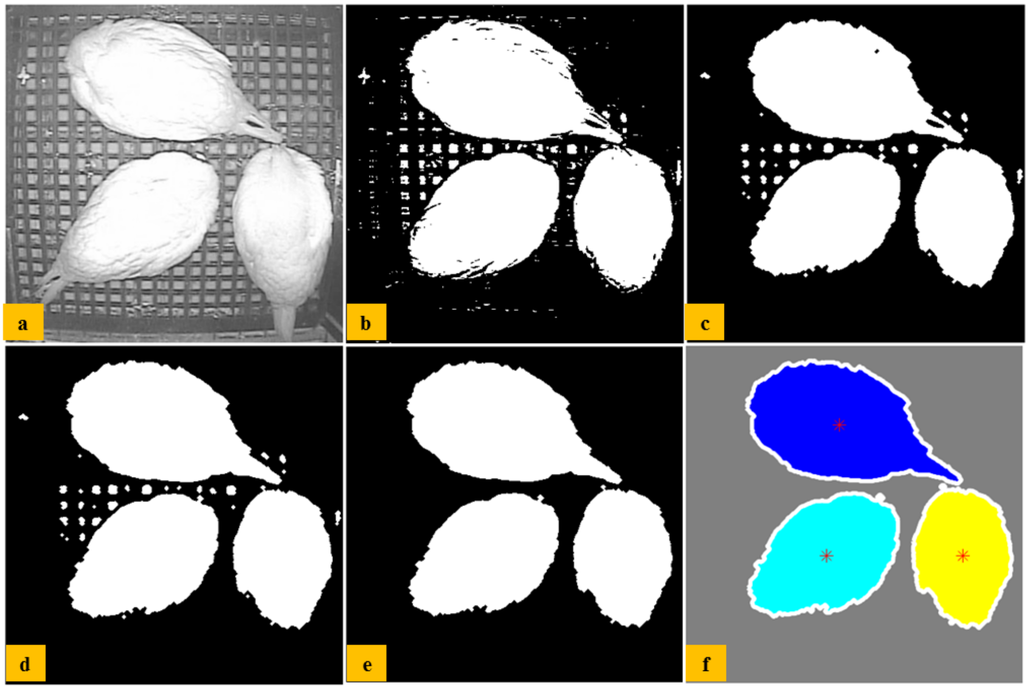

Figure 10. Image processing procedures. (a) RGB image of birds, (b) binary image of birds without enhancement, (c) binary image of birds with morphological opening operation, (d) binary image of birds with morphological closing operation, (e) binary image of birds with small objects removed, and (f) detected blobs in the binary image[10].

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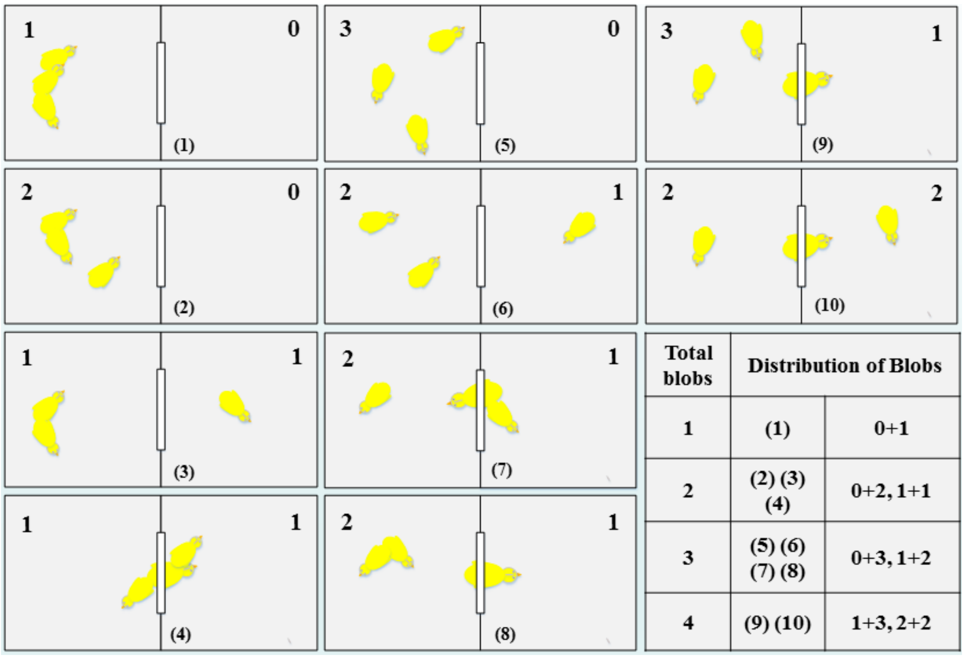
[10] Figure from paper: Choice between fluorescent and poultry-specific LED lights by pullets and laying hens. Liu *et al.* (2017)



Total blobs	Distribution of Blobs	
1	(1)	0+1
2	(2) (3) (4)	0+2, 1+1
3	(5) (6) (7) (8)	0+3, 1+2
4	(9) (10)	1+3, 2+2

Figure 11. Representative distributions of birds in the light preference test compartments. Numbers in parentheses are scenario ID's. For each scenario, three birds were present in two adjoining compartments. The small rectangular in the center represents the passageway between the compartments. The number in each corner of the compartment box represents the number of blobs detected in that compartment[11].

Expected Outcomes and Practical Implications

The experiments covered in this dissertation were conducted in controlled environment. They were expected to yield science-based data that would help guide the design and placement of perches in enriched hen housing systems and the selection of lighting type or source in egg production. In some cases, the experiments fill knowledge gaps on the subjects, and in others they provide new data toward clarifying or verifying inconsistent results reported in the literature. In either case, this research should prove conducive to the decision-making process for improving resource use efficiency and animal welfare associated with egg production.

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[11] Figure from paper: Choice between fluorescent and poultry-specific LED lights by pullets and laying hens. Liu *et al.* (2017)

References

Abrahamsson, P., & Tauson, R. (1993). Effect of perches at different positions in conventional cages for laying hens of two different strains. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *43*(4), 228–235. https://doi.org/10.1080/09064709309410171

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. *FAO ESA Working Paper No. 12-03*.

American Humane. (2016). Animal welfare standards for laying hens - cage-free. *American Humane Association.*

American Humane. (2017). Animal welfare standards for laying hens - enriched colony housing. *American Humane Association.*

Appleby, M. C. (1995). Perch length in cages for medium hybrid laying hens. *British Poultry Science*, *36*(1), 23–31. https://doi.org/10.1080/00071669508417749

Appleby, M. C. (2003). The EU ban on battery cages: history and prospects. *The State of the Animals II* (Vol. 2003).

Appleby, M. C., & Hughes, B. O. (1990). Cages modified with perches and nests for the improvement of bird welfare. *World's Poultry Science Journal*, *46*(1), 38–40. https://doi.org/10.1079/WPS19900007

Appleby, M. C., & Hughes, B. O. (1995). The Edinburgh modified gage for laying hens. *British Poultry Science*, *36*(5), 707–718. https://doi.org/10.1080/00071669508417815

Appleby, M. C., Smith, S. F., & Hughes, B. O. (1992). Individual perching behaviour of laying hens and its effects in cages. *British Poultry Science*, *33*(2), 227–238. https://doi.org/10.1080/00071669208417462

Appleby, M. C., Smith, S. F., & Hughes, B. O. (1993). Nesting, dust bathing and perching by laying hens in cages: Effects of design on behaviour and welfare. *British Poultry Science*, *34*(5), 835–847. https://doi.org/10.1080/00071669308417644

Barnett, J. L., Tauson, R., Downing, J. a, Janardhana, V., Lowenthal, J. W., Butler, K. L., & Cronin, G. M. (2009). The effects of a perch, dust bath, and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens. *Poultry Science*, *88*(3), 456–470. https://doi.org/10.3382/ps.2008-00168

Baxter, M., Joseph, N., Osborne, V. R., & Bedecarrats, G. Y. (2014). Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poultry Science*, *93*(5), 1289–1297. https://doi.org/10.3382/ps.2013-03799

Borille, R., Garcia, R. G., Nääs, I. A., Caldara, F. R., & Santana, M. R. (2015). Monochromatic light-emitting diode (LED) source in layers hens during the second production cycle. *Rodrigo Borille et Al. R. Bras. Eng. Agríc. Ambiental*, *1919*(99), 877–881. https://doi.org/10.1590/1807-1929/agriambi.v19n9p877-881

Borille, R., Garcia, R., Royer, A., Santana, M., Colet, S., Naas, I., Castilho, V. (2013). The use of light-emitting diodes (LED) in commercial layer production. *Revista Brasileira de Ciência Avícola*, *15*(2), 135–140. https://doi.org/10.1590/S1516-635X2013000200009

Boshouwers, F. M. G., & Nicaise, E. (1993). Artificial light sources and their influence on physical activity and energy expenditure of laying hens. *British Poultry Science*, *34*(1), 11–19. https://doi.org/10.1080/00071669308417558

Braastad, B. O. (1990). Effects on behaviour and plumage of a key-stimuli floor and a perch in triple cages for laying hens. *Applied Animal Behaviour Science*, *27*(1–2), 127–139. https://doi.org/10.1016/0168-1591(90)90012-3

Cao, J., Liu, W., Wang, Z., Xie, D., Jia, L., & Chen, Y. (2008). Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. *The Journal of Applied Poultry Research*, *17*(2), 211–218. https://doi.org/10.3382/japr.2007-00043

Carson, J. R., Junnila, W. A., & Bacon, B. F. (1958). Sexual maturity and productivity in the chicken as affected by the quality of illumination during the growing period. *Poultry Science*, *37*(1), 102–112. https://doi.org/10.3382/ps.0370102

Cordiner, L. S., & Savory, C. J. (2001). Use of perches and nestboxes by laying hens in relation to social status, based on examination of consistency of ranking orders and frequency of interaction. *Applied Animal Behaviour Science*, *71*(4), 305–317. https://doi.org/10.1016/S0168-1591(00)00186-6

Council Directive 1999/74/EC. (1999). Laying down minimum standards for the protection of laying hens. *Official Journal of the European Communities*, 53–57.

Donaldson, C. J., Ball, M. E. E., & O'Connell, N. E. (2012). Aerial perches and free-range laying hens: The effect of access to aerial perches and of individual bird parameters on keel bone injuries in commercial free-range laying hens. *Poultry Science*, *91*(2), 304–315. https://doi.org/10.3382/ps.2011-01774

Donaldson, C. J., & O'Connell, N. E. (2012). The influence of access to aerial perches on fearfulness, social behaviour and production parameters in free-range laying hens. *Applied Animal Behaviour Science*, *142*(1–2), 51–60.

Duncan, E. T., Appleby, M. C., & Hughes, B. O. (1992). Effect of perches in laying cages on welfare and production of hens. *British Poultry Science*, *33*(1), 25–35. https://doi.org/10.1080/00071669208417441

Enneking, S. a., Cheng, H. W., Jefferson-Moore, K. Y., Einstein, M. E., Rubin, D. a., & Hester, P. Y. (2012). Early access to perches in caged white leghorn pullets. *Poultry Science*, *91*(9), 2114–2120. https://doi.org/10.3382/ps.2012-02328

Er, D., Wang, Z., Cao, J., & Chen, Y. (2007). Effect of monochromatic light on the egg quality of laying hens. *The Journal of Applied Poultry Research*, *16*(4), 605–612. https://doi.org/10.3382/japr.2006-00096

FAO. (2009). How to feed the world in 2050. *Insights from an Expert Meeting at FAO*, *2050*(1), 1–35. https://doi.org/10.1111/j.1728-4457.2009.00312.x

Glatz, P. C., & Barnett, J. L. (1996). Effect of perches and solid sides on production, plumage and foot condition of laying hens housed in conventional cages in a naturally ventilated shed. *Australian Journal of Experimental Agriculture*, *36*(3), 269–275. Retrieved from http://www.publish.csiro.au/paper/EA9960269

Gongruttananun, N. (2011). Influence of red light on reproductive performance, eggshell ultrastructure, and eye morphology in Thai-native hens. *Poultry Science*, *90*(12), 2855–2863. https://doi.org/10.3382/ps.2011-01652

Gunnarsson, S., Yngvesson, J., Keeling, L. J., & Forkman, B. (2000). Rearing without early access to perches impairs the spatial skills of laying hens. *Applied Animal Behaviour Science*, *67*(3), 217–228. https://doi.org/10.1016/S0168-1591(99)00125-2

Habinski, A. M., Caston, L. J., Casey-Trott, T. M., Hunniford, M. E., & Widowski, T. M. (2016). Development of perching behavior in 3 strains of pullets reared in furnished cages. *Poultry Science*, (2000), pew377. https://doi.org/10.3382/ps/pew377

HÄne, M., Huber-Eicher, B., & FrÖhlich, E. (2000). Survey of laying hen husbandry in Switzerland. *World's Poultry Science Journal*, *56*(1), 21–31. https://doi.org/10.1079/WPS20000003

Harrison, P. C. (1972). Extraretinal photocontrol of reproductive responses of Leghorn hens to photoperiods of different length and spectrum. *Poultry Science*, *51*(6), 2060–4.

Harrison, P., McGinnis, J., Schumaier, G., & Lauber, J. (1969). Sexual maturity and subsequent reproductive performance of white leghorn chickens subjected to different parts of the light spectrum. *Poultry Science*, *48*(3), 878–83.

Hassan, M. R., Sultana, S., Choe, H. S., & Ryu, K. S. (2013). Effect of monochromatic and combined light colour on performance, blood parameters, ovarian morphology and reproductive hormones in laying hens. *Italian Journal of Animal Science*, *12*(3), e56. https://doi.org/10.4081/ijas.2013.e56

Hassan, M. R., Sultana, S., S.Choe, H., & S. Ryu, K. (2014). Effect of combinations of monochromatic lED light color on the performance and behavior of laying hens. *The Journal of Poultry Science*, *51*(3), 321–326. https://doi.org/10.2141/jpsa.0130105

Hester, P. Y. (2014). The effect of perches installed in cages on laying hens. *World's Poultry Science Journal*, *70*(2), 247–264. https://doi.org/10.1017/S0043933914000270

Hester, P. Y., Enneking, S. A., Haley, B. K., Cheng, H. W., Einstein, M. E., & Rubin, D. A. (2013). The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poultry Science*, *92*(8), 1972–1980. https://doi.org/10.3382/ps.2013-03008

Hester, P. Y., Enneking, S. a, Jefferson-Moore, K. Y., Einstein, M. E., Cheng, H. W., & Rubin, D. a. (2013). The effect of perches in cages during pullet rearing and egg laying on hen performance, foot health, and plumage. *Poultry Science*, *92*(2), 310–320. https://doi.org/10.3382/ps.2012-02744

HFAC. (2017). Animal care standards for egg laying hens - 2017 Standards. *Humane Farm Animal Care*.

Huber-Eicher, B., Suter, A., & Spring-Stahli, P. (2013). Effects of colored light-emitting diode illumination on behavior and performance of laying hens. *Poultry Science*, *92*(4), 869–873. https://doi.org/10.3382/ps.2012-02679

Hughes, B. O., Wilson, S., Appleby, M. C., & Smith, S. F. (1993). Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. *Research in Veterinary Science*, *54*(2), 202–206. https://doi.org/10.1016/0034-5288(93)90057-M

Jiang, S., Hester, P. Y., Hu, J. Y., Yan, F. F., Dennis, R. L., & Cheng, H. W. (2014). Effect of perches on liver health of hens. *Poultry Science*, *93*(7), 1618–1622. https://doi.org/10.3382/ps.2013-03659

Kamanli, S., Durmus, I., Demir, S., & Tarim, B. (2015). Effect of different light sources on performance and egg quality traits in laying hens. *Europ.Poult.Sci.*, *79*(August). https://doi.org/10.1399/eps.2015.109

Käppeli, S., Gebhardt-Henrich, S. G., Fröhlich, E., Pfulg, A., & Stoffel, M. H. (2011). Prevalence of keel bone deformities in Swiss laying hens. *British Poultry Science*, *52*(5), 531–536. https://doi.org/10.1080/00071668.2011.615059

Kavtarashvili, A. S., Novotorov, E. N., Volkonskaya, T. N., & Ridzhal, S. P. (2007). Productive qualities of chickens under various lighting spectra. *Russian Agricultural Sciences*, *33*(2), 118–120. https://doi.org/10.3103/S1068367407020152

Lewis, P. D., Caston, L., & Leeson, S. (2007). Green light during rearing does not significantly affect the performance of egg-type pullets in the laying phase. *Poultry Science*, *86*(4), 739–743. https://doi.org/10.1093/ps/86.4.739

Lewis, P. D., & Morris, T. R. (1998). Responses of domestic poultry to various light sources. *World's Poultry Science Journal*, *54*(1), 7–25. https://doi.org/10.1079/WPS19980002

Lewis, P. D., & Morris, T. R. (2000). Poultry and coloured light. *World's Poultry Science Journal*, *56*(3), 189–207. https://doi.org/10.1079/WPS20000015

Lewis, P. D., (2006). Poultry lighting – the theory and practice.

Li, D. Y., Wu, N., Tu, J. B., Hu, Y. D., Yang, M. Y., Yin, H. D., Zhu, Q. (2015). Expression patterns of melatonin receptors in chicken ovarian follicles affected by monochromatic light. *Genetics and Molecular Research*, *14*(3), 10072–10080. https://doi.org/10.4238/2015.August.21.14

Li, D., Zhang, L., Yang, M., Yin, H., Xu, H., Trask, J. S., Zhu, Q. (2014). The effect of monochromatic light-emitting diode light on reproductive traits of laying hens. *The Journal of Applied Poultry Research*, *23*(3), 367–375. https://doi.org/10.3382/japr.2013-00746

Liu, K. & Xin, H. (2017). Effects of horizontal distance between perches on perching behaviors of Lohmann hens. *Applied Animal Behavioral Science*. https//dx.doi.org/10.1016/j.applanim.2017.05.001

Liu, K., Xin, H., & Settar, P. (2017). Effects of light-emitting diode light versus fluorescent light on growing performance, activity levels and well-being of non-beak-trimmed W-36 pullets. *Animal*. https://doi.org/10.1017/S1751731117001240

Long, H., Zhao, Y., Wang, T., Ning, Z., & Xin, H. (2016a). Effect of light-emitting diode vs. fluorescent lighting on laying hens in aviary hen houses: Part 1 – Operational characteristics of lights and production traits of hens. *Poultry Science*, *95*(1), 1–11. https://doi.org/10.3382/ps/pev121

Long, H., Zhao, Y., Xin, H., Hansen, H., Ning, Z., & Wang, T. (2016b). Effect of light-emitting diode (LED) vs. fluorescent (FL) lighting on laying hens in aviary hen houses: Part 2 – Egg quality, shelf-life and lipid composition. *Poultry Science*, *95*(1), 115–124. https://doi.org/10.3382/ps/pev306

Min, J. K., Hossan, M. S., Nazma, A., Jae, C. N., Han, T. B., Hwan, K. K., Ok, S. S. (2012). Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biology Research*, *5*(2), 69–74. https://doi.org/10.3184/175815512X13350270679453

Mobarkey, N., Avital, N., Heiblum, R., & Rozenboim, I. (2010). The role of retinal and extra-retinal photostimulation in reproductive activity in broiler breeder hens. *Domestic Animal Endocrinology*, *38*(4), 235–243. https://doi.org/10.1016/j.domaniend.2009.11.002

Moinard, C., Morisse, J. P., & Faure, J. M. (1998). Effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. *British Poultry Science, 39*(2), 198–202. https://doi.org/10.1080/00071669889123

Newberry, R. C., Estevez, I., & Keeling, L. J. (2001). Group size and perching behaviour in young domestic fowl. *Applied Animal Behaviour Science*, *73*(2), 117–129. https://doi.org/10.1016/S0168-1591(01)00135-6

Olsson, I. A. S., & Keeling, L. J. (2000). Night-time roosting in laying hens and the effect of thwarting access to perches. *Applied Animal Behaviour Science*, *68*(3), 243–256. https://doi.org/10.1016/S0168-1591(00)00097-6

Olsson, I. A. S., & Keeling, L. J. (2002). The push-door for measuring motivation in hens : laying hens are motivated to perch at night. *Animal Welfare*, *11*, 11–19.

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. https://doi.org/10.1016/j.visres.2008.06.018

Panel, E., & Ahaw, A. W. (2015). Scientific Opinion on welfare aspects of the use of perches for laying hens. *EFSA Journal*, *13*(6), 4131. https://doi.org/10.2903/j.efsa.2015.4131

Parvin, R., Mushtaq, M. M. H., Kim, M. J., & Choi, H. C. (2014). Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. *World's Poultry Science Journal*, *70*(3), 543–556. https://doi.org/10.1017/S0043933914000592

Pelletier, N., Ibarburu, M., & Xin, H. (2014). Comparison of the environmental footprint of the egg industry in the United States in 1960 and 2010. *Poultry Science*, *93*(2), 241–255. https://doi.org/10.3382/ps.2013-03390

Pickel, T., Scholz, B., & Schrader, L. (2010). Perch material and diameter affects particular perching behaviours in laying hens. *Applied Animal Behaviour Science*, *127*(1–2), 37–42. https://doi.org/10.1016/j.applanim.2010.08.005

Pickel, T., Scholz, B., & Schrader, L. (2011). Roosting behaviour in laying hens on perches of different temperatures: Trade-offs between thermoregulation, energy budget, vigilance and resting. *Applied Animal Behaviour Science*, *134*(3–4), 164–169. https://doi.org/10.1016/j.applanim.2011.07.003

Prayitno, D. S., Phillips, C. J., & Omed, H. (1997). The effects of color of lighting on the behavior and production of meat chickens. *Poultry Science*, *76*(3), 452–7.

Prescott, N. B., Wathes, C. M., & Jarvis, J. R. (2003). Light, vision and the welfare of poultry. *Animal Welfare*, *12*(2), 269–288.

Pyrzak, R., Snapir, N., Goodman, G., Arnon, E., & Perek, M. (1986). The influence of light quality on initiation of egg laying by hens. *Poultry Science*, *65*(1), 190–193. https://doi.org/10.3382/ps.0650190

Pyrzak, R., Snapir, N., Goodman, G., & Perek, M. (1987). The effect of light wavelength on the production and quality of eggs of the domestic hen. *Theriogenology*, *28*(6), 947–960. https://doi.org/10.1016/0093-691X(87)90045-8

Rozenboim, I., Biran, I., Chaiseha, Y., Yahav, S., Rosenstrauch, A., Sklan, D., & Halevy, O. (2004). The effect of a green and blue monochromatic light combination on broiler growth and development. *Poultry Science*, *83*(5), 842–845. https://doi.org/10.1093/ps/83.5.842

Rozenboim, I., Zilberman, E., & Gvaryahu, G. (1998). New monochromatic light source for laying hens. *Poultry Science*, *77*(11), 1695–1698. https://doi.org/10.1093/ps/77.11.1695

Saunders, J. E., Jarvis, J. R., & Wathes, C. M. (2008). Calculating luminous flux and lighting levels for domesticated mammals and birds. *Animal*, *2*(6), 921–932. https://doi.org/10.1017/S1751731108002012

Savory, C. J. (2004). Laying hen welfare standards: A classic case of "power to the people." *Animal Welfare*, *13*, 153–158.

Schumaier, G., Harrison, P. C., & McGinnis, J. (1968). Effect of colored fluorescent light on growth, cannibalism, and subsequent egg production of single comb white leghorn pullets. *Poultry Science*, *47*(5), 1599–1602. https://doi.org/10.3382/ps.0471599

Siopes, T. D. (1984). The effect of full-spectrum fluorescent lighting on reproductive traits of caged turkey hens. *Poultry Science*, *63*(6), 1122–1128. https://doi.org/10.3382/ps.0631122

Struelens, E., & Tuyttens, F. A. M. (2009). Effects of perch design on behaviour and health of laying hens. *Animal Welfare*, *18*(4), 533–538.

Struelens, E., Tuyttens, F. A. M., Ampe, B., Ödberg, F., Sonck, B., & Duchateau, L. (2009). Perch width preferences of laying hens. *British Poultry Science*, *50*(4), 418–423. https://doi.org/10.1080/00071660903110885

Sultana, S., Hassan, M. R., Choe, H. S., & Ryu, K. S. (2013). The effect of monochromatic and mixed LED light colour on the behaviour and fear responses of broiler chicken. *Avian Biology Research*, *6*(3), 207–214. https://doi.org/10.3184/175815513X13739879772128

Svobodová, J., Tůmová, E., Popelářová, E., & Chodová, D. (2016). Effect of light colour on egg production and egg contamination. *Czech Journal of Animal Science*, *60*(No. 12), 550–556. https://doi.org/10.17221/8597-CJAS

Tauson, R. (1984). Effects of a perch in conventional cages for laying hens. *Acta Agriculturae Scandinavica*, *34*(2), 193–209. https://doi.org/10.1080/00015128409435389

Tauson, R., & Abrahamsson, P. (1994). Foot and skeletal disorders in laying hens: effects of perch design, hybrid, housing system and stocking density. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *44*(2), 110–119. https://doi.org/10.1080/09064709409410189

UEP. (2017). Animal husbandry guidelines for U.S. egg laying flocks. 2017 edition. *United Egg Producers.*

United Nations. (2008). World population prospects : The 2008 revision. *Population Newsletter*, *87*, 1–20. https://doi.org/21 February 2014

USDA. (2017). Chickens and Eggs - 2016 Summary. *USDA, National Agricultural Statistics Service.*

Valkonen, E., Rinne, R., & Valaja, J. (2009). Effects of perch on feed consumption and behaviour of caged laying hens. *Agricultural and Food Science*, *18*(3–4), 257–267.

Vandenbert, C., & Widowski, T. M. (2000). Hens' preferences for high-intensity high-pressure sodium or low-intensity incandescent lighting. *The Journal of Applied Poultry Research*, *9*(2), 172–178. https://doi.org/10.1093/japr/9.2.172

Wall, H., & Tauson, R. (2007). Perch arrangements in small-group furnished cages for laying hens. *The Journal of Applied Poultry Research*, *16*(3), 322–330. https://doi.org/10.1093/japr/16.3.322

Wechsler, B., & Huber-Eicher, B. (1998). The effect of foraging material and perch height on feather pecking and feather damage in laying hens. *Applied Animal Behaviour Science*, *58*(1–2), 131–141. https://doi.org/10.1016/S0168-1591(97)00137-8

Weeks, C. A., & Nicol, C. J. (2006). Behavioural needs, priorities and preferences of laying hens. *World's Poultry Science Journal*, *62*(2), 296–307. https://doi.org/10.1079/WPS200598

Widowski, T. M., & Duncan, I. J. H. (1996). Laying hens do not have a preference for high-frequency versus low-frequency compact fluorescent light sources. *Canadian Journal of Animal Science*, *76*(2), 177–181.

Widowski, T. M., Keeling, L. J., & Duncan, I. J. H. (1992). The preferences of hens for compact fluorescent over incandescent lighting. *Canadian Journal of Animal Science*, *72*(2), 203–211. https://doi.org/10.4141/cjas92-027

Woodard, a E., Moore, J. a, & Wilson, W. O. (1969). Effect of wave length of light on growth and reproduction in Japanese quail (Coturnix coturnix japonica). *Poultry Science*, *48*(1), 118–123. https://doi.org/10.3382/ps.0480118

Xie, D., Wang, Z. X., Dong, Y. L., Cao, J., Wang, J. F., Chen, J. L., & Chen, Y. X. (2008). Effects of monochromatic light on immune response of broilers. *Poultry Science*, *87*(8), 1535–1539. https://doi.org/10.3382/ps.2007-00317

Xin, H., & Liu, K. (2017). Precision livestock farming in egg production. *Animal Frontiers*, *7*(1), 24. https://doi.org/10.2527/af.2017.0105

Yan, F. F., Hester, P. Y., & Cheng, H. W. (2014). The effect of perch access during pullet rearing and egg laying on physiological measures of stress in White Leghorns at 71 weeks of age. *Poultry Science*, *93*(6), 1318–1326. https://doi.org/10.3382/ps.2013-03572

Yang, Y. F., Jiang, J. S., Pan, J. M., Ying, Y. B., Wang, X. S., Zhang, M. L., Chen, X. H. (2016). The relationship of spectral sensitivity with growth and reproductive response in avian breeders (*Gallus gallus*). *Scientific Reports*, *6*(January), 19291. https://doi.org/10.1038/srep19291

CHAPTER 2

PERCH-SHAPE PREFERENCE AND PERCHING BEHAVIORS OF YOUNG LAYING HENS

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A manuscript submitted to *Applied Animal Behavior Science*

Abstract

Provision of perches in enriched colony or cage-free hen housing facilitates birds' ability to express natural behaviors, thus enhancing animal welfare. Although considerable research has been conducted on poultry perches, there still exists the need to further investigate perching behavior and preference of laying hens to perch exposure and perch types. This study aimed to assess preference of young laying hens for round *vs.* hexagon perches and to characterize temporal perching behaviors of the young hens brought to an enriched colony setting from a cage pullet-rearing environment. A total of 42 Lohmann white hens in six equal groups, 17 weeks of age at the experiment onset, were used in the study. Each group of hens was housed in a wire-mesh floor pen equipped with two 120 cm long perches (one round perch at 3.2 cm dia. and one hexagon perch at 3.1 cm circumscribed dia., placed 40 cm apart and 30 cm above the floor). Each group was monitored continuously for 9 weeks. Perching behaviors during the monitoring period, including perching time (PT), perch visit (PV), and perching bird number (PBN), were recorded and analyzed daily using an automated perching monitoring system. Results showed that the experimental hens performed comparable choice for round *vs.* hexagon perches (p = 0.587-0.987). Specifically, 50.1 ± 4.3% *vs.* 49.9 ± 4.3% of daily PT, 49.7 ± 1.0% *vs.* 50.3 ± 1.0% of daily PV, and 47.7 ± 4.1% *vs.* 52.3 ± 4.1% of dark-period PBN were on round *vs.* hexagon perches. Results thus revealed that the laying hens showed no preference between the round and hexagon perches. This study also revealed that the young laying hens (without prior perching experience) showed increasing use of perches over time. It took up to 5-6 weeks of perch exposure for young hens to approach stabilization of perching behaviors in the enriched colony setting.

Keywords: Perch utilization, Perch preference, Alternative housing, Behavior and welfare, Automated monitoring

Nomenclature

PT	Perching time – time spent perching; min/bird
PV	Perch visit – times of jumping on and off perch; times/bird
PBN	Perching bird number – number of simultaneous perching birds
EU	European Union
ECH	Enriched colony housing
WOA	Weeks of age
LED	Light-emitting diode
WPE	Weeks of perch exposure
VBA	Visual basic for application
PTR	Perching time ratio – proportion of perching time for a given period, %
PF	Perching frequency – perch visit per unit time for a given period, times/bird-h
PTP	Perching time proportion – proportion of perching time for a given period relative to the daily total, %
PVP	Perch visit proportion – proportion of perch visit for a given period relative to the daily total, %
PBP	Perching bird proportion - proportion of simultaneous perching birds relative to the group total, %

Introduction

Laying hens are highly motivated to perch, thus provision of perches in hen housing can accommodate hen's natural behavior needs, enhancing animal welfare (Olsson and Keeling, 2002; Cooper and Albentosa, 2003; Weeks and Nicol, 2006). Switzerland first established legislation in 1980s that banned the use of conventional cages by 1992 and required all housing systems to provide a minimum of 14 cm of elevated perch space per hen (HÄne *et al.*, 2000; Käppeli *et al.*, 2011). Thereafter, the EU Directive banned conventional cages from 2012 and set forth the minimum standards that perches must have no sharp edges and perch space must be at least 15 cm per hen in alternative hen housing systems (Council Directive 1999/74/EC, 1999). To date, most laying hens are housed in conventional cages in the United States (approximately 85%) and many other major egg-producing countries (e.g., China, Mexico, Japan, Indian, Brazil). Because of the EU's ban on conventional cages, enriched colony housing (ECH) became a popular alternative hen housing system. In 2014, 58% of the laying hens in the EU were housed in ECH systems (Personal Communication with Hans-Wilhelm Windhorst, University of Vechta, Germany, 2017). ECH has also found adoption by some egg producers in the United States and Canada. In the ECH system, perch is one of the most essential enrichments for the laying hens.

Many studies have investigated the effects of perch provision on production performance, health, and well-being of laying hens over the past four decades (Struelens and Tuyttens, 2009; Hester, 2014). Benefits of providing perches to laying hens include stimulating leg muscle deposition and bone mineralization (Enneking *et al.*, 2012; Hester *et al.*, 2013a), increasing certain bone volume and strength (Hughes *et al.*, 1993; Appleby and Hughes, 1990; Barnett *et al.*, 2009), reducing abdominal fat deposition (Jiang *et al.*, 2014), and reducing fearfulness and aggression (Donaldson and O'Connell, 2012). On the contrary, detrimental effects associated with perches include keel bone deformities, foot disorders, and bone fractures (Appleby *et al.*, 1993; Tauson and Abrahamsson, 1994; Donaldson *et al.*, 2012). Studies have also shown inconsistent results related to the impact of perches on feather condition or mortality rates of laying hens. Duncan *et al.* (1992), Glatz and Barnett (1996), and Wechsler and Huber-Eicher (1998) reported beneficial impacts, whereas Tauson (1984), Moinard *et al.* (1998), and Hester *et al.* (2013b) reported detrimental impacts. These inconsistent results, to a large extent, could be attributed to differences in perch design, spatial arrangement, or timing of birds introduction to perch in the studies (Struelens and Tuyttens, 2009; Hester, 2014).

In general, an ideal perch should be suitable in meeting the digital tendon locking mechanism (a mechanism that maintains the distal and other interphalangeal joints of the digits in a flexed position) of the hen's feet (Quinn and Baumel, 1990). The EU Directive has required that perches must have no sharp edges (Council Directive 1999/74/EC, 1999). Consequently, round perches are most commonly used in alternative housing systems. Pickel *et al.* (2011) found that peak force on the footpads of hens was greater when standing on the perches with sharp edges (square perch) as compared to round perches. This finding provided certain scientific evidence for the requirement of no sharp edges. Because the extra force on the footpads may lead to severe foot disorders such as bumble foot and toe pad hyperkeratosis. However, the peak force on the keel bone of hens was much greater when resting on round *vs.* square perches (Pickel *et al.*, 2011), which could contribute to development of more keel bone deformity. It should be noted that the pressure peaks on the keel bone were approximately 5 times higher compared with the pressure peaks on a single footpad (Pickel *et al.*, 2011). In addition, round perches might be less adequate in terms of providing the stability necessary to accommodate the hen's landing or long-term roosting. For instance, Duncan *et al.* (1992) found that hens' feet slipped back and forth on round perches but not on square perches. Therefore, a hexagon perch, combining the shape features and advantages of both square and round perches, might prove to be more attractive to hens because of its potential to improve hens' ability to grasp the perch and reduce the chance of peak pressure (stress) on the keel bone and footpads. A review of literature did not reveal research information regarding hen's comparative use of round *vs.* hexagon perches.

Some studies showed that early assess to perches had positive effects on musculoskeletal health of pullets as well as subsequent long-term health of hens (Hester *et al.*, 2013a; Yan *et al.*, 2014; Habinski *et al.*, 2016). Similarly, research found that rearing pullets without early access to perches, in some ways, would impair the spatial cognitive skills of hens (Gunnarsson *et al.*, 2000), thus may be detrimental to their subsequent perching ability and long-term welfare. However, raising pullets in conventional cages without perches is most typical management practice in current commercial ECH systems. Thus there still exists a need to further investigate and characterize perching behaviors of young laying hens introduced to ECH systems with perch exposure.

The objectives of this study were a) to assess hens' preference for perch shape between round and hexagon perches, and b) to quantify and characterize temporal perching behaviors of young laying hens after transferred from pullet-rearing cage into enriched colony setting. The results are expected to contribute to scientific information on laying hen perch design and responses of novice birds to perch introduction.

Materials and Methods

The study was conducted in an environment-controlled animal research laboratory located at Iowa State University, Ames, Iowa, USA. The experimental protocol had been approved by the Iowa State University Institutional Animal Care and Use Committee (Log # 5-12-7364-G).

Experimental Birds and Management

A total of 42 Lohmann white laying hens in two successive batches (21 hens per batch) were used in the study. The birds were reared in a commercial pullet-rearing cage house until the commencement of the experiment when they were at 17 weeks of age (**WOA**). All the birds had similar conditions, including body weight (1200 - 1250 g), feather coverage (no damage/loss), feet and keel bone conditions (no abnormal sign), and no prior perching experience at the experiment onset. For each batch, the birds were randomly assigned to three groups, with seven birds per group (experimental unit).

Three identical enriched experimental pens (P1, P2, and P3) were used in the study. These experimental pens (Fig. 1), each measuring 120 × 120 × 120 cm (L×W×H), had a wire-mesh floor (2.5 × 2.5 cm wire-mesh, 2057 cm^2/bird space allowance), a 120 × 30 × 40 cm elevated nest box (45 cm above floor, 514 cm^2/bird), two 60 × 15 × 10 cm rectangular feeders (installed outside of the left and right sidewalls), two nipple drinkers (on the rear wall at 40 cm above floor), and two parallel 120 cm long metal perches (a 3.2 cm dia. round perch and a 3.1 cm circumscribed circle dia. hexagon perch, giving a minimum of 17 cm perch space per bird). Both perches were installed on adjustable brackets, 30 cm above the floor and 40 cm away from the respective sidewall, with a horizontal space of 40 cm between the two perches. The adjustable brackets allowed for quick relocation and placement of perches.

The hexagon perches were oriented to present a flat surface on the top (Fig. 2a). All resource allowances, including perch, floor, feeder, nest, and nipple drinkers met or exceeded those in the legislation or recommendations for the hens. The experimental room was equipped with mechanical ventilation and heating/cooling to maintain desired temperature of 21ºC throughout the experiment.

Lighting scheme applied in the study followed the commercial management guidelines (Table 1), including light, dim (dawn and dusk), and dark periods. Artificial light was the only light source throughout the experiment and light was provided with compact fluorescent lamps for daytime light (20 lux) and light-emitting diode (**LED**) lights for the dim (1-2 lux) periods. Light intensity was measured and adjusted using a light meter (Model EA31, FLIR Systems Inc., Wilsonville, OR, USA[12]), and maintained at comparable levels at the same spot of the respective perch.

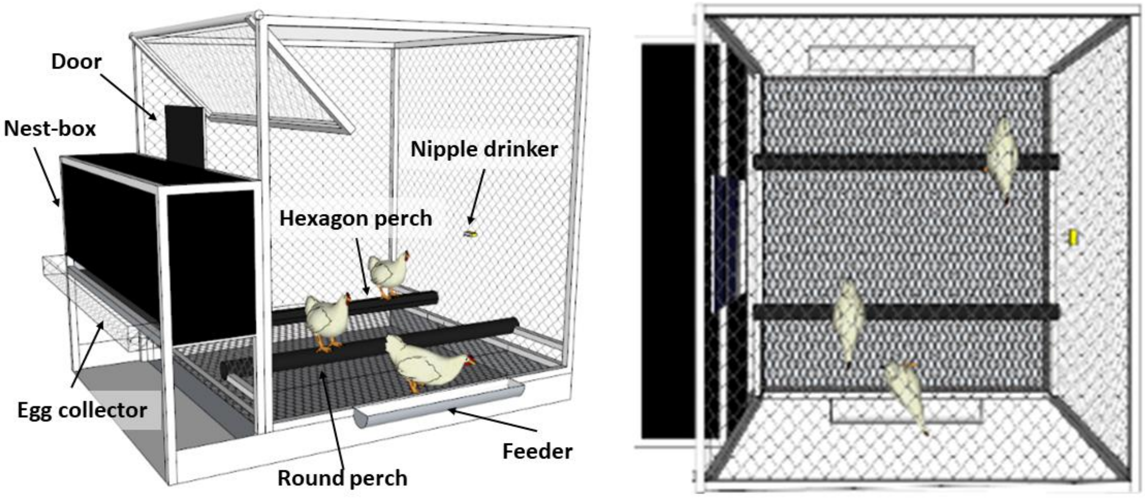

Figure 1: A schematic representation of the experimental pens. (a) side view, (b) top view.

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[12] Mention of product or company name is for presentation clarity and does not imply endorsement by the authors or Iowa State University, nor exclusion of other suitable products.

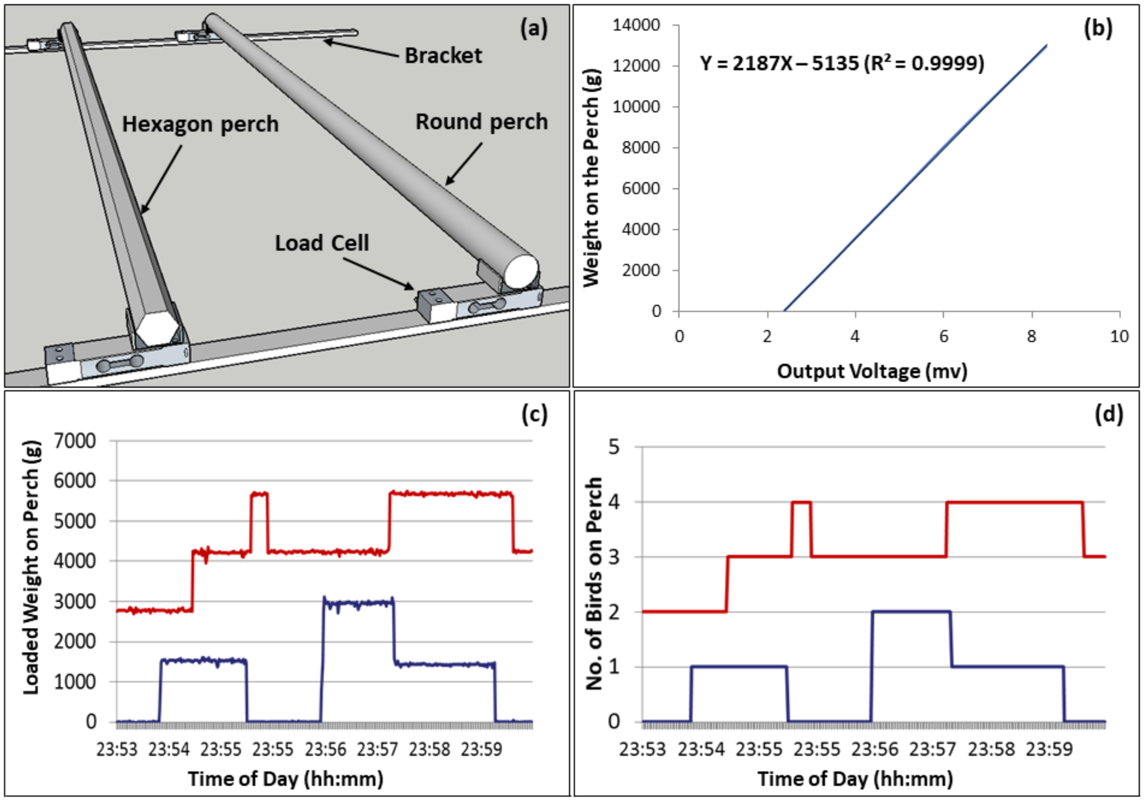


Figure 2. An automated perching monitoring system. (a) weighting perches, (b) linear response of loadcell scale output to load weight, (c) load weight of perching hens on each perch, (d) number of perching birds on each perch.

Table 1. Light schedule for laying hens used in the study

WOA [1]	WPE [2]	Dawn (1-2 lux)	Light (20 lux)	Dusk (1-2 lux)	Dark (0 lux)	Light hour (h/day)
17	1	08:45-09:00	09:00-21:00	21:00-21:15	21:15-08:45	12
18	2	08:15-08:30	08:30-21:30	21:30-21:45	21:45-08:15	13
19	3	07:45-08:00	08:00-22:00	22:00-22:15	22:15-07:45	14
20	4	07:30-07:45	07:45-22:15	22:15-22:30	22:30-07:30	14.5
21	5	07:15-07:30	07:30-22:30	22:30-22:45	22:45-07:15	15
22	6	07:15-07:30	07:30-22:45	22:45-23:00	23:00-07:15	15.25
23	7	07:00-07:15	07:15-22:45	22:45-23:00	23:00-07:00	15.5
24	8	07:00-07:15	07:15-23:00	23:00-23:15	23:15-07:00	15.75
25	9	06:45-07:00	07:00-23:00	23:00-23:15	23:15-06:45	16

[1] WOA = weeks of age

[2] WPE = week(s) of perch exposure

All birds underwent a 9-week test period (17-25 WOA). During this test period, the round and hexagon perches were continuously provided and the birds had free access to both. The locations of the two perches were swapped once a week (at the end of each week) to avoid potential location effect (Table 2). The nest box door was blocked to restrict hen access during the dark period. Feed (commercial corn and soy diets) and water were available *ad-libitum* for hens throughout the test. Feeders were replenished and eggs were collected once a day at 17:00 h. The experimental pens were cleaned right after relocation of the perches. Wood shavings were placed under the wire-mesh floor to absorb the manure moisture and for easier cleaning.

Table 2. Perch arrangements in the study

WOA [1]	WPE [2]	Batch 1						Batch 2					
		P1 [3]		P2		P3		P1		P2		P3	
		L [4]	R	L	R	L	R	L	R	L	R	L	R
17	1	C [5]	H	H	C	H	C	H	C	C	H	C	H
18	2	C	H	H	C	H	C	H	C	C	H	C	H
19	3	H	C	C	H	C	H	C	H	H	C	H	C
20	4	H	C	C	H	H	C	C	H	H	C	C	H
21	5	C	H	H	C	C	H	H	C	C	H	H	C
22	6	C	H	C	H	H	C	H	C	H	C	C	H
23	7	H	C	C	H	H	C	C	H	H	C	C	H
24	8	C	H	H	C	C	H	H	C	C	H	H	C
25	9	H	C	H	C	C	H	C	H	C	H	H	C

[1] WOA = weeks of age
[2] WPE = week(s) of perch exposure
[3] P1, P2, and P3: testing pen 1, 2, and 3, respectively
[4] L, R: left and right side of the testing pen, respectively
[5] C, H: circular (round) and hexagon perch, respectively

Automated Perching Monitoring System

A real-time, sensor-based perching monitoring system was built by incorporating six pairs of load-cell sensors (Model 642C, Revere Transducers Inc., Tustin, CA, USA) supporting six metal perches, coupled with a LabVIEW-based data acquisition system (version 7.1, National Instrument Corporation, Austin, TX, USA). This monitoring system consisted of a compact FieldPoint controller (NI cFP-2020, National Instrument Corporation) and two 8-channel thermocouple input modules (NI cFP-TC-120, National Instrument Corporation), collecting data at 1 Hz sampling rate. Each pair of load-cell sensors was fitted with the adjustable brackets and coupled to a metal perch, forming the weighing perch (Fig. 2a). The data acquisition system automatically read analog voltage outputs of the weighing perches and converted the electronic signals to load weight using pre-defined calibration equations (Fig. 2b), thereby providing real-time measurement of load weight on the perches (Fig. 2c). The load weight of perching birds on each perch was then converted to the number of perching birds on the corresponding perch (Fig. 2d) by using a series of determined weight thresholds (Table 3). With using this system, perching behaviors of the experimental birds were continuously monitored throughout the test period, covering the first day to nine weeks of perch exposure (**WPE**).

Table 3. Determination of number of birds on each perch based on the threshold values

PBN [1]	Threshold values for load weight on each perch (g)	
	Period 1 [2]	Period 2 [3]
1	1000 - 1550	1150 - 1750
2	2200 - 2900	2500 - 3300
3	3400 - 4300	3850 - 4850
4	4600 - 5600	5200 - 6400
5	5800 - 6950	6500 - 7900
6	7050 - 8250	7950 - 9400
7	8250 - 9600	9400 - 11000

[1] PBN = perching bird number.
[2] Birds at 17-19 weeks of age (WOA) with body weight ranging from 1200 g to 1350 g.
[3] Birds at 20-25 WOA with body weight ranging from 1350 g to 1550 g.

Characterization of Temporal Perching Behaviors

With the knowledge of the time-series (1-s intervals) numbers of perching birds on each perch, perching behaviors of birds were quantified daily using an automated VBA program in Excel (Microsoft Office 2016, Redmond, WA, USA). Three primary perching behavior responses were determined, including a) perching time (**PT**) – time spent perching, min/bird; b) perch visit (**PV**) – times of jumping on and off perch, times/bird; and c) perching birds number (**PBN**) – number of simultaneous perching birds. From the three primary responses, five derived behavior parameters were obtained for each period (light, dim, dark, or entire day) of the day. The derived responses included 1) perching time ratio (**PTR**) – proportion of perching time for a given period, %; 2) perching frequency (**PF**) – perch visit per hour for a given period, times/bird-h; 3) perching time proportion (**PTP)** – proportion of perching time for a given period relative to the daily total, %; 4) perch visit proportion (**PVP**) – proportion of perch visit for a given period relative to daily total, %; and 5) perching bird proportion (**PBP**) – proportion of simultaneous perching birds relative to the group total, %.

In this study, birds were not individually identified; thus all behavior variables were presented as group averages.

Statistical Analysis

All statistical analyses of the perching behavior variables were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, NC, USA). Proportion values of daily PT, daily PV, and dark-period PBN for the respective perch were first analyzed to assess preference between round and hexagon perches. Then data of all the behavior variables for both perch types were pooled to characterize temporal perching behaviors of the young hens. All analyses were implemented with generalized linear mixed models using GLIMMIX procedure. A Gaussian distribution was specified for the analyses of PT, PV, and PF, whereas a beta distribution was specified for proportion data (PTR, PTP, PVP, and PBP). All the models were of the following form:

$$Y_{ijkd} = \mu + W_i + B_j + P_k + (WB)_{ij} + (BP)W_{ijk} + D(WBP)_{ijkd} + e_{ijkd}$$

Where Y_{ijkd} denotes the independent observation on day d at i WPE in pen k of batch j; μ is the overall mean; W_i is the WPE effect (fixed); B_j is the batch effect (fixed); P_k is the pen effect (fixed); $(WB)_{ij}$ is the interaction effect (fixed) of WPE and batch; $(BP)W_{ijk}$ is the interaction effect (random) of batch and pen within each WPE; $D(WBP)_{ijkd}$ is the day effect (random) within each WPE for each batch and pen combination, adjusted with first-order autoregressive or AR (1) covariance structure; and e_{ijkd} is the random error with a normal distribution with mean μ and variance σ^2 $[N \sim (\mu, \sigma^2)]$.

Evaluation of the perch preference was accomplished by testing the null hypothesis that the proportion of daily PT, daily PV, or dark-period PBN on respective perch equaled 0.5. As the beta distributions used a logit link function, it was to test whether the intercept equaled zero. Data at 1 WPE were excluded from the analysis of perch preference due to the infrequent perch use (acclimatization). In addition, Tukey-Kramer tests were used for pairwise comparisons among different WPEs for all the behavior variables. Effects were considered significant at $p < 0.05$. Normality and homogeneity of variance of data were examined by residual diagnostics. Unless otherwise specified, data are presented as least squares means along with the standard error of the mean (**SE**).

Results

Preference of Laying Hens between Round and Hexagon Perches

The experimental hens showed no preference for round *vs*. hexagon perches based on daily perching time (PT), daily perch visit (PV), and dark-period perching bird number (PBN) (Fig. 3). Specifically, the hens showed a daily PT of 50.1 ± 4.3% ($p = 0.980$), daily PV of 49.7 ± 1.0% ($p = 0.744$), and dark-period PBN of 47.7 ± 4.1% ($p = 0.587$) for the round perch. The corresponding values for the hexagon perch were daily PT of 49.9 ± 4.3% ($p = 0.980$), daily PV of 50.3 ± 1.0% ($p = 0.744$), and dark-period PBN of 52.3 ± 4.1% ($p = 0.587$). Because of the no preference with the perches, the response variables were pooled in the presentation and analysis of diurnal and temporal perching behaviors of the hens in the following sections.

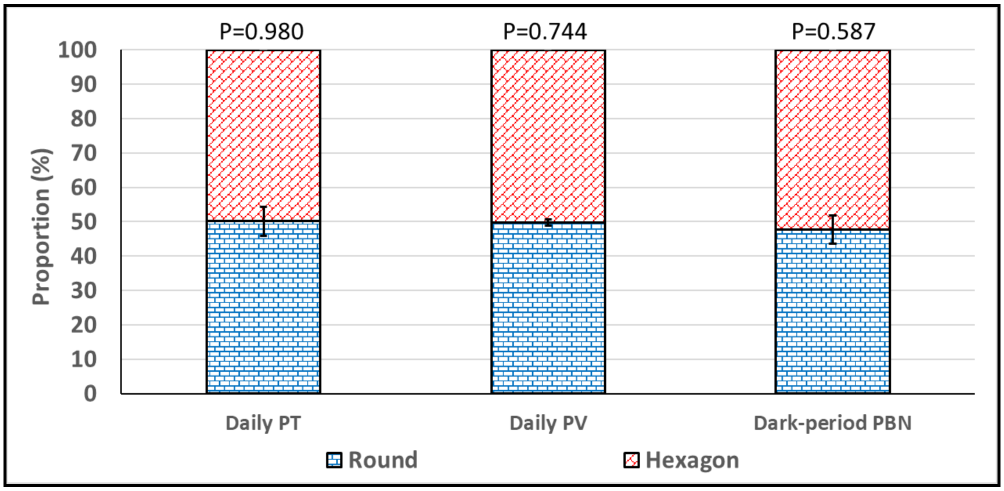
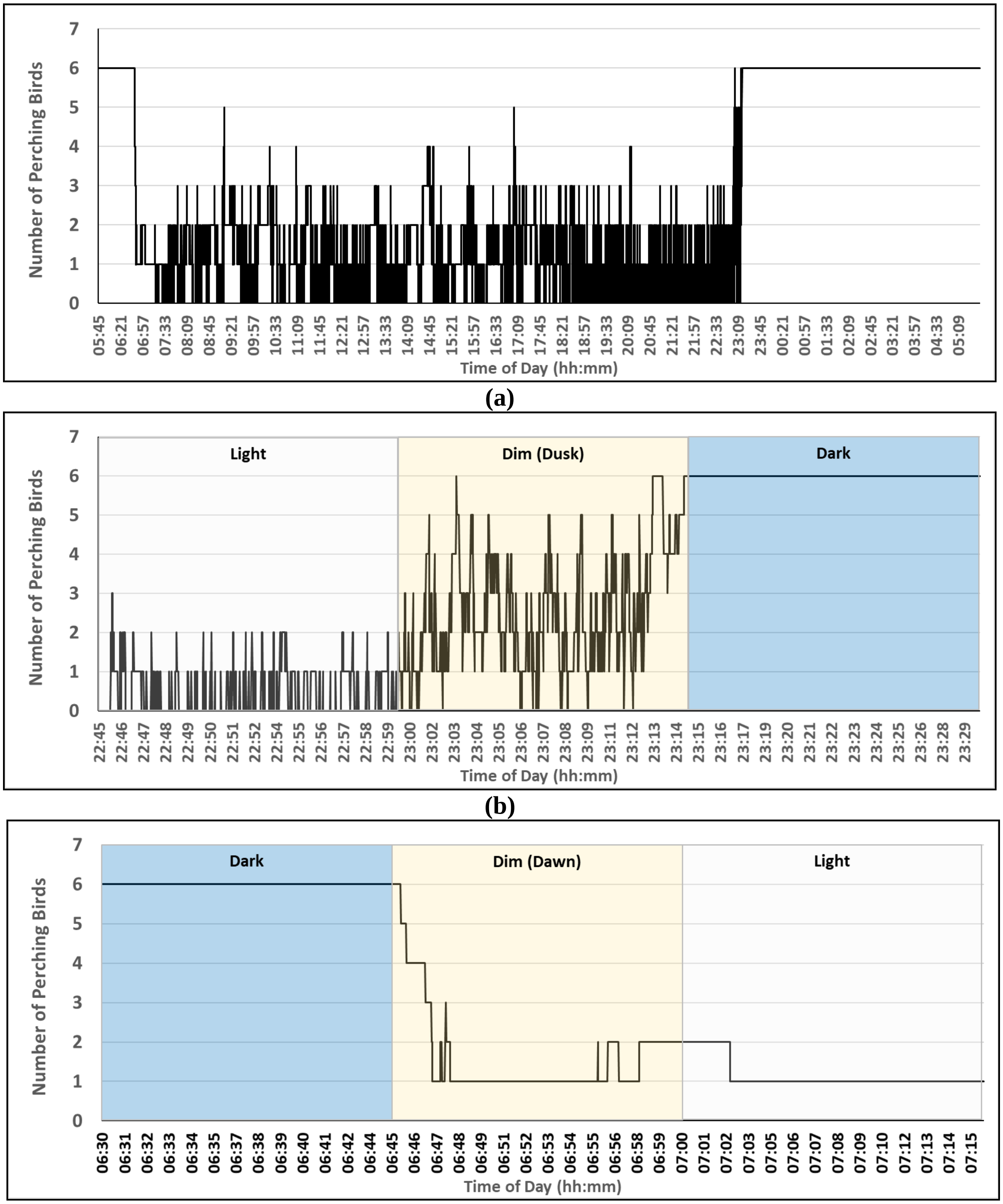


Figure 3. Proportions of perch use by hens between round and hexagon perches. Data are presented as least squares means ± SE. PT = perching time (min/bird), PV = perch visit (times/bird), PBN = perching bird number.

Diurnal and Temporal Perching Behavior of Laying Hens

Diurnal Perching Pattern

A representative diurnal perching pattern of laying hens at 9 WPE (25 WOA) is illustrated in Figure 4. Six out of the seven hens perched simultaneously during the dark period, with all perching hens continuously roosting on perches throughout the dark period (23:15 h - 6:45 h, Fig. 4a). In contrast, only one, two, or three hens (occasionally, four or five hens) perched simultaneously during the light period, with hens jumping on and off the perches frequently throughout the light period (7:00 h - 23:00 h, Fig. 4a). During the transition of light to dark period, hens jumped on and off the perches more frequently throughout the dusk-dimming period (started at 23:00 h until total dark at 23:15 h, Fig. 4b). Immediately following lights off, hens' activity stabilized and subsequent movement ceased. During the transition of dark to light period, hens got off the perches in the early part (first 2-3 min) of the dawn-dimming period (started at 6:45 h until full light at 7:00 h, Fig. 4c).



(a)

(b)

(c)

Figure 4. Diurnal perching pattern of hens at nine weeks of perch exposure: (a) diurnal pattern, (b) during dusk transition period, and (c) during dawn transition period.

Temporal Perching Time

Perching time (PT) and PT proportion (PTP) of laying hens at 1-9 WPE are shown in Table 4, categorized for each period (light, dim, dark, and entire day) of the day. PT ratio (PTR) of laying hens at 1-9 WPE for each period are shown in Figure 5. Over this 9-week period of perch exposure, the hens were observed to perch, on average, 2.8 ± 0.7% to 9.7 ± 1.1% of the light period, 6.3 ± 1.8% to 19.9 ± 2.0% of the dim period, 26.2 ± 6.9% to 75.5 ± 2.6% of the dark period, and 14.6 ± 3.2% to 30.7 ± 1.3% of the entire day. Dark-period PT of hens accounted for 78.7 ± 2.5% to 87.8 ± 1.7% % of the daily PT, followed by light-period PT, 11.0 ± 1.2% to 19.9% ± 1.9% of the daily PT. Although the dark period was shortened by 4 hr during the 9-week period of perch exposure, daily PT increased over time due to the increasing PTR during the light and dark periods. Daily PT tended to approach stabilization after 1-2 WPE, whereas light-period PTR and dark-period PTR continued to increase until approaching stabilization at 5-6 WPE.

Table 4. Weekly average perching time and percentage of daily total for different periods of the day during a 9-week perch exposure of laying hens [1]

WPE [2]	Light		Dark		Dim		Daily
	PT [3] (min/bird)	PTP [4] (%)	PT (min/bird)	PTP (%)	PT (min/bird)	PTP (%)	PT (min/bird)
1	18.8 ± 4.4^{c}	18.5 ± 5.0^{ab}	189.6 ± 43.0^{b}	79.9 ± 4.9^{a}	2.2 ± 0.8^{b}	1.9 ± 0.5^{a}	210.8 ± 46.0^{b}
2	47.9 ± 5.9^{ab}	16.0 ± 3.7^{ab}	289.4 ± 43.2^{ab}	81.7 ± 3.5^{a}	4.2 ± 1.0^{ab}	1.5 ± 0.3^{a}	341.1 ± 55.6^{ab}
3	44.5 ± 6.3^{b}	12.8 ± 1.6^{ab}	319.0 ± 33.3^{ab}	85.8 ± 2.0^{a}	3.9 ± 0.6^{ab}	1.1 ± 0.2^{a}	367.0 ± 39.1^{ab}
4	43.7 ± 3.2^{b}	11.0 ± 1.2^{b}	349.8 ± 14.9^{a}	87.8 ± 1.7^{a}	4.6 ± 0.4^{ab}	1.2 ± 0.2^{a}	397.9 ± 19.5^{a}
5	52.9 ± 3.1^{b}	13.1 ± 1.3^{ab}	346.7 ± 10.0^{a}	85.5 ± 1.8^{a}	5.1 ± 0.4^{ab}	1.2 ± 0.2^{a}	404.5 ± 10.9^{a}
6	56.1 ± 3.9^{ab}	13.4 ± 1.3^{ab}	354.5 ± 10.0^{a}	85.1 ± 1.8^{a}	6.1 ± 0.7^{a}	1.5 ± 0.2^{a}	416.9 ± 10.9^{a}
7	64.4 ± 6.8^{ab}	14.9 ± 1.4^{ab}	355.4 ± 10.0^{a}	83.8 ± 1.9^{a}	5.6 ± 0.6^{a}	1.3 ± 0.2^{a}	425.4 ± 10.9^{a}
8	84.0 ± 7.5^{a}	19.0 ± 1.6^{a}	346.6 ± 10.0^{a}	79.5 ± 2.2^{a}	6.1 ± 0.6^{a}	1.4 ± 0.2^{a}	436.6 ± 10.9^{a}
9	89.4 ± 10.5^{a}	19.9 ± 1.9^{a}	346.7 ± 12.1^{a}	78.7 ± 2.5^{a}	6.2 ± 1.0^{a}	1.4 ± 0.2^{a}	442.3 ± 18.4^{a}

[1] Data are least squares means ± SE. Within each column, values with different superscripts are significantly different at $p < 0.05$.

[2] WPE = weeks of perch exposure.

[3] PT = perching time – time spent perching (min/bird).

[4] PTP = perching time proportion – proportion of perching time for a given period relative to the daily total (%).

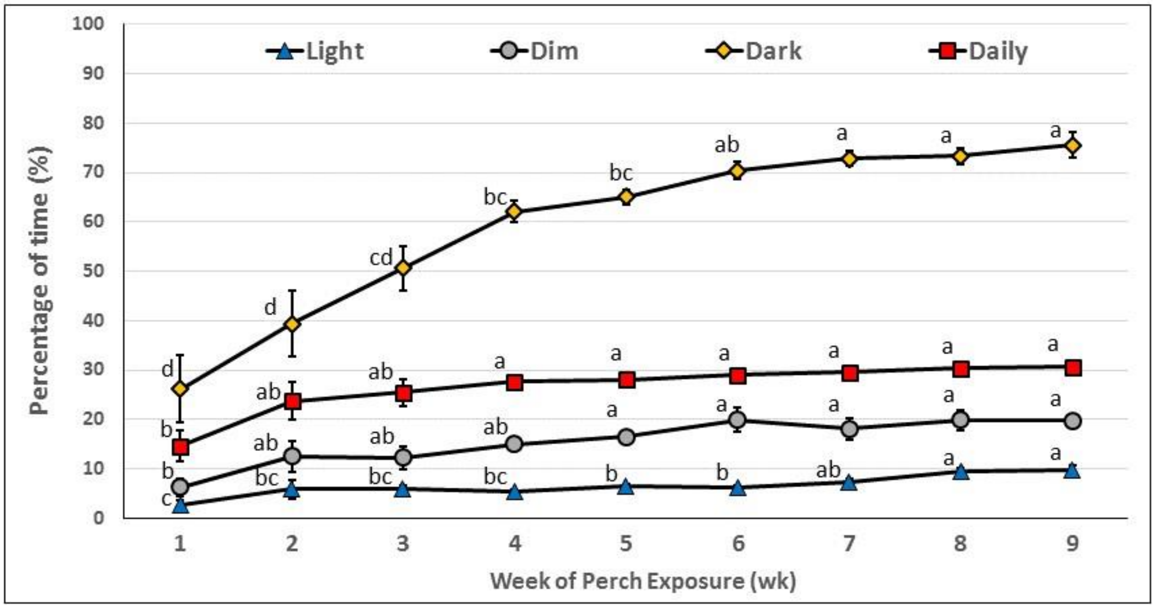


Figure 5. Temporal profiles of perching time ratio for light, dim, dark periods and the entire day. Data are presented as least squares means ± SE. For each curve, values with different superscripts are significantly different at $p < 0.05$.

Temporal Perch Visit

Perch visit (PV) and PV proportion (PVP) of laying hens at 1-9 WPE are shown in Table 5, categorized for each period (light, dim, dark, and entire day) of the day. Perching frequency (PF) of the hens at 1-9 WPE for each period is shown in Figure 6. Over this 9-week period of perch exposure, the hens were observed to perch, on average, 4.9 ± 0.5 to 8.6 ± 0.5 times/bird-h, 10.5 ± 2.0 to 22.2 ± 1.9 times/bird-h, 0.1 ± 0.0 to 0.2 ± 0.0 times/bird-h, and 2.6 ± 0.3 to 5.9 ± 0.4 times/bird-h for the light, dim, and dark periods and the entire day, respectively. Light-period PV of hens accounted for 87.2 ± 4.5% to 92.5 ± 3.2% of the daily PV, followed by dim-period PV, 6.6 ± 0.4% to 9.3% ± 0.4% of the daily PV. Although light period was extended by 4 hr during the 9-week period of perch exposure, daily PV did not significantly increase after 2 WPE.

Table 5. Weekly average perch visit and percentage of daily total for different periods of the day during a 9-week perch exposure of laying hens [1]

WPE [2]	Light		Dark		Dim		Daily
	PV [3] (times/bird)	PVP [4] (%)	PV (times/bird)	PVP (%)	PV (times/bird)	PVP (%)	PV (times/bird)
1	54.2 ± 5.2^{c}	87.2 ± 4.5	1.9 ± 0.1^{a}	3.6 ± 0.4^{a}	5.3 ± 0.8^{b}	9.3 ± 0.4^{a}	61.8 ± 8.0^{c}
2	81.2 ± 4.8^{b}	89.4 ± 3.7	1.8 ± 0.2^{a}	2.0 ± 0.2^{b}	7.7 ± 0.5^{ab}	8.6 ± 0.4^{ac}	90.5 ± 7.0^{bc}
3	98.9 ± 6.6^{ab}	91.1 ± 3.4	1.3 ± 0.3^{ab}	1.2 ± 0.2^{b}	8.4 ± 0.6^{a}	7.7 ± 0.4^{ab}	108.4 ± 8.2^{ab}
4	116.0 ± 3.4^{a}	91.5 ± 3.3	1.4 ± 0.3^{ab}	1.0 ± 0.3^{b}	9.5 ± 0.4^{a}	7.4 ± 0.4^{bc}	127.1 ± 3.8^{a}
5	121.3 ± 6.2^{a}	92.1 ± 3.2	1.2 ± 0.2^{ab}	0.9 ± 0.2^{c}	9.1 ± 0.5^{a}	6.9 ± 0.4^{b}	131.6 ± 5.9^{a}
6	125.2 ± 6.8^{a}	92.5 ± 3.2	1.2 ± 0.2^{ab}	0.9 ± 0.2^{c}	8.9 ± 0.4^{a}	6.6 ± 0.4^{b}	135.4 ± 5.4^{a}
7	130.8 ± 9.9^{a}	92.0 ± 3.2	1.0 ± 0.1^{b}	0.8 ± 0.1^{c}	10.3 ± 0.7^{a}	7.2 ± 0.4^{bc}	142.2 ± 9.1^{a}
8	130.7 ± 7.3^{a}	92.1 ± 3.2	1.0 ± 0.1^{b}	0.7 ± 0.1^{c}	10.2 ± 0.4^{a}	7.2 ± 0.4^{bc}	141.8 ± 6.0^{a}
9	125.6 ± 9.4^{a}	91.0 ± 3.4	1.2 ± 0.2^{ab}	0.8 ± 0.2^{c}	11.1 ± 0.8^{a}	8.0 ± 0.4^{ab}	137.9 ± 9.2^{a}

[1] Data are presented as least squares means ± SE. Within each column, values with different superscripts are significantly different at $p < 0.05$.

[2] WPE = weeks of perch exposure.

[3] PV = perch visit – times of jumping on and off perch (times/bird).

[4] PVP = perch visit proportion – proportion of perch visit for a given period relative to daily total (%).

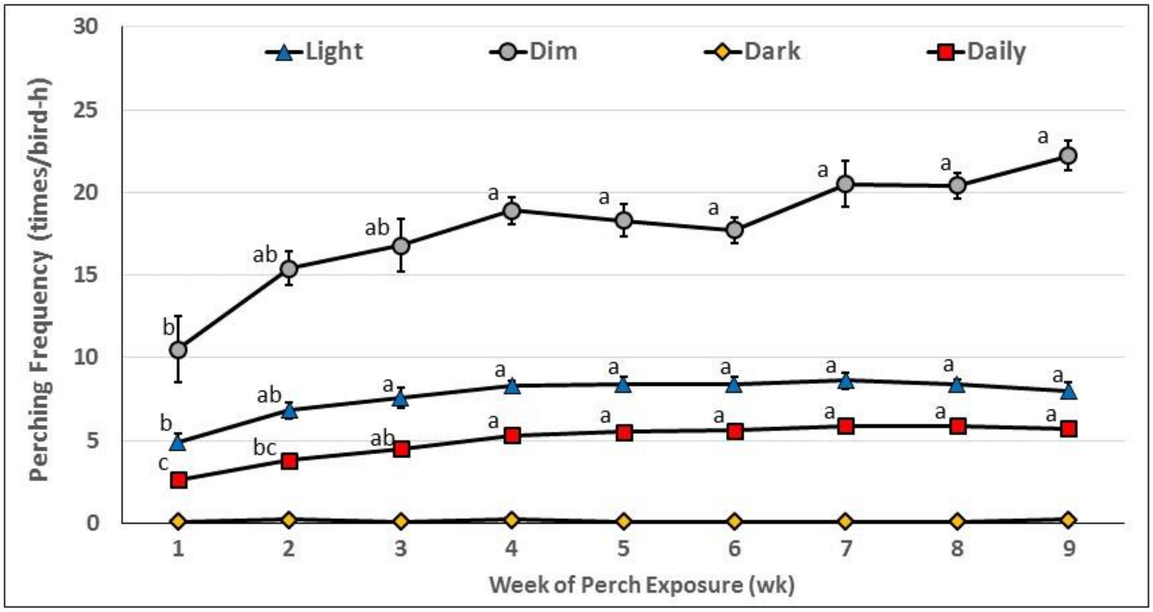


Figure 6. Temporal profiles of perching frequency for the light, dim and dark periods and the entire day. Data are presented as least squares means ± SE. For each curve, values with different superscripts are significantly different at $p < 0.05$.

Temporal Proportion of Hens Perching during the Dark Period

Perching bird proportion (PBP) of laying hens during the dark period at 1-9 WPE is shown in Figure 7. Dark-period PBP increased over time during the 9-week period of perch exposure. Specifically, from 1 to 9 WPE, dark-period PBP averaged 34.8 ± 7.4%, 49.7 ± 4.8%, 58.2 ± 4.7%, 67.4 ± 2.3%, 69.9 ± 1.9%, 73.3 ± 1.5%, 75.6 ± 1.5%, 76.0 ± 1.6%, and 78.7 ± 1.9%, respectively. Dark-period PBP approached stabilization at 4 WPE.

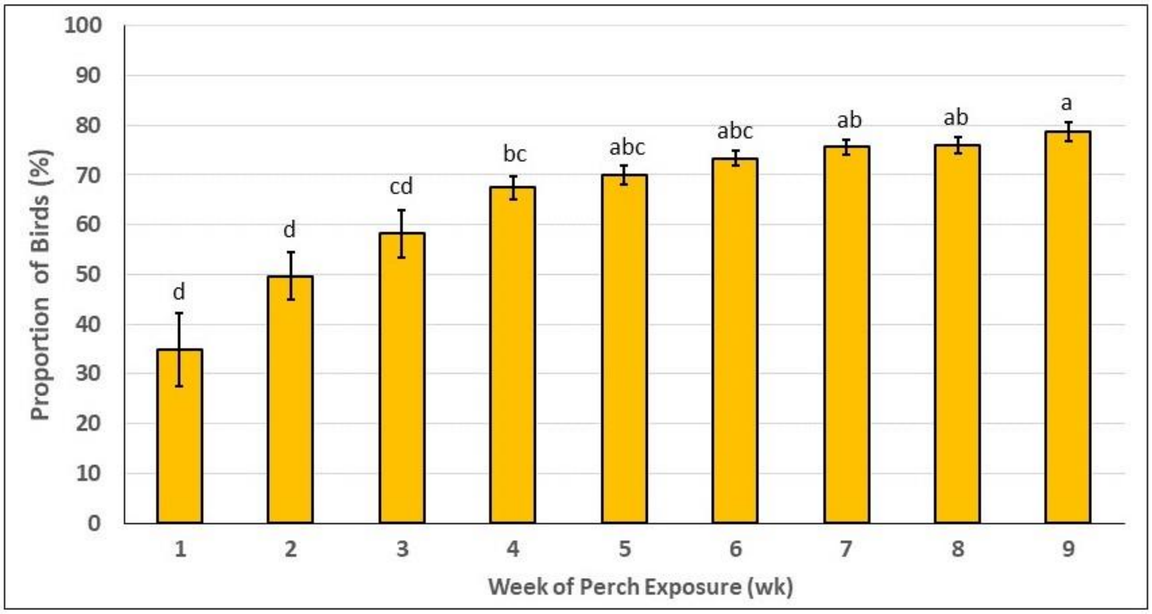


Figure 7. Proportion of birds perching during the dark period. Data are presented as least squares means ± SE. Values with different superscripts are significantly different at $p < 0.05$.

Discussion

According to our literature review, this study is the first effort that assessed preference between round and hexagon perches, and continuously monitored and characterized temporal perching behaviors of young laying hens (17-25 WOA) after transferred to an enriched colony housing from a cage-rearing pullet house (no perches). By taking advantage of the automated sensor-based perching monitoring system, perch utilization by the hens were continuously recorded at 1-9 WPE. The young hens without prior perching experience were found to use the perches increasingly with WPE. It took them up to 5-6 weeks to get used to or maximize the use of the perches. These hens did not show preference between the round perch and the hexagon perch.

Perch-Shape Preference of Laying Hens

Limited published studies existed regarding perching behavior and preference of laying hens subjected to different shapes of perches; and no information was found about behavioral responses of hens to hexagon perch in the literature. In the current study, laying hens showed no preference between the round and hexagon perches with regards to perching time, perch visit, and the number of perching birds on the respective perch. This outcome coincides with the finding of an earlier study by Lambe and Scott (1998) who reported that hens showed no difference in time spent on round *vs.* rectangular perches or single *vs.* double wooden perches. Likewise, an earlier study found that hens showed no perch size preference (1.5, 3.0, 4.5, 6.0, 7.5, 9.0, or 10.5 cm perch width) as judged by the perch use at night (Struelens *et al.*, 2009). In contrast, several earlier studies found certain perch features being preferred by laying hens. For instance, Struelens *et al.* (2008) found hens like to roost on high perches at night when given the opportunity to do so. Appleby *et al.* (1992) found that a perch with a slightly rough surface was preferred by hens. Studies have also found detrimental impacts (keel bone deformities, foot disorders and bone fractures) of using perches (Appleby *et al.*, 1993; Tauson and Abrahamsson, 1994; Donaldson *et al.*, 2012). To overcome these detriments, Scholz *et al.* (2014) and Stratmann *et al.* (2015) investigated soft-surface perches that were shown to provide the most stable footing on perching and reduce the risk of perch-related keel bone injury. The benefit of the soft-surface perches arose from the compressible materials absorbing kinetic energy during collisions and increasing the spread of pressure on the keel bone during perching. Future research may focus on furthering the perch surface materials as opposed to perch shape.

Diurnal and Temporal Perching Behavior of Laying Hens

The diurnal perching patterns of laying hens observed in the current study agreed well with observations in earlier studies. Yeates (1963) investigated activity pattern of White Leghorn fowls in relation to photoperiod and found that the time when birds went up to perches in the evening and came down from perches in the morning were associated with the changes in light intensity. Lambe and Scott (1998) found much more movement of the hens on and off perches during the light period as compared to the dark period, and hens frequently became very active, jumping on and off perches as dark period approached. Olsson and Keeling (2000) also found that hens started to get onto perch immediately after lights-off, and more than 90% of the hens were on perch within 10 min. Likewise, Struelens *et al.* (2008) found hens immediately started to take their roosting positions on perches when lights were dimmed in the evening. In comparison, little information was reported regarding when and how birds got off the perch upon lights-on in the morning. In the current study, majority of the hens were observed to get off the perches at the beginning of the dawn-dimming period, which could be attributed to the intrinsic motivation of feeding and drinking of the birds after a relatively long period of resting/sleeping in the dark period.

Laying hens are highly motivated to perch at night (Weeks and Nicol, 2006). Studies have shown that perching-experienced birds in cages/pens roosted on perches to a very high degree (80-100%) after dark when perch space was sufficient (Tauson, 1984; Appleby *et al.*, 1992; Duncan *et al.*, 1992; Appleby *et al.*, 1993; Abrahamsson and Tauson, 1993; Tauson and Abrahamsson, 1994; Appleby and Hughes, 1995; Appleby, 1995; Wall and Tauson, 2007; Pickel *et al.*, 2010; Pickel *et al.*, 2011; Liu and Xin, 2017). In the current study, on average 78.7% of the hens perched during the dark period at 9 WPE, which was consistent with the findings from the cited studies. In contrast, a few studies also reported relatively low proportions of birds that perched at night despite unlimited perch space. For instance, the proportion of birds perching during the dark period was about 65-70% as reported by Valkonen *et al.* (2009) and about 60% as reported by Tauson and Abrahamsson (1996). A couple of studies reported even lower proportions, e.g., 30-60% by Barnett *et al.* (2009) and 18.4% by Cordiner and Savory (2001). In all these cited studies, hens were found to perform considerably high preference in using nest box instead of roosting on perches at night (Tauson and Abrahamsson, 1996; Cordiner and Savory, 2001; Barnett *et al.*, 2009; Valkonen *et al.*, 2009). In the current study, the nest box was only accessible during the light period.

On the other hand, although the novice young hens (without prior perching experience) increased perching at night in the current study, some birds always remained on the floor during the dark period. This result paralleled the findings of several earlier studies. A large variation in time spent perching among individual birds at night (dark period) has been reported (Lambe and Scott, 1998) and some individual birds did not use the perches at all (Appleby and Hughes, 1990; Appleby *et al.*, 1992; Lambe and Scott, 1998). Moreover, Appleby and Hughes (1990) and Appleby *et al.* (1992) found that the birds roosted on the floor tended to be the same individuals. The perch monitoring system utilized in the current study was not designed or intended to determine or discern perching behavior of individual birds. The birds roosting on the floor at night in the current study and the cited studies might have been attributed to the dominance hierarchy among group-housed hens. Dominance hierarchy influences spatial distribution of birds on perches (Lill, 1968), and the subdominant birds may not be allowed to use perch at night. Floor-roosting may also be associated with the antipredator behavior of chickens (Hu *et al.*, 2016). Hu *et al.* (2016) found that the degree of protective behavior of hens has decreased during domestication, which might have contributed to the reduced proportion of hens perching at night.

Perch utilization during the light period observed in this study (10% of the light period at 9 WPE) was much lower than that reported in earlier studies (ranging between 25-50%). Tauson (1984) reported hens perching 25-50% of the daytime, while others reported hens spending about 25% of the daytime on perches (Braastad, 1990; Appleby *et al.*, 1992; Appleby *et al.*, 1993; Abrahamsson and Tauson, 1993; Cordiner and Savory, 2001; Valkonen *et al.*, 2009). Yet, some studies reported that hens spent about 32-38% of the daytime on perches (Hughes *et al.*, 1993; Appleby and Hughes, 1995; Appleby, 1995; Wechsler and Huber-Eicher, 1998; Newberry *et al.*, 2001; Barnett *et al.*, 2009). More studies reported that hens spent about 47-51% of the daytime on perches (Appleby & Hughes, 1990; Barnett *et al.*, 1997; Appleby and Hughes, 1990; Struelens *et al.*, 2009). For all these cited studies, the results were derived from manual observations, i.e., live observation or off-site observation of recorded videos, which covered limited parts of the light period (daytime) at certain ages (e.g., a couple of hours a day at each age). As a result, these results might not be inclusive enough to represent the actual daily usage, especially considering variations observed in perching behavior through the light period. When comparing the results in the current study with our earlier study that investigated perching behavior of hens as affected by horizontal space between parallel perches using the same automated perching monitoring system (Liu and Xin, 2017), hens in the current study spent much lower proportion of the daytime on perches (i.e., 10% *vs.* 21%) but had much higher perching frequency (8.0 *vs.* 1.9 times/bird-h). It should be noted that there were three distinct differences between the earlier study and the current study that may have influenced the perch utilization. First, hens in the earlier study were chosen from a commercial aviary house and were experienced in using perches, whereas pullets used in the current study came from pullet-rearing cages and had no prior perching experience. Second, birds in the earlier study were older (68 WOA), whereas birds in the current study were much younger (17-25 WOA) that were presumably more energetic. Third, stocking density was higher in the earlier study than in the current study (11 hens/m^2 *vs.* 5 hens/m^2).

In terms of the temporal perching behavior, the results of the current study agreed well with the findings of earlier studies. In general, perch use increased significantly with WPE within the first 1-2 weeks after the birds were introduced to perches. Hens tended to use the perch consistently throughout the subsequent WPE. Newberry *et al.* (2001) found that daytime perch utilization varied with bird age, with the total proportion of birds perching increasing from 27.5% in the youngest birds (3-6 WOA) to 47.4% when the birds were at 12-15 WOA. Faure and Jones (1982a) found that White Leghorn birds without perching experience took two days to get used to using perch when the perch was first introduced at 17 WOA. In addition, Duncan *et al.* (1992) found that overall time spent in daytime perching was relatively consistent over the laying cycle. In contrast, Faure and Jones (1982b) found when providing perches to 15-week old pullets, repeated perch exposure increased the time spent on perches in daytime by the perching birds but did not affect the non-perching birds. Individual variance of perch use was not determined in the current study. Therefore, we were unable to tell perching or lack thereof by individual birds nor could we determine perching variance among the individual birds.

Conclusions

A total of 42 Lohmann White hens in six groups, 17 weeks of age without prior perching experience at the experiment onset, were used in the study to a) assess perch preference of the hens between a round perch (3.2 cm dia.) and a hexagon perch (3.1 cm circumscribed dia.), and b) quantify temporal perching behavior of the hens introduced to an enriched colony setting from conventional cages. Perch utilization by the hens were continuously recorded at 1-s intervals throughout a 9-week testing period. The number/proportion of hens perching, perching time, and perch visit, perching frequency were quantified. The following conclusions were drawn.

- The laying hens showed no preference for the perch shape of round or hexagon.
- The young hens without prior perching experience showed increasing perching behaviors with time of perch exposure. In general, perch visit or perching frequency tended to stabilize after 1-2 weeks of perch exposure (WPE); perching bird proportion during the dark period stabilized after 4 WPE, whereas the perching time during the light and dark periods stabilized after 5-6 WPE.

Acknowledgements

Funding for the study was in part provided by the Egg Industry Center located at Iowa State University. We would like to thank the cooperative egg producer for the generous donation of the hens and feed used in the study. Thanks are also extended to the Agriculture Experiment Station (AES) Consulting Group at Iowa State University for the consistent assistance in statistical consultation for the study. Lastly, author Kai Liu wishes to thank

China Scholarship Council (CSC) for providing part of the financial support for his PhD study at Iowa State University.

References

Abrahamsson, P., & Tauson, R. (1993). Effect of perches at different positions in conventional cages for laying hens of two different strains. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *43*(4), 228–235. https://doi.org/10.1080/09064709309410171

Appleby, M. C. (1995). Perch length in cages for medium hybrid laying hens. *British Poultry Science*, *36*(1), 23–31. https://doi.org/10.1080/00071669508417749

Appleby, M. C., & Hughes, B. O. (1990). Cages modified with perches and nests for the improvement of bird welfare. *World's Poultry Science Journal*, *46*(1), 38–40. https://doi.org/10.1079/WPS19900007

Appleby, M. C., & Hughes, B. O. (1995). The Edinburgh modified gage for laying hens. *British Poultry Science*, *36*(5), 707–718. https://doi.org/10.1080/00071669508417815

Appleby, M. C., Smith, S. F., & Hughes, B. O. (1992). Individual perching behaviour of laying hens and its effects in cages. *British Poultry Science*, *33*(2), 227–238. https://doi.org/10.1080/00071669208417462

Appleby, M. C., Smith, S. F., & Hughes, B. O. (1993). Nesting, dust bathing and perching by laying hens in cages: Effects of design on behaviour and welfare. *British Poultry Science*, *34*(5), 835–847. https://doi.org/10.1080/00071669308417644

Barnett, J. L., Glatz, P. C., Newman, E. A., & Cronin, G. M. (1997). Effects of modifying layer cages with perches on stress physiology, plumage, pecking and bone strength of hens. *Australian Journal of Experimental Agriculture*, *37*(5), 523.

Barnett, J. L., Tauson, R., Downing, J. a, Janardhana, V., Lowenthal, J. W., Butler, K. L., & Cronin, G. M. (2009). The effects of a perch, dust bath, and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens. *Poultry Science*, *88*(3), 456–470. https://doi.org/10.3382/ps.2008-00168

Braastad, B. O. (1990). Effects on behaviour and plumage of a key-stimuli floor and a perch in triple cages for laying hens. *Applied Animal Behaviour Science*, *27*(1–2), 127–139. https://doi.org/10.1016/0168-1591(90)90012-3

Cooper, J. J., & Albentosa, M. J. (2003). Behavioural priorities of laying hens. *Avian and Poultry Biology Reviews*, *14*(3), 127–149. https://doi.org/10.3184/147020603783637508

Cordiner, L. S., & Savory, C. J. (2001). Use of perches and nestboxes by laying hens in relation to social status, based on examination of consistency of ranking orders and frequency of interaction. *Applied Animal Behaviour Science*, *71*(4), 305–317. https://doi.org/10.1016/S0168-1591(00)00186-6

Council Directive 1999/74/EC. (1999). Laying down minimum standards for the protection of laying hens. *Official Journal of the European Communities*, 53–57.

Donaldson, C. J., Ball, M. E. E., & O’Connell, N. E. (2012). Aerial perches and free-range laying hens: The effect of access to aerial perches and of individual bird parameters on keel bone injuries in commercial free-range laying hens. *Poultry Science*, *91*(2), 304–315. https://doi.org/10.3382/ps.2011-01774

Donaldson, C. J., & O’Connell, N. E. (2012). The influence of access to aerial perches on fearfulness, social behaviour and production parameters in free-range laying hens. *Applied Animal Behaviour Science*, *142*(1–2), 51–60. https://doi.org/10.1016/j.applanim.2012.08.003

Duncan, E. T., Appleby, M. C., & Hughes, B. O. (1992). Effect of perches in laying cages on welfare and production of hens. *British Poultry Science*, *33*(1), 25–35. https://doi.org/10.1080/00071669208417441

Enneking, S. a., Cheng, H. W., Jefferson-Moore, K. Y., Einstein, M. E., Rubin, D. a., & Hester, P. Y. (2012). Early access to perches in caged White Leghorn pullets. *Poultry Science*, *91*(9), 2114–2120. https://doi.org/10.3382/ps.2012-02328

Faure, J. M., & Bryan Jones, R. (1982a). Effects of age, access and time of day on perching behaviour in the domestic fowl. *Applied Animal Ethology*, *8*(4), 357–364. https://doi.org/10.1016/0304-3762(82)90068-2

Faure, J. M., & Jones, R. B. (1982b). Effects of sex, strain and type of perch on perching behaviour in the domestic fowl. *Applied Animal Ethology*, *8*(3), 281–293. https://doi.org/10.1016/0304-3762(82)90211-5

Glatz, P. C., & Barnett, J. L. (1996). Effect of perches and solid sides on production, plumage and foot condition of laying hens housed in conventional cages in a naturally ventilated shed. *Australian Journal of Experimental Agriculture*, *36*(3), 269–275. Retrieved from http://www.publish.csiro.au/paper/EA9960269

Gunnarsson, S., Yngvesson, J., Keeling, L. J., & Forkman, B. (2000). Rearing without early access to perches impairs the spatial skills of laying hens. *Applied Animal Behaviour Science*, *67*(3), 217–228. https://doi.org/10.1016/S0168-1591(99)00125-2

Habinski, A. M., Caston, L. J., Casey-Trott, T. M., Hunniford, M. E., & Widowski, T. M. (2016). Development of perching behavior in 3 strains of pullets reared in furnished cages. *Poultry Science*, (2000), pew377. https://doi.org/10.3382/ps/pew377

HÄne, M., Huber-Eicher, B., & FrÖhlich, E. (2000). Survey of laying hen husbandry in Switzerland. *World's Poultry Science Journal*, *56*(1), 21–31. https://doi.org/10.1079/WPS20000003

Hester, P. Y. (2014). The effect of perches installed in cages on laying hens. *World's Poultry Science Journal*, *70*(2), 247–264. https://doi.org/10.1017/S0043933914000270

Hester, P. Y., Enneking, S. A., Haley, B. K., Cheng, H. W., Einstein, M. E., & Rubin, D. A. (2013). The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poultry Science*, *92*(8), 1972–1980. https://doi.org/10.3382/ps.2013-03008

Hester, P. Y., Enneking, S. a, Jefferson-Moore, K. Y., Einstein, M. E., Cheng, H. W., & Rubin, D. a. (2013). The effect of perches in cages during pullet rearing and egg laying on hen performance, foot health, and plumage. *Poultry Science*, *92*(2), 310–320. https://doi.org/10.3382/ps.2012-02744

Hu, J. Y., Hester, P. Y., Makagon, M. M., Vezzoli, G., Gates, R. S., Xiong, Y. J., & Cheng, H. W. (2016). Cooled perch effects on performance and well-being traits in caged White Leghorn hens. *Poultry Science*, pew248. https://doi.org/10.3382/ps/pew248

Hughes, B. O., Wilson, S., Appleby, M. C., & Smith, S. F. (1993). Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. *Research in Veterinary Science*, *54*(2), 202–206. https://doi.org/10.1016/0034-5288(93)90057-M

Jiang, S., Hester, P. Y., Hu, J. Y., Yan, F. F., Dennis, R. L., & Cheng, H. W. (2014). Effect of perches on liver health of hens. *Poultry Science*, *93*(7), 1618–1622. https://doi.org/10.3382/ps.2013-03659

Käppeli, S., Gebhardt-Henrich, S. G., Fröhlich, E., Pfulg, A., & Stoffel, M. H. (2011). Prevalence of keel bone deformities in Swiss laying hens. *British Poultry Science*, *52*(5), 531–536. https://doi.org/10.1080/00071668.2011.615059

Lambe, N. R., & Scott, G. B. (1998). Perching behaviour and preferences for different perch designs among laying hens. *Animal Welfare*, *7*(2), 203–216.

Lill, A. (1968). Spatial organisation in small flocks of domestic fowl. *Behaviour*, *32*(4), 258–290. https://doi.org/10.1163/156853968X00225

Liu, K., & Xin, H. (2017). Effects of horizontal distance between perches on perching behaviors of Lohmann Hens. *Applied Animal Behaviour Science*, *194*, 54–61. https://doi.org/10.1016/j.applanim.2017.05.001

Moinard, C., Morisse, J. P., & Faure, J. M. (1998). Effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. *British Poultry Science*, *39*(2), 198–202. https://doi.org/10.1080/00071669889123

Newberry, R. C., Estevez, I., & Keeling, L. J. (2001). Group size and perching behaviour in young domestic fowl. *Applied Animal Behaviour Science*, *73*(2), 117–129. https://doi.org/10.1016/S0168-1591(01)00135-6

Olsson, I. A. S., & Keeling, L. J. (2000). Night-time roosting in laying hens and the effect of thwarting access to perches. *Applied Animal Behaviour Science*, *68*(3), 243–256. https://doi.org/10.1016/S0168-1591(00)00097-6

Olsson, I. A. S., & Keeling, L. J. (2002). The push-door for measuring motivation in hens : laying hens are motivated to perch at night. *Animal Welfare*, *11*, 11–19.

Pickel, T., Scholz, B., & Schrader, L. (2011). Roosting behaviour in laying hens on perches of different temperatures: Trade-offs between thermoregulation, energy budget, vigilance and resting. *Applied Animal Behaviour Science*, *134*(3–4), 164–169. https://doi.org/10.1016/j.applanim.2011.07.003

Pickel, T., Schrader, L., & Scholz, B. (2011). Pressure load on keel bone and foot pads in perching laying hens in relation to perch design. *Poultry Science*, *90*(4), 715–724. https://doi.org/10.3382/ps.2010-01025

Quinn, T. H., & Baumel, J. J. (1990). The digital tendon locking mechanism of the avian foot (Aves). *Zoomorphology*, *109*(5), 281–293. https://doi.org/10.1007/BF00312195

Scholz, B., Kjaer, J. B., & Schrader, L. (2014). Analysis of landing behaviour of three layer lines on different perch designs. *British Poultry Science*, *55*(4), 419–426. https://doi.org/10.1080/00071668.2014.933175

Stratmann, A., Fröhlich, E. K. F., Harlander-Matauschek, A., Schrader, L., Toscano, M. J., Würbel, H., & Gebhardt-Henrich, S. G. (2015). Soft perches in an aviary system reduce incidence of keel bone damage in laying hens. *PLOS ONE*, *10*(3), e0122568. https://doi.org/10.1371/journal.pone.0122568

Struelens, E., & Tuyttens, F. A. M. (2009). Effects of perch design on behaviour and health of laying hens. *Animal Welfare*, *18*(4), 533–538.

Struelens, E., Tuyttens, F. A. M., Ampe, B., Ödberg, F., Sonck, B., & Duchateau, L. (2009). Perch width preferences of laying hens. *British Poultry Science*, *50*(4), 418–423. https://doi.org/10.1080/00071660903110885

Struelens, E., Tuyttens, F. A. M., Duchateau, L., Leroy, T., Cox, M., Vranken, E., Sonck, B. (2008). Perching behaviour and perch height preference of laying hens in furnished cages varying in height. *British Poultry Science*, *49*(4), 381–389. https://doi.org/10.1080/00071660802158332

Tauson, R. (1984). Effects of a Perch in Conventional Cages for Laying Hens. *Acta Agriculturae Scandinavica*, *34*(2), 193–209.

Tauson, R., & Abrahamsson, P. (1994). Foot and skeletal disorders in laying hens: effects of perch design, hybrid, housing system and stocking density. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *44*(2), 110–119. https://doi.org/10.1080/09064709409410189

Tauson, R., & Abrahamsson, P. (1996). Foot and keel bone disorders in laying hens: effects of artificial perch material and hybrid. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *46*(4), 239–246. https://doi.org/10.1080/09064709609415876

Valkonen, E., Rinne, R., & Valaja, J. (2009). Effects of perch on feed consumption and behaviour of caged laying hens. *Agricultural and Food Science*, *18*(3–4), 257–267.

Wall, H., & Tauson, R. (2007). Perch arrangements in small-group furnished cages for laying hens. *The Journal of Applied Poultry Research*, *16*(3), 322–330. https://doi.org/10.1093/japr/16.3.322

Wechsler, B., & Huber-Eicher, B. (1998). The effect of foraging material and perch height on feather pecking and feather damage in laying hens. *Applied Animal Behaviour Science*, *58*(1–2), 131–141. https://doi.org/10.1016/S0168-1591(97)00137-8

Weeks, C. A., & Nicol, C. J. (2006). Behavioural needs, priorities and preferences of laying hens. *World's Poultry Science Journal*, *62*(2), 296–307.

Yan, F. F., Hester, P. Y., & Cheng, H. W. (2014). The effect of perch access during pullet rearing and egg laying on physiological measures of stress in White Leghorns at 71 weeks of age. *Poultry Science*, *93*(6), 1318–1326. https://doi.org/10.3382/ps.2013-03572

Yeates, N. T. M. (1963). The activity pattern in poultry in relation to photoperiod. *Animal Behaviour*, *11*(2–3), 287–289. https://doi.org/10.1016/S0003-3472(63)80112-8

CHAPTER 3

EFFECTS OF HORIZONTAL DISTANCE BETWEEN PERCHES ON PERCHING BEHAVIORS OF LOHMANN HENS

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A paper published in *Applied Animal Behavior Science* (2017) 194: 54-61
Available online at: https://doi.org/10.1016/j.applanim.2017.05.001

Abstract

Perching is a highly-motivated natural behavior of laying hens that has been considered as one of the essential welfare requirements. The objective of the study was to evaluate perching behaviors of laying hens as affected by horizontal distance (HD) between parallel perches. A total of 48 Lohmann white hens in three groups (16 hens/group) were used, 68 weeks of age at the experiment onset. For each group, hens were housed in an enriched wire-mesh floor pen (120 cm L×120 cm W×120 cm H) equipped with two round galvanized tube perches (120 cm long × 32 mm diameter, an average of 15 cm perch space/hen). HD was varied sequentially at 60, 40, 30, 25, 20 and 15 cm and then in reverse order. A real-time monitoring system was developed to continuously record hen's perching behaviors. The number or proportion of perching hens, perching duration, and perching trip and frequency were analyzed using an automated VBA (Visual Basic for Applications) program developed in Microsoft Excel. Heading direction of the perching hens and pattern of the perch occupancy were determined manually by video observation. Results showed that reduction of HD to 25 cm did not restrain hens' perching behaviors, whereas HD of 20 or 15 cm restrained perching to some extent. Specifically, at HD of 25 cm, hens perched interlacing with one another to maximize use of the perches during the dark period. As a result, the proportion of perching hens and perching duration for HD of 25 cm were not reduced as compared to HD of 30-60 cm. However, the proportion of perching hens was significantly reduced at HD of 15 cm (p = 0.001-0.025). HD of 15 and 20 cm also significantly reduced daily perching time of the hens. In contrast, perching trip or frequency and heading direction of the perching hens were not influenced by HD (15-40 cm) except for HD of 60 cm. The results suggest that although 30 cm is the recommended minimum HD, 25 cm may be considered for situations where additonal perches are necessary to meet all hens' perching needs.

Keywords. animal welfare, perching behavior, horizontal distance, laying hens, commercial guideline, weighing perch

Introduction

Perching is a highly-motivated natural behavior of laying hens (Olsson and Keeling, 2002; Cooper and Albentosa, 2003; Weeks and Nicol, 2006); thus provision of perches in hen housing can accommodate hen's natural behavior, hence enhancing animal welfare. Consequently, perches are typically used in alternative hen housing systems, such as enriched colony and cage-free houses. Perching behaviors of laying hens have drawn extensive attention of researchers and egg producers over the past four decades. A number of studies have been conducted to investigate perch design (e.g., type, shape, texture and material) and spatial perch arrangement (e.g., height, angle and relative location). These studies mainly focused on the effects of perch provision on production performance (e.g., body weight, egg production and egg quality, feed usage and efficiency), health and welfare (e.g., skeletal and feet health, feather condition and physiological stress), and perching behaviors (e.g., perch use and preference) of laying hens (Struelens and Tuyttens, 2009; Hester, 2014).

Results of studies from both laboratory and commercial settings have shown benefits as well as detriments of providing perches to laying hens. For example, use of perches can stimulate leg muscle deposition and bone mineralization (Enneking *et al.*, 2012; Hester *et al.*, 2013a), increase certain bone volume and strength (Hughes *et al.*, 1993; Appleby and Hughes, 1990; Barnett *et al.*, 2009), reduce abdominal fat deposition (Jiang *et al.*, 2014), and reduce fearfulness and aggression (Donaldson and O'Connell, 2012). However, keel bone deformities, foot disorders (e.g., bumble foot) and bone fractures have also been reported to be associated with perches (Appleby *et al.*, 1993; Tauson and Abrahamsson, 1994; Donaldson *et al.*, 2012). Moreover, controversies occur when contradictory results are derived from different experiments. For instance, some studies showed beneficial impacts of perches on feather condition or mortality of laying hens (Duncan *et al.*, 1992; Glatz and Barnett, 1996; Wechsler and Huber-Eicher, 1998), whereas others showed detrimental impacts (Tauson, 1984; Moinard *et al.*, 1998; Hester *et al.*, 2013b). More inconsistent results came from the studies that investigated perch use and preference of laying hens, especially when involving various perch shapes, sizes, textures, materials or spatial arrangements (Struelens and Tuyttens, 2009; Hester, 2014). To date, neither the egg industry nor the scientific community has designed a perfect perching system. Thus continually exploring proper perch design is warranted.

Switzerland first established legislation to improve welfare of laying hens in that conventional cages were banned in 1992 and all housing systems must provide at least 14 cm of elevated perches per hen (HÄne *et al.*, 2000; Käppeli *et al.*, 2011). Thereafter, the EU Directive set forth the minimum standards, which states that perch must have no sharp edges and perch space must be at least 15 cm per hen in alternative hen housing systems. In addition, horizontal distance between perches and between perch and wall should be at least 30 and 20 cm, respectively (Council Directive 1999/74/EC, 1999). However, ambiguities and debates exist due to unclear statement in perch design and lack of substantive scientific information. Some researchers criticized that this directive was more about satisfying public opinion than to meet laying hen’s actual need (Savory, 2004). To meet the recommended minimum lineal space requirement of 15 cm, multiple parallel perches are typically used in alternative laying-hen facilities. However, a few recently published studies found that perches were not equally attractive to the hens in commercial aviary systems in that perches installed in higher tiers of the system were the most preferred, whereas perches in lower tiers were infrequently used at night (Brendler and Schrader, 2016; Campbell *et al.*, 2016). Thus incorporating more perches to the higher tiers of multi-tier cage-free system by moderately reducing the horizontal distance between perches might still improve laying hen welfare by meeting more hens' perching needs. However, research does not exist in the literature that investigates the effects of horizontal distance between the parallel perches in meeting hen's actual perching needs.

Therefore, the objective of the study was to investigate the behavioral responses of Lohmann white laying hens to a range of horizontal distance (**HD**) between parallel perches (i.e., 15, 20, 25, 30, 40 and 60 cm) with regards to the proportion of hens perching during the dark period (**PHP**, %), perching duration (**PD**, i.e., time spent on the perch, min/hen), perching trip (**PT**, i.e., times of jumping on and off the perch, times/hen) and perching frequency (**PF**, i.e., number of perching trips per unit time, times/hen-hr), proportion of perching hens with heads toward the opposite perch (**PHO**, %), and the pattern of perch occupancy (**PPO**). The results will contribute to scientific evidence for setting or refining guidelines on HD of perches for laying hens in alternative hen-housing systems.

Materials and Methods

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (Log # 5-12-7364-G).

Experimental Animal and Husbandry

The study was conducted in an environment-controlled animal research lab located at Iowa State University, Ames, Iowa, USA. A total of 48 Lohmann LSL White laying hens provided by a cooperative egg producer were used in the study. The hens had been housed in a commercial aviary house until onset of the experiment when they were 68 weeks of age.

All the hens were considered to have had prior perching experience in the aviary house because they returned to the system at night and moved between the system and the litter floor during the day (as reported by the farm staff). The hens also had similar physiological and welfare conditions at the experiment onset, namely, comparable body weight (ranging from 1450 to 1550 g), feather coverage (slight to moderate feather damage/loss), feet health (no obvious foot disorders) and keel bone condition (slight to moderate keel bone deformity; keel bone fracture was not diagnosed). The hens were randomly assigned to three groups, 16 hens per group.

Three identical experimental pens (pen 1, 2 and 3) were used in the study. These experimental pens (Fig. 1), each measuring 120 cm L × 120 cm W × 120 cm H, had a wire-mesh (2.5 cm × 2.5 cm) floor (900 cm^2/bird space allowance), four wire-mesh (2.5 cm × 5.0 cm) sidewalls, an elevated nest box (120 cm L × 30 cm W × 40 cm H, 225 cm^2/bird; 45 cm above floor), two linear feeders (100 cm long, 12.5 cm per bird; installed outside the sidewalls), two nipple drinkers (1 nipple per 8 hens; 40 cm above floor, on the rear wall at 40 cm above floor), and two round galvanized tube perches (120 cm long × 32 mm diameter, 15 cm perch space per bird). The nest box had a door that only allowed hens to access it during the light period. The perches were designed to be adjustable so that HD between perches could be set accordingly. Both perches were installed at 30 cm above the floor which was within the height range in commercial aviary systems (19-32 cm above the floor). All the resource allowances, including perch, floor, feeder, nesting and nipple drinkers, were either higher than or comparable to those in the legislation or commercial guidelines for the hens.

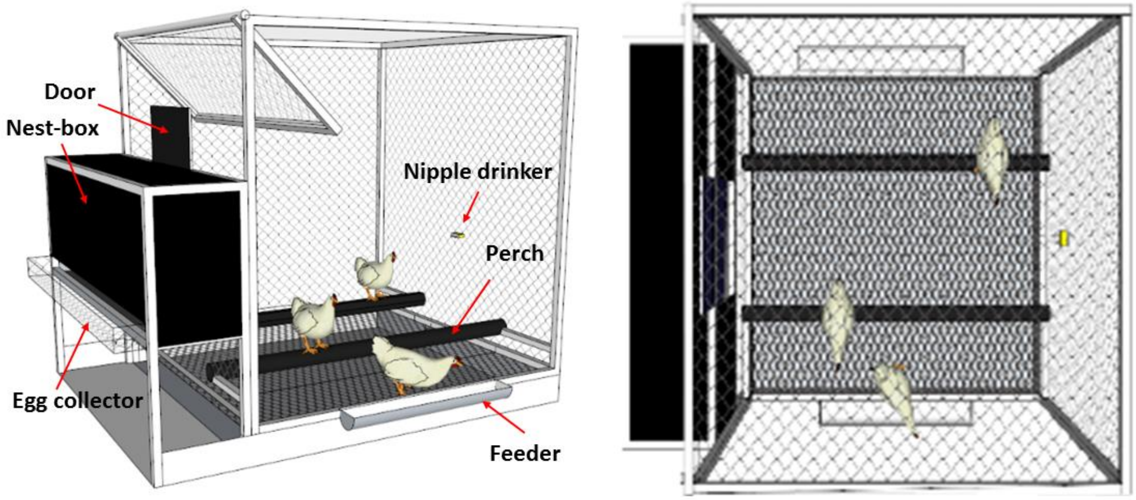

Figure 1. Side view (left) and top view (right) of the schematic drawing of the experimental pen.

Lighting scheme of the study followed the commercial management guidelines, namely, 16-h light at 15 lux (06:00 h-22:00 h), 7.5-h dark at 0 lux (22:15 h-05:45 h), and 0.5-h dim at 1-2 lux (05:45 h-06:00 h and 22:00 h-22:15 h). Light was provided by compact fluorescent lamps and light-emitting diode (LED) night lights for light and dim periods (i.e., dawn and dusk), respectively. Light intensity was measured using a light meter (0 to 20000 lux, model EA31, FLIR Systems Inc., Wilsonville, OR, USA[13]) and maintained at about 15 lux at bird head level (20 lux at perch height level) during the light period. The experimental room was equipped with mechanical ventilation and heating/cooling to maintain desired temperature of 21ºC. *Ad-lib* feed (commercial corn and soy diets) and water were available for hens throughout the test. Feeders were replenished and eggs were collected once a day at 18:00 h. The experiment pens were cleaned twice a week (i.e., removal of manure under the floor, feed waste, and dust or manure on the perch surface).

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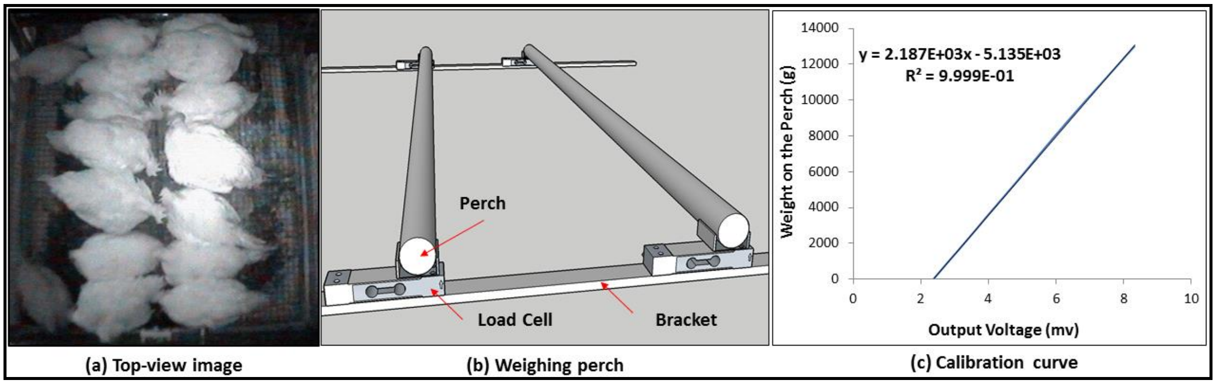
[13] Mention of product or company name is for presentation clarity and does not imply endorsement by the authors or Iowa State University, nor exclusion of other suitable products.

Testing System

A real-time vision-based monitoring system was built by incorporating three infrared night-vision cameras (GS831SM/B, Gadspot Inc. Corp., Tainan City, Taiwan, China) with a commercial surveillance software (MSH-Video surveillance system, S-VIDIA Inc., Santa Clara, CA, USA). It could record top-view images (Fig. 2a) from all three cameras simultaneously at 1 frame per second (FPS), and was used to record hen's perching behaviors during dark period to determine the heading directions and patterns of perch occupancy by hens.

A real-time sensor-based perching monitoring system was built by incorporating six pairs of load-cell sensors (5 to 100 kg ± 30 g, model 642C, Revere Transducers Inc., Tustin, CA, USA) supporting the six perches with a LabView-based data acquisition system (version 7.1, National Instrument Corporation, Austin, TX, USA). This monitoring system consisted of a compact FieldPoint controller (NI cFP-2020, National Instrument Corporation, Austin, TX, USA) and two 8-channel thermocouple input modules (NI cFP-TC-120, National Instrument Corporation, Austin, TX, USA) that was running at the sampling rate of 1 Hz. Each pair of load-cell sensors coupled with a tube perch made up a weighing perch (Fig. 2b). The analog voltage outputs of the load-cells were converted to weight values using pre-defined calibration curves (Fig. 2c, an example of the calibration curve). Consequently, real-time weight on the perch (i.e., total weight of perching birds) could be measured and recorded.



(a) Top-view image (b) Weighing perch (c) Calibration curve

Figure 2. Data acquisition system for hen behavior monitoring.

Experimental Procedures

The three groups of hens were randomly assigned to the three experimental pens. All treatments were applied simultaneously to all three groups. Specifically, all hens were allowed to acclimate in their respective pen for two weeks before the commencement of the test. During acclimation period, HD between the two perches was kept at 60 cm, which was considered non-restraining to perching behavior of the hens. Thus behavioral measurements at HD of 60 cm were used as the reference (control) in this experiment. Behavioral responses of laying hens to changing HD was then examined by decreasing HD sequentially from 60 to 40, 30, 25, 20 and 15 cm, and then increasing it by following the reverse order. The number of days tested for each HD is listed in Table 1, ranging from 2 to 6 d, depending on the behavioral responses of the hens to the changing HD (e.g., hens tended to have more rapid responses in step-down procedure than in step-up procedure due to the carry-over effect). In the analysis, only data associated with the last one day (in step-down procedure) or two days (in step-up procedure) at each HD were analyzed.

Table 1. Horizontal distance (HD) between perches implemented in the study

Arrangement Order	HD (cm)			Number of Days Tested [1]	Number of Days Analyzed [1]
	Pen 1	Pen 2	Pen 3		
1	60	60	60	5	1
2	40	40	40	2	1
3	30	30	30	2	1
4	25	25	25	2	1
5	20	20	20	2	1
6	15	15	15	3	1
7	15	15	15	3	2
8	20	20	20	3	2
9	25	25	25	6	2
10	30	30	30	3	2
11	40	40	40	4	2
12	60	60	60	5	2

[1] The number of test days for each HD depended on the behavioral responses of hens to the changing HD to minimize or remove the carry-over effect. Days with incomplete dataset were excluded.

Data Processing

There was almost no movement after birds settled down on the perches during the dark period. Thus images recorded within the first 5 min of each hour after light-off were manually analyzed to determine the number of perching hens, heading direction and relative position of each perching hen during the dark period. Thereafter, PHP and PHO were calculated. The PPO was qualitatively compared among HD arrangements in terms of the relative positions of perching hens.

The weight data from the weighing perches were analyzed using an automated VBA program developed in Microsoft Excel (Microsoft Office 2016, Redmond, WA, USA). By implementing the program, first, the total weight of hens (**TW**) on each perch was converted to the number of perching hens (**NP**) by using a series of weight thresholds. With body weight of each hen ranging from 1450 g to 1550 g, NP = 1 when 1200 g < TW < 1800 g; NP = 2 when 2650 g < TW < 3350 g; NP = 3 when 4100 g < TW < 4900 g; NP = 4 when 5550 g

< TW < 6450 g; NP = 5 when 7000 g < TW < 8000 g; NP = 6 when 8450 g < TW < 9550 g; NP = 7 when 9800 g < TW < 11100 g; and NP = 8 when 11250 g < TW < 12150 g, which was the maximum number of hens on a single perch in the study. Then PD, PT, and PF were calculated for each specific period, i.e., entire day (24 h), light period (16 h, 06:00 h-22:00 h), dark period (7.5 h, 22:15 h-05:45 h), and dim period (0.5 h, 05:45 h-06:00 h and 22:00 h-22:15 h).

Statistical Analysis

All statistical analyses were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, NC, USA). Pen was the experimental unit for the study. The PHP, PHO and all other proportion data were analyzed with generalized linear mixed models using GLIMMIX procedure, specified with a beta distribution and a logit link function. The PD, PT and PF data were analyzed using MIXED procedure with linear mixed models. All the models were expressed as

$$Y_{ijk} = \mu + P_i + D_j + (P \times D)_{ij} + T(P \times D)_{ijk} + e_{ijk}$$

Where Y_{ijk} denotes the independent observation for pen i on the day k of HD$_j$; μ is the overall mean; P_i is the pen effect (fixed); D_j is the HD effect (fixed); $(P\times D)_{ij}$ is the interaction effect (random) of pen and HD; $T(P\times D)_{ijk}$ is the day effect (random) for each HD tested within each pen, adjusted with a first-order autoregressive or AR (1) covariance structure; and e_{ijk} is the random error with $N \sim (0, \sigma^2)$. The DDFM=KENWARDROGER option was applied to the standard error and degrees-of-freedom corrections. Tukey-Kramer tests were used for pairwise comparisons of behavioral variables among different HDs. Effects were considered significant at $p < 0.05$. Normality and homogeneity of variance of data were examined by residual diagnostics. Unless otherwise specified, data are presented as least squares means along with SEM. Finally, Pearson correlations among all behavioral variables were investigated by implementing the CORR procedure.

Results

Pattern of Perch Occupancy

Representative PPOs by hens during the dark period at HD of 15, 20, 25, 30, 40 and 60 cm between perches are shown in Figure 3, in which 9, 11, 13, 14, 13 and 13 out of the total 16 hens, respectively, perched during the dark period. Two distinct perching patterns were classified based on the relative positions of the perching hens, i.e., interlaced and random. For the interlaced pattern (at HD of 15, 20 and 25 cm), use of two perches was interrelated. Perches were occupied by either 6 or 7 hens (almost fully occupied) at HD of 25 cm, with perching hens interlacing with one another (i.e., a hen on one perch fitted her head or tail into the gap between the two hens on the opposite perch). In comparison, only part of each perch could be used at HD of 20 or 15 cm because the narrow horizontal space did not allow two hens at the same spot of the respective perch. For the random pattern (at HD of 30, 40 and 60 cm), HD was sufficient to accommodate two hens at the same spot of the respective perch without interfering each other.

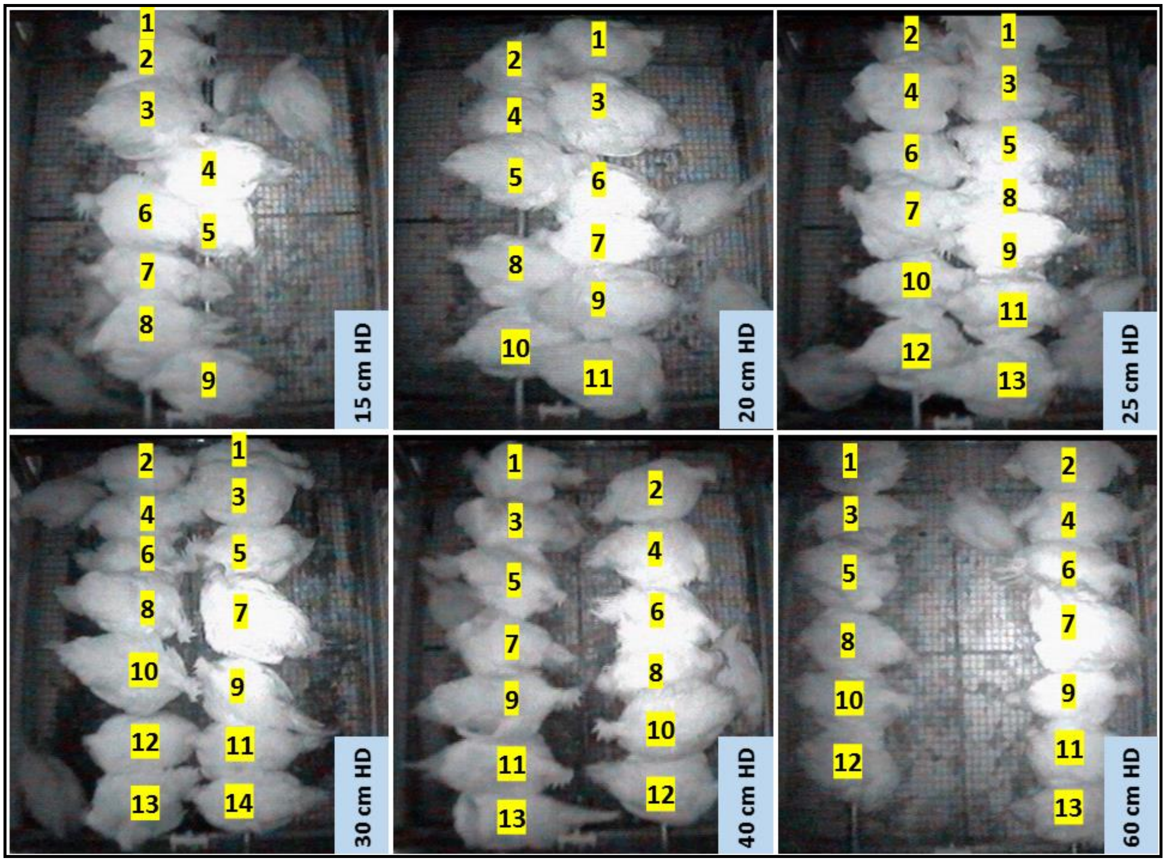


Figure 3. Representative patterns of perch occupancy by perching hens during the dark period at horizontal distance of 15, 20, 25, 40, and 60 cm between perches.

Perching Proportion and Heading Direction

PHP was significantly affected by HD (P = 0.002). As shown in Figure 4a, fewer hens perched simultaneously as HD decreased, although the overall perch length allowance remained the same. More specifically, 55.4 ± 2.9%, 69.5 ± 1.7%, 77.1 ± 1.8%, 74.7 ± 1.9%, 78.1 ± 1.9% and 78.6 ± 1.9% of the hens were perching simultaneously during the dark period at HD of 15, 20, 25, 30, 40 and 60 cm, respectively. The PHP values at HD of 20, 25, 30, 40 and 60 cm were significantly larger than the value at 15 cm (p = 0.025, 0.002, 0.005, 0.002 and 0.001, respectively). However, no difference was observed among the PHP values at HD of 20, 25, 30, 40 and 60 cm (p = 0.059-1.000), although the PHP at HD of 20 cm tended to be lower than that for HD of 60 cm (p = 0.059).

PHO was also significantly influenced by HD (p = 0.026). As shown in Figure 4b, 52.7 ± 5.2%, 65.7 ± 5.2%, 67.4 ± 5.2%, 57.0 ± 5.2%, 52.1 ± 5.2% and 37.2 ± 5.2% of the perching hens had their heads facing the opposite perch at HD of 15, 20, 25, 30, 40 and 60 cm, respectively. The PHO values at HD of 20 and 25 cm were significantly greater than that for HD of 60 cm (p = 0.031 and 0.023, respectively), while no difference was noticed among the values at HD of 15, 20, 25, 30 and 40 cm (p = 0.168-1.000).

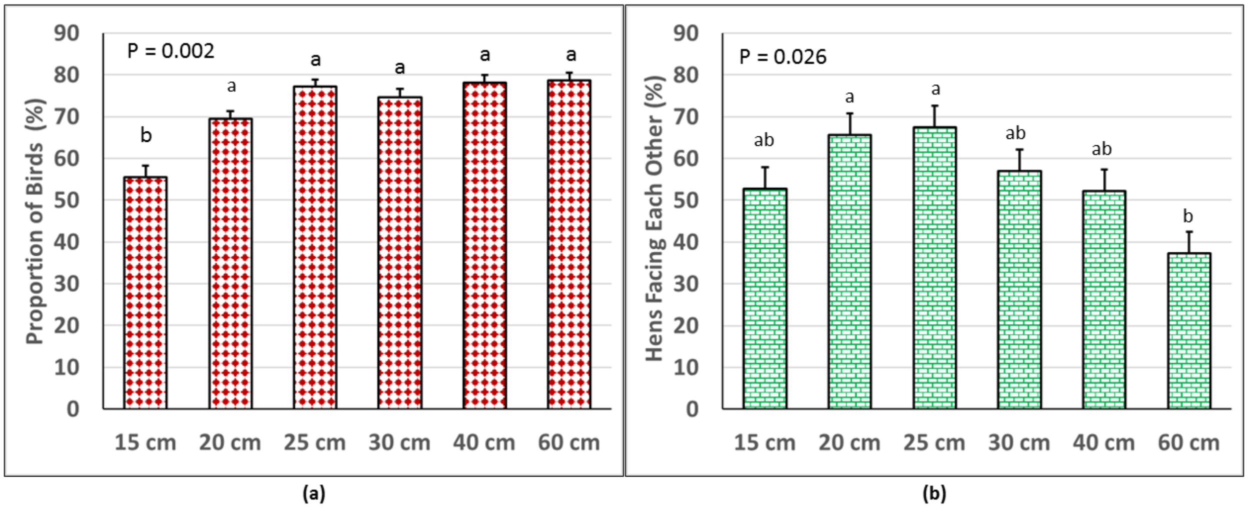


Figure 4. (a) Proportion of hens perching during dark period, and (b) proportion of perching hens with heads toward the opposite perch (i.e., facing each other). Bars with different letters are significantly different at $p < 0.05$.

Perching Duration

Daily PD and PD during dark and dim periods were significantly affected by HD (p = 0.002, 0.002 and 0.005, respectively), whereas PD of light period was not as much (p = 0.054). As shown by the data in Table 2, the daily PD at HD of 15 cm (441.3 ± 19.2 min/hen) was significantly lower than those at HD of 25, 30, 40 and 60 cm (p = 0.030, 0.050, 0.006 and 0.002, respectively), although there was no difference in daily PD between HDs of 15 and 20 cm (p = 0.320). There was also no difference in daily PD for pairwise comparison among HDs of 20, 25, 30, 40 and 60 cm (p = 0.097-0.994) with the exception of 20 cm *vs*. 60 cm (496.8 ± 16.4 *vs.* 595.0 ± 16.9 min/hen, p = 0.020).

The PD data were also summarized for the light, dark and dim periods, which accounted for 34.1% to 40.5%, 56.7% to 63.1% and 2.7% to 3.0% of the daily PD, respectively. These proportion values at HD of 15, 20, 25, 30, 40 and 60 cm were not significantly different from one another regardless of the period (p = 0.108, 0.101 and 0.338 for light, dark, and dim period, respectively). During the light period, the PD value at HD of 60 cm tended to be greater than that at 20 cm (p = 0.053), and no significant difference was observed between any other two HD's (p = 0.101-1.000). During the dark period, the PD value at HD of 15 cm was significantly smaller than the values at 20, 25, 30, 40 and 60 cm (p = 0.047, 0.003, 0.006, 0.002 and 0.001, respectively). Meanwhile, the PD value at HD of 20 cm tended to be smaller than the values at 40 and 60 cm (p = 0.058 and 0.074); however, the PD values were not significantly different between any other two HD's (p = 0.231-1.000). During the dim period, PD at HD of 15 cm was significantly smaller than those at 40 and 60 cm (p = 0.006 and 0.009, respectively). Meanwhile, PD at HD of 20 cm tended to be smaller than that at 40 cm (p = 0.064), and PD's were not significantly different between any other two HD's (p = 0.110-0.999).

Perching Trip and Frequency

PT of the hens also tended to be affected by HD for the entire day and light period (p = 0.057 and 0.057, respectively). As shown in Table 3, for both the entire day and light period, PTs at HD of 30 cm were significantly greater than those at 60 cm (p = 0.051 and 0.043, respectively), whereas PTs at other HDs were not different from one another (p =

0.091-1.000 and 0.109-1.000, respectively). There was essentially no PT during the dark period. No difference in PT during the dim periods was observed among different HDs ($p = 0.138$-1.000). When comparing PTs among different periods, PT during the light period accounted for about 90% of the daily PT, whereas only about 6% to 9% of the daily PT occurred during the dim period (0.5 h). At HD of 20, 25 and 30 cm, significantly higher proportions of daily PT occurred during the light period and lower proportions of daily PT during the dim period as compared to HD of 60 cm ($p = 0.003$ and 0.005, respectively). However, PF averaged 1.3-2.0 times/hr-hen during the light period, contrasting 4.0-5.2 times/hr-hen during the dim period, and negligible during the dark period.

Correlations between Perching Behavior Variables

Pearson correlations among all the perching behavior variables are shown in Table 4. Daily PD and PD during the dark and dim periods were highly correlated to PHP ($r = 0.91$, $p < 0.001$; $r = 0.99$, $p < 0.001$; and $r = 0.66$, $p < 0.001$, respectively). Daily PT was highly correlated to light-period PT ($r = 1.00$, $p < 0.001$). In addition, PHO during the dark period, PD during the light period, and PT during the dark and dim periods were slightly correlated to some of the other parameters ($r < 0.6$). Otherwise, no correlations existed among the variables.

Table 2. Perching duration of hens at different horizontal distances

Behavioral Parameters	Horizontal Distance between Perches						P-value
	15 cm	20 cm	25 cm	30 cm	40 cm	60 cm	
Perching duration (min/bird-period)							
Daily	441.3 ± 19.2^{c}	496.8 ± 16.4^{bc}	540.5 ± 16.8^{ab}	528.4 ± 16.8^{ab}	569.4 ± 16.9^{ab}	595.0 ± 16.9^{a}	0.002
Light	178.2 ± 12.3	174.7 ± 10.2	186.7 ± 10.2	181.0 ± 10.3	201.3 ± 10.4	225.8 ± 10.4	0.054
Dark	250.0 ± 13.9^{b}	308.8 ± 9.0^{a}	340.1 ± 9.1^{a}	333.5 ± 9.3^{a}	351.0 ± 9.4^{a}	353.2 ± 9.4^{a}	0.002
Dim	12.6 ± 0.6^{b}	14.1 ± 0.6^{ab}	14.8 ± 0.6^{ab}	14.5 ± 0.6^{ab}	16.7 ± 0.6^{a}	16.5 ± 0.6^{a}	0.005
Time budget of perching within each period (%)							
Daily	30.6 ± 1.3^{c}	34.5 ± 1.1^{bc}	37.5 ± 1.2^{ab}	36.7 ± 1.2^{ab}	39.5 ± 1.2^{ab}	41.3 ± 1.2^{a}	0.002
Light	18.6 ± 1.3	18.2 ± 1.1	19.4 ± 1.1	18.9 ± 1.1	21.0 ± 1.1	23.5 ± 1.1	0.054
Dark	55.6 ± 3.1^{b}	68.6 ± 2.0^{a}	75.6 ± 2.0^{a}	74.1 ± 2.1^{a}	78.0 ± 2.1^{a}	78.5 ± 2.1^{a}	0.002
Dim	42.1 ± 2.0^{b}	47.0 ± 1.9^{ab}	49.4 ± 1.9^{ab}	48.3 ± 1.9^{ab}	55.8 ± 1.9^{a}	54.9 ± 1.9^{a}	0.005
Proportion of perching duration for each period (%)							
Light	40.5 ± 1.8	35.6 ± 1.2	34.8 ± 1.2	34.1 ± 1.3	35.5 ± 1.3	38.2 ± 1.3	0.108
Dark	56.7 ± 1.8	61.5 ± 1.2	62.4 ± 1.2	63.1 ± 1.3	61.6 ± 1.3	59.0 ± 1.3	0.101
Dim	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.7 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	0.338

Data presented as least squares means ± SEM, n = 9. SEM and degrees-of-freedom corrections were applied to the statistical analyses.
Row means with different superscript letters differed significantly at $p < 0.05$.

Table 3. Perching trip and frequency of hens at different horizontal distances

Behavioral Parameters	Horizontal Distance between Perches						P-value
	15 cm	20 cm	25 cm	30 cm	40 cm	60 cm	
Perching trips (times/bird-period)							
Daily	33.0 ± 2.8^{ab}	28.8 ± 2.4^{ab}	31.0 ± 2.2^{ab}	34.0 ± 2.2^{a}	32.8 ± 2.2^{ab}	23.3 ± 2.2^{b}	0.057
Light	30.5 ± 2.7^{ab}	26.8 ± 2.3^{ab}	28.8 ± 2.2^{ab}	31.9 ± 2.1^{a}	30.0 ± 2.1^{ab}	21.2 ± 2.1^{b}	0.057
Dark	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.499
Dim	2.6 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.6 ± 0.2	2.1 ± 0.2	0.048
Perching frequency (times/bird-hr)							
Daily	1.4 ± 0.1^{ab}	1.2 ± 0.1^{ab}	1.3 ± 0.1^{ab}	1.4 ± 0.1^{a}	1.4 ± 0.1^{ab}	1.0 ± 0.1^{b}	0.057
Light	1.9 ± 0.2^{ab}	1.7 ± 0.1^{ab}	1.8 ± 0.1^{ab}	2.0 ± 0.1^{a}	1.9 ± 0.1^{ab}	1.3 ± 0.1^{b}	0.058
Dark	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.506
Dim	5.1 ± 0.3	4.0 ± 0.3	4.0 ± 0.3	4.2 ± 0.3	5.2 ± 0.3	4.2 ± 0.3	0.048
Proportion of perching trips for each period (%)							
Light	91.7 ± 0.7^{ab}	92.8 ± 0.3^{a}	92.9 ± 0.3^{a}	93.6 ± 0.3^{a}	91.5 ± 0.3^{ab}	90.6 ± 0.3^{b}	0.003
Dark	0.2 ± 0.2	0.3 ± 0.2	0.5 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.420
Dim	8.1 ± 0.6^{ab}	6.9 ± 0.3^{b}	6.6 ± 0.3^{b}	6.4 ± 0.3^{b}	8.1 ± 0.3^{ab}	8.9 ± 0.3^{a}	0.005

Data presented as least squares means ± SEM, n = 9. SEM and degrees-of-freedom corrections were applied to the statistical analyses

Row means with different superscript letters differed significantly at $p < 0.05$.

Table 4. Pearson correlation coefficient between behavioral parameters

Parameters		PHP	PHO	PD				PT/PF			
		Dark	Dark	Daily	Light	Dark	Dim	Daily	Light	Dark	Dim
PHP	Dark	-	0.33^{*}	0.91^{***}	0.18	0.99^{***}	0.66^{***}	-0.49^{***}	-0.48^{***}	0.20	-0.37^{**}
PHO	Dark		-	0.16	-0.29^{*}	0.32^{*}	0.33^{*}	-0.27^{*}	-0.25	-0.02	-0.31^{*}
PD	Daily			-	0.56^{***}	0.92^{***}	0.72^{***}	-0.45^{***}	-0.44^{***}	0.13	-0.31^{*}
	Light				-	0.19	0.37^{**}	-0.07	-0.08	-0.03	0.07
	Dark					-	0.66^{***}	-0.49^{***}	-0.47^{***}	0.17	-0.39^{**}
	Dim						-	-0.61^{***}	-0.60^{***}	0.20	-0.39^{**}
PT/PF	Daily							-	1.00^{***}	-0.16	0.46^{***}
	Light								-	-0.17	0.40^{**}
	Dark									-	-0.18
	Dim										-

Correlation values with single (*), double (**) or triple asterisks (***) was significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Discussion

A weighing perch first came about in the early 1980s to automatically measure body weight in commercial poultry production (Turner *et al.*, 1984). Inspired by this idea, the current study investigated perch use of laying hens by using sensor-based weighing perches that allowed for continuous and automated perching monitoring and analysis. Compared with previously published perching studies that typically used labor-intensive and time-consuming manual methods in live or off-site video observation (Struelens *et al.*, 2009; Chen *et al.*, 2014; Campbell *et al.*, 2016; Brendler and Schrader, 2016; Habinski *et al.*, 2016), the current study provided more objective, repeatable and complete quantification on perching behavior of laying hens (number/proportion of hens perching at night, perching duration, and perching trip/frequency). However, the heading direction of perching hens and the pattern of perch occupancy had to be manually determined in the current study as the automated image processing of the video recorded during the dark period was not as accurate or reliable.

In the current study, perch occupancy was classified into interlaced and random patterns according to the relative positions of the hens on the parallel perches. When HD (e.g., 25 cm) was insufficient to accommodate two parallel hens at the same perch location on the respective perch, the hens maximized the perch availability by interlacing with other hens so that more hens could perch simultaneously. However, the effectiveness of this behavioral adjustment was limited as HD was further reduced (e.g., 20 and 15 cm). Perch occupancy of the cross-wise perch designs have been investigated in a couple of previous studies. For instance, adding a short cross-wise perch to an existing long perch to increase perch space from 12 to 15 cm per bird did not increase perch use as the crossing space was not efficiently used by hens (Wall and Tauson, 2007). Likewise, a perch of 30 cm cross-wise to another perch (i.e., 30, 45 or 60 cm) did not allow more hens to perch simultaneously at night as hens didn’t use it optimally (Struelens *et al.*, 2008). With limited results available, it is somewhat difficult to fully understand the behavioral mechanisms of hens in utilizing perches of various arrangements. However, it is certain that simply providing enough perch length without considering the relative positions of the perches may not satisfy the perching needs of the hens. It should be noted that besides HD, other factors, such as domestication, thermal condition, dominance relationship, and genetic/breed may also affect perching patterns of the hens by changing their inter-individual spacing during perching (Eklund and Jensen, 2011).

Allowing hens to perch simultaneously at night is one of the most important criteria in assessing perch availability as laying hens are highly motivated to perch and display signs of unrest and frustration when access to perch is denied (Olsson and Keeling, 2000; Olsson and Keeling, 2002). A recently published study found that hens even chose to crowd (over 100% of perch capacity) perches on the higher tiers of the aviary system when the perch space was limited (Campbell *et al.*, 2016). In other studies involving Lohmann LSL, Lohmann Brown, Hy-Line White, Hy-Line Brown and Shaver hens, approximately 80% to 100% of hens in furnished cages perched at night when the available perch space was as low as 12-15 cm per bird (Tauson, 1984; Tauson and Abrahamsson, 1994; Olsson and Keeling, 2000; Wall and Tauson, 2007). For the current study with 15 cm perch space per bird provided, the maximum proportion of hens perching during the dark period was 78.6 ± 1.9% at HD of 60 cm. When the perch availability was not restrained by HD, there were 2-3 hens that did not perch at night even though the perches were not fully occupied. This lower perching proportion compared to other studies may have partially attributed to the age of the hens (68 weeks at the experimental onset). Aged hens are heavier and tend to have inferior physical conditions (e.g., keel bone deformity and/or fractures and foot disorders); as a result they may be less motivated to perch (Käppeli *et al.*, 2011; Petrik *et al.*, 2015; Stratmann *et al.*, 2015). The hens used in the current study had slight to moderate keel bone deformity and might have had some keel bone fractures, although they were not examined. In addition, genetic differences between the hens in the current study and those reported in the literature might have contributed to the lower proportion values observed in the current study. Faure and Jones (1982) reported high genetic variance in hen's perching behavior.

In the current study, the proportion of perching hens with their heads toward the opposite perch (each other) during dark period was significantly larger at HD of 20 or 25 cm than that at 60 cm, although no difference was detected among HDs of 15, 20, 25, 30 and 40 cm. A previous study showed that hens in groups of three tended to orientate away from each other at distances greater than 25 cm but toward each other at distance less than 25 cm when they were on the floor (Keeling and Duncan, 1989). Result of the current study was consistent with the finding by Keeling and Duncan (1989). The explanation for the perching hens to face each other could be that the hens may exercise the instinct of protecting themselves by facing to, as opposed to away from, each other, especially at the closer distances. However, the similar proportions among HDs of 15-40 cm could be that the hens had less moving ability on the perches as compared to the floor (Stampfli *et al.*, 2013). Studies have shown that hens rest or sleep on perch at night (Hester, 2014). Therefore, it is possible that heading direction of the perching hens at night has no behavioral significance to the birds; and the heading direction may simply depend on the relative positions of the hens at the moment of jumping on the perch. Consequently, with a narrower HD, hens needed to mount each perch from the outside, leading to a higher proportion of facing each other.

In terms of PD and PT, no other study could be found involving continuous measurements of perch use by laying hens. As mentioned earlier, HD of 60 cm was used during the acclimation period and considered an unrestrained condition for the hens to express perching behaviors. The PPO's showed qualitatively that HD of 15 or 20 cm is insufficient to meet the hens' perching needs due to reduced perch availability as compared to HD of 25-60 cm. Comparisons of PHP values also quantitatively showed that HD of 15 and 20 cm reduced the proportion of perching hens as compared to HD of 60 cm ($p = 0.001$ and 0.059, respectively). The PD data further strengthened afore-stated observation, as the results showed that daily PD and dark-period PD at HDs of 15 and 20 cm were much smaller than that at 60 cm. On the other hand, light-period PD was not affected by HD, which might have resulted from the circadian behavior pattern of the hens as they are less motivated to perch during the light period. Specifically, the hens spent about 18% to 24% of time on the perches during light period (16 h), accounting for about 35% to 40% of the daily PD. These values were comparable to those reported in other studies in that hens in furnished cages spent approximately 20% to 25% of their time on the perch during the daytime (Tauson, 1984; Tauson and Abrahamsson, 1994; Appleby *et al.*, 1993). As for PT, values for daily, light, dim and dark periods were relatively consistent across all the HD regimens of the study. Some previous studies found much more movements on and off perches during daylight as compared to at night (Lambe and Scott, 1998), which was quantitatively verified in the current study showing that over 90% of the perching trips (on and off perch) occurred during the light period. However, the most active perching behaviors occurred during the dim period in terms of PF (4.0-5.2 *vs.* 1.3-2.0 times/hen-hr for dim *vs.* light period). The most active perching activities during the dim period presumably arose from the hens needing to have serval attempts or compete before eventually accommodating themselves on the perches.

Perch could benefit laying hens by providing the opportunities of weight-loaded exercise (Wilson *et al.*, 1993). Thus a proper perch system needs to not only allow all hens to perch at night but also encourage more perching trips during daytime. With the increasing adoption of alternative housing systems for egg production nowadays, scientists are finding new interests on perch use and the resultant effects on pullets and laying hens, especially in commercial systems (Yan *et al.*, 2013; Campbell *et al.*, 2016; Habinski *et al.*, 2016; Brendler and Schrader, 2016). However, almost all the studies focused their measurements on the number or proportion of perching hens, with limited ability to quantify the actual perching duration and perching trip/frequency. According to the Pearson correlation analysis of the current study, PHP during the dark period, PT during the light and dim periods, and PD during the light period should be quantified to provide a comprehensive assessment on perching behaviors. Engineering techniques that target for precision livestock farming applications, e.g., a weighing perch system as used in the current study, offers a promising alternative to human labors, especially as the traditional methods based on human observations become less applicable to large-scale commercial settings.

Conclusions

With a group size of 16 hens provided with an average 15 cm perch length per bird, HD of 25 cm between parallel perches was shown to be the lower threshold to accommodate the hen’s perching behaviors. HD of 20 or 15 cm was shown to be insufficient, hence restraining the perching. Hens were observed to show most frequent perching activities during the dim period. The implication is that although 30 cm is the recommended minimum horizontal distance between perches, 25 cm may be considered if reducing HD from 30 to 25 cm would allow placement of more perches to meet the perching needs of all hens.

Acknowledgements

Funding for the study was in part provided by the Egg Industry Center located at Iowa State University. We would like to thank the cooperative egg producer for the generous donation of the hens and feed used in the study. Thanks are also extended to the Agriculture Experiment Station (AES) Consulting Group at Iowa State University for the consistent assistance in statistical consultation for the study. Lastly, author Kai Liu wishes to thank China Scholarship Council (CSC) for providing part of the financial support for his PhD study at Iowa State University.

References

Appleby, M. C., & Hughes, B. O. (1990). Cages modified with perches and nests for the improvement of bird welfare. *World's Poultry Science Journal*, *46*(1), 38–40. https://doi.org/10.1079/WPS19900007

Appleby, M. C., Smith, S. F., & Hughes, B. O. (1993). Nesting, dust bathing and perching by laying hens in cages: Effects of design on behaviour and welfare. *British Poultry Science*, *34*(5), 835–847. https://doi.org/10.1080/00071669308417644

Barnett, J. L., Tauson, R., Downing, J. a, Janardhana, V., Lowenthal, J. W., Butler, K. L., & Cronin, G. M. (2009). The effects of a perch, dust bath, and nest box, either alone or in combination as used in furnished cages, on the welfare of laying hens. *Poultry Science*, *88*(3), 456–470. https://doi.org/10.3382/ps.2008-00168

Brendler, C., & Schrader, L. (2016). Perch use by laying hens in aviary systems. *Applied Animal Behaviour Science*, *182*(1), 9–14. https://doi.org/10.1016/j.applanim.2016.06.002

Campbell, D. L. M., Makagon, M. M., Swanson, J. C., & Siegford, J. M. (2016). Perch use by laying hens in a commercial aviary. *Poultry Science*, *95*(8), 1736–1742. https://doi.org/10.3382/ps/pew111

Chen, D., Bao, J., Meng, F., & Wei, C. (2014). Choice of perch characteristics by laying hens in cages with different group size and perching behaviours. *Applied Animal Behaviour Science*, *150*(31172246), 37–43. https://doi.org/10.1016/j.applanim.2013.10.007

Cooper, J. J., & Albentosa, M. J. (2003). Behavioural priorities of laying hens. *Avian and Poultry Biology Reviews*, *14*(3), 127–149. https://doi.org/10.3184/147020603783637508

Council Directive 1999/74/EC. (1999). Laying down minimum standards for the protection of laying hens. *Official Journal of the European Communities*, 53–57.

Donaldson, C. J., Ball, M. E. E., & O’Connell, N. E. (2012). Aerial perches and free-range laying hens: The effect of access to aerial perches and of individual bird parameters on keel bone injuries in commercial free-range laying hens. *Poultry Science*, *91*(2), 304–315. https://doi.org/10.3382/ps.2011-01774

Donaldson, C. J., & O’Connell, N. E. (2012). The influence of access to aerial perches on fearfulness, social behaviour and production parameters in free-range laying hens. *Applied Animal Behaviour Science*, *142*(1–2), 51–60. https://doi.org/10.1016/j.applanim.2012.08.003

Duncan, E. T., Appleby, M. C., & Hughes, B. O. (1992). Effect of perches in laying cages on welfare and production of hens. *British Poultry Science*, *33*(1), 25–35. https://doi.org/10.1080/00071669208417441

Eklund, B., & Jensen, P. (2011). Domestication effects on behavioural synchronization and individual distances in chickens (Gallus gallus). *Behavioural Processes*, *86*(2), 250–256. https://doi.org/10.1016/j.beproc.2010.12.010

Enneking, S. a., Cheng, H. W., Jefferson-Moore, K. Y., Einstein, M. E., Rubin, D. a., & Hester, P. Y. (2012). Early access to perches in caged White Leghorn pullets. *Poultry Science*, *91*(9), 2114–2120. https://doi.org/10.3382/ps.2012-02328

Faure, J. M., & Jones, R. B. (1982). Effects of sex, strain and type of perch on perching behaviour in the domestic fowl. *Applied Animal Ethology*, *8*(3), 281–293. https://doi.org/10.1016/0304-3762(82)90211-5

Glatz, P. C., & Barnett, J. L. (1996). Effect of perches and solid sides on production, plumage and foot condition of laying hens housed in conventional cages in a naturally ventilated shed. *Australian Journal of Experimental Agriculture*, *36*(3), 269–275. Retrieved from http://www.publish.csiro.au/paper/EA9960269

Habinski, A. M., Caston, L. J., Casey-Trott, T. M., Hunniford, M. E., & Widowski, T. M. (2016). Development of perching behavior in 3 strains of pullets reared in furnished cages. *Poultry Science*, (2000), pew377. https://doi.org/10.3382/ps/pew377

HÄne, M., Huber-Eicher, B., & FrÖhlich, E. (2000). Survey of laying hen husbandry in Switzerland. *World's Poultry Science Journal*, *56*(1), 21–31. https://doi.org/10.1079/WPS20000003

Hester, P. Y. (2014). The effect of perches installed in cages on laying hens. *World's Poultry Science Journal*, *70*(2), 247–264. https://doi.org/10.1017/S0043933914000270

Hester, P. Y., Enneking, S. A., Haley, B. K., Cheng, H. W., Einstein, M. E., & Rubin, D. A. (2013). The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poultry Science*, *92*(8), 1972–1980. https://doi.org/10.3382/ps.2013-03008

Hester, P. Y., Enneking, S. a, Jefferson-Moore, K. Y., Einstein, M. E., Cheng, H. W., & Rubin, D. a. (2013). The effect of perches in cages during pullet rearing and egg laying on hen performance, foot health, and plumage. *Poultry Science*, *92*(2), 310–320. https://doi.org/10.3382/ps.2012-02744

Hughes, B. O., Wilson, S., Appleby, M. C., & Smith, S. F. (1993). Comparison of bone volume and strength as measures of skeletal integrity in caged laying hens with access to perches. *Research in Veterinary Science, 54*(2), 202–206. https://doi.org/10.1016/0034-5288(93)90057-M

Jiang, S., Hester, P. Y., Hu, J. Y., Yan, F. F., Dennis, R. L., & Cheng, H. W. (2014). Effect of perches on liver health of hens. *Poultry Science*, *93*(7), 1618–1622. https://doi.org/10.3382/ps.2013-03659

Käppeli, S., Gebhardt-Henrich, S. G., Fröhlich, E., Pfulg, A., & Stoffel, M. H. (2011). Prevalence of keel bone deformities in Swiss laying hens. *British Poultry Science, 52*(5), 531–536. https://doi.org/10.1080/00071668.2011.615059

Keeling, L. J., & Duncan, I. J. H. (1989). Inter-individual distances and orientation in laying hens housed in groups of three in two different-sized enclosures. *Applied Animal Behaviour Science*, *24*(4), 325–342. https://doi.org/10.1016/0168-1591(89)90060-9

Lambe, N. R., & Scott, G. B. (1998). Perching behaviour and preferences for different perch designs among laying hens. *Animal Welfare*, *7*(2), 203–216.

Moinard, C., Morisse, J. P., & Faure, J. M. (1998). Effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. *British Poultry Science*, *39*(2), 198–202. https://doi.org/10.1080/00071669889123

Olsson, I. A. S., & Keeling, L. J. (2000). Night-time roosting in laying hens and the effect of thwarting access to perches. *Applied Animal Behaviour Science*, *68*(3), 243–256. https://doi.org/10.1016/S0168-1591(00)00097-6

Olsson, I. A. S., & Keeling, L. J. (2002). The push-door for measuring motivation in hens : laying hens are motivated to perch at night. *Animal Welfare*, *11*, 11–19.

Petrik, M. T., Guerin, M. T., & Widowski, T. M. (2015). On-farm comparison of keel fracture prevalence and other welfare indicators in conventional cage and floor-housed laying hens in Ontario, Canada. *Poultry Science*, *94*(4), 579–585. https://doi.org/10.3382/ps/pev039

Savory, C. J. (2004). Laying hen welfare standards: A classic case of "power to the people." *Animal Welfare*, *13*, 153–158.

Stampfli, K., Buchwalder, T., Frohlich, E. K. F., & Roth, B. A. (2013). Design of nest access grids and perches in front of the nests: Influence on the behavior of laying hens. *Poultry Science*, *92*(4), 890–899. https://doi.org/10.3382/ps.2011-02046

Stratmann, A., Fröhlich, E. K. F., Gebhardt-Henrich, S. G., Harlander-Matauschek, A., Würbel, H., & Toscano, M. J. (2015). Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Applied Animal Behaviour Science*, *165*, 112–123. https://doi.org/10.1016/j.applanim.2015.01.012

Struelens, E., & Tuyttens, F. A. M. (2009). Effects of perch design on behaviour and health of laying hens. *Animal Welfare*, *18*(4), 533–538.

Struelens, E., Tuyttens, F. A. M., Ampe, B., Ödberg, F., Sonck, B., & Duchateau, L. (2009). Perch width preferences of laying hens. *British Poultry Science*, *50*(4), 418–423. https://doi.org/10.1080/00071660903110885

Struelens, E., Van Poucke, E., Duchateau, L., Ödberg, F., Sonck, B., & Tuyttens, F. A. M. (2008). Effect of cross-wise perch designs on perch use in laying hens. *British Poultry Science*, *49*(4), 402–408. https://doi.org/10.1080/00071660802210687

Tauson, R. (1984). Effects of a perch in conventional cages for laying hens. *Acta Agriculturae Scandinavica*, *34*(2), 193–209. https://doi.org/10.1080/00015128409435389

Tauson, R., & Abrahamsson, P. (1994). Foot and skeletal disorders in laying hens: effects of perch design, hybrid, housing system and stocking density. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *44*(2), 110–119. https://doi.org/10.1080/09064709409410189

Turner, M. J. B., Gurney, P., Crowther, J. S. W., & Sharp, J. R. (1984). An automatic weighing system for poultry. *Journal of Agricultural Engineering Research*, *29*(1), 17–24. https://doi.org/10.1016/0021-8634(84)90056-8

Wall, H., & Tauson, R. (2007). Perch arrangements in small-group furnished cages for laying hens. *The Journal of Applied Poultry Research*, *16*(3), 322–330. https://doi.org/10.1093/japr/16.3.322

Wechsler, B., & Huber-Eicher, B. (1998). The effect of foraging material and perch height on feather pecking and feather damage in laying hens. *Applied Animal Behaviour Science*, *58*(1–2), 131–141. https://doi.org/10.1016/S0168-1591(97)00137-8

Weeks, C. A., & Nicol, C. J. (2006). Behavioural needs, priorities and preferences of laying hens. *World's Poultry Science Journal*, *62*(2), 296–307. https://doi.org/10.1079/WPS200598

Wilson, S., Hughes, B. O., Appleby, M. C., & Smith, S. F. (1993). Effects of perches on trabecular bone volume in laying hens. *Research in Veterinary Science*, *54*(2), 207–211. https://doi.org/10.1016/0034-5288(93)90058-N

Yan, F. F., Hester, P. Y., Enneking, S. A., & Cheng, H. W. (2013). Effects of perch access and age on physiological measures of stress in caged White Leghorn pullets. *Poultry Science*, *92*(11), 2853–2859. https://doi.org/10.3382/ps.2013-03271

CHAPTER 4

EFFECTS OF LIGHT-EMITTING DIODE LIGHT V. FLUORESCENT LIGHT ON GROWING PERFORMANCE, ACTIVITY LEVELS AND WELL-BEING OF NON-BEAK-TRIMMED W-36 PULLETS

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A paper published in *Animal* (2017)
Available online at: https://doi.org/10.1017/S1751731117001240

Abstract

More energy-efficient, readily-dimmable, long-lasting, and more affordable light-emitting diode (LED) lights are increasingly finding applications in poultry production facilities. Despite anecdotal evidence about the benefits of such lighting on bird performance and behavior, concrete research data are lacking. In this study, a commercial poultry-specific LED light (dim-to-blue, controllable correlated color temperature or CCT from 4500K to 5300K) and a typical compact fluorescent (CFL) light (soft white, CCT = 2700K) were compared with regards to their effects on growing performance, activity levels, and feather and comb conditions of non-beak-trimmed W-36 pullets during a 14-week rearing period. A total of 1280-day-old pullets in two successive batches, 640 birds each, were used in the study. For each batch, pullets were randomly assigned to four identical litter-floor rooms equipped with perches, two rooms per light regimen, 160 birds per room. BW, BW uniformity (BWU), BW gain (BWG), and cumulative mortality rate (CMR) of the pullets were determined biweekly from day-old to 14 weeks of age (WOA). Activity levels of the pullets at 5-14 WOA were delineated by movement index. Results revealed that pullets under the LED and CFL lights had comparable BW (1140 ± 5 g *vs.* 1135 ± 5 g, p = 0.41), BWU (90.8 ± 1.0% *vs.* 91.9 ± 1.0%, p = 0.48), and CMR (1.3 ± 0.6% *vs.* 2.7 ± 0.6%, p = 0.18) at 14 WOA despite some varying BWG during the rearing. Circadian activity levels of the pullets were higher under the LED light than under the CFL light, possibly resulting from differences in spectrum and/or perceived light intensity between the two lights. No feather damage or comb wound was apparent in either light regimen at the end of the rearing period. The results contribute to understanding the impact of emerging LED lights on pullets rearing which is a critical component of egg production.

Keywords: Poultry Lighting, Growing Performance, Activity Level, Feather Condition, Animal Behavior

Introduction

Light is a crucial environmental factor that affects bird's behaviors, development, production performance, health, well-being, and possibly product quality of modern egg production (Lewis and Morris, 1998). Extensive research on poultry lighting has been conducted over the past eight decades, which has contributed to understanding of poultry responses to lighting, improved energy efficiency in lighting, and general management practices of modern egg production. Today, more energy-efficient, readily-dimmable, long-lasting, and more affordable light-emitting diode (**LED**) lights are increasingly finding applications in poultry production facilities (Parvin *et al.*, 2014). There have been some anecdotal claims about the benefits of such lighting on bird performance and behavior; however data from controlled research are lacking.

Many lighting effects on poultry have been well understood by both scientific and industrial communities. For example, activity levels of birds are known to be positively correlated to light intensity (Boshouwers and Nicaise, 1993; Deep *et al.*, 2012). Sexual development and maturity of pullets are known to be associated with changes in day length and red light spectrum (Smith and Noles, 1963; Min *et al.*, 2012; Baxter *et al.*, 2014). However, certain aspects remain to be fully investigated and understood. For instance, a few studies reported that blue lights were associated with improving broiler growth, calming the birds (e.g., reducing aggressive interaction and locomotion), and enhancing immune response (Prayitno *et al.*,1997; Rozenboim *et al.*, 2004; Cao *et al.*, 2008; Xie *et al.*, 2008; Sultana *et al.*, 2013). However, the underlying mechanisms were not clearly delineated in these studies. In contrast, some studies reported no effects of different light sources on growth performance of pullets and broilers (Schumaier *et al.*,1968; Pyrzak *et al.*, 1986; Baxter *et al.*, 2014; Huth and Archer, 2015; Olanrewaju *et al.*, 2016). A long-term field study with commercial aviary hen houses revealed no differences in egg weight, egg production, feed use, and mortality rate of DeKalb white hens between a commercial LED light and CFL light (Long *et al.*, 2016). In addition, studies found that different genetic breeds of birds responded differently to lights. For example, W-36 laying hens were reported to have the highest feed intake at 5 lux but lowest at 100 lux (Ma *et al.*, 2016), whereas ISA Brown hens showed most feeding in the brightest (200 lux) and least in the dimmest light (<1 lux) (Prescott and Wathes, 2002). Thus further investigation of poultry lighting is warranted.

Poultry and humans have different light spectral sensitivities (Prescott *et al.*, 2003; Saunders *et al.*, 2008) in that humans have three types of retinal cone photoreceptors, but poultry have five that are sensitive to ultraviolet, short-, medium-, and long-wavelength lights (Osorio and Vorobyev, 2008). Compared to humans, poultry can perceive light not only through their retinal cone photoreceptors in the eyes, but via extra retinal photoreceptors in the brain (e.g., pineal and hypothalamic glands) (Mobarkey *et al.*, 2010). Retinal cone photoreceptors produce the perception of light colors by receiving lights at the peak sensitivities of about 415, 450, 550, and 700 nm, and are more related to poultry activities (e.g., feeding, drinking, and locomotion) and growth (Lewis and Morris, 2000). In contrast, the extra retinal photoreceptors can only be activated by long-wavelength lights (e.g., red) that can penetrate the skull and deep tissue of poultry, and are more related to sexual development and maturity (Lewis and Morris, 2000). It has been demonstrated that red lights can pass through hypothalamic extra retinal photoreceptors, thus stimulate reproductive axis by controlling the secretion of gonadotrophin receptor hormone (**GnRH**) and stimulating the release of LH and FSH (Lewis and Morris, 2000). As different light sources (e.g., incandescent, high pressure sodium or **HPS**, fluorescent, and LED lights) usually have different spectral characteristics, retinal and extra retinal photoreceptors of poultry may be stimulated differently when exposed to different light sources, thus causing different impacts on birds.

Despite the increasing LED light applications in egg production facilities, current lighting guidelines or recommendations (e.g., Hy-Line Commercial Layers Management Guideline) were established based on conventional incandescent and/or CFL lights and measured based on human vision. As a result, existing guidelines may not accurately reflect the operational characteristics and impact of the LED lights, hence the need for more research regarding the impact of LED lights on poultry and the corresponding lighting strategy. Meanwhile, concerns over animal welfare have led to increasing adoption of alternative housing systems such as enriched colony and cage-free aviary housing. However, there exist a number of challenges in such alternative housing systems, such as incidences of floor eggs, aggressive pecking and cannibalism, and resultant high mortality rate. With the important role that light plays in controlling hen behaviors, fine-tuning of lighting conditions and management strategies is expected to have a profound impact on alleviating some of these challenges.

Lighting experience during rearing period is very important for pullets as it can have profound impact on their growth and development (e.g., BW, BW uniformity, mortality rate, and skeleton health), behaviors (e.g., aggressive pecking and cannibalism), subsequent lay performance (e.g., egg production rate and egg quality), and well-being (Lanson and Sturkie, 1961; Zappia and Rogers, 1983; Nicol *et al.*, 2013; Hy-Line International, 2016). With the emergence of various LED lights intended for poultry production, science-based information is necessary to optimize lighting characteristics. Just as CFL lamps have been replacing incandescent lamps, LED lights are expected to replace CFL lamps and become the predominant lighting source in the foreseeable future. Thus, it is of socio-economic as well as scientific importance to quantify and compare the growing performance and behavioral responses of pullets to LED *vs.* CFL lighting conditions.

The objective of this study was to evaluate the effects of a commercial Dim-to-Blue® poultry-specific LED light (dim-to-blue, controllable correlated color temperature or **CCT** from 4500K to 5300K) *vs.* a typical CFL light (soft white, CCT = 2700K) with regards to growing performance (BW, BW uniformity or **BWU**, BW gain or **BWG**, cumulative mortality rate or **CMR**), activity levels, and feather and comb conditions of pullets. The results will contribute to the scientific basis of improving lighting guidelines for pullet rearing and egg production.

Materials and Methods

This study was conducted at the Hy-Line International Research Farm Facility located in Dallas Center, Iowa, USA. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (Log #: 3-15-7982-G).

Experimental Pullets and Husbandry

A total of 1280 Hy-Line W-36 non-beak-trimmed pullets in two successive batches were used in the study. For each batch, 640 pullets were individually identified with wing-bands, randomly assigned to four identical litter-floor rooms, 160 pullets per room at stocking density of 10 birds per m^2 (967 cm^2 per bird). The pullet-rearing rooms (Fig. 1), each measuring 4.3 × 3.6 × 2.4 m (L × W × H), had a concrete floor covered with wood shavings (4-5 cm in depth), two round auto-fill feeders (51 cm in diameter), 14 nipple drinkers (adjustable height), and a wooden gable perch set (90 cm L × 140 cm W × 67 cm H) that had five parallel perches (90 cm in length and 1.6 cm in diameter) in three tiers. Four cameras were installed on the ceiling of each room, evenly distributed, covering the entire floor area with top views (Fig. 1). The rooms were equipped with mechanical ventilation (one variable speed fan per room, up to 1495 m^3/hr airflow rate) and supplemental heating to ensure thermal comfort conditions throughout the rearing period. Room temperature and relative humidity (**RH**) were set according to the Hy-Line Commercial Layers Management Guideline (Hy-Line International, 2016), i.e., 33-35°C from placement to day 3, decreased to 31-33°C from day 4 to day 7, and then gradually reduced by 2°C per week until 21°C by day 36; 40-60% RH. The pullets had *ad-lib* access to feed and water. Corn and soy diets were formulated to meet the nutritional recommendations based on BW (Hy-Line International, 2016), i.e., starter-1 diet [20.00% CP, 2977-3087 kcal/kg ME, 1.00% Ca, and 0.50% available phosphorus] for BW below 176-184 g, starter-2 diet [18.25% CP, 2977-3087 kcal/kg ME, 1.0% Ca, and 0.49% available phosphorus] for BW below 413-427 g, grower diet [17.50% CP, 2977-3087 kcal/kg ME, 1.0% Ca, and 0.47% available phosphorus] for BW below 947-973 g, and developer diet [16.00% CP, 2977-3131 kcal/kg ME, 1.0% Ca, and 0.45% available phosphorus] for BW below 1154-1186 g (Hy-Line International, 2016). Standard vaccination program (e.g., Marek's disease, Newcastle disease, infectious bronchitis, infectious bursal disease, avian encephalomyelitis, and fowl pox) recommended for pullet production was also followed (Hy-Line International, 2016).

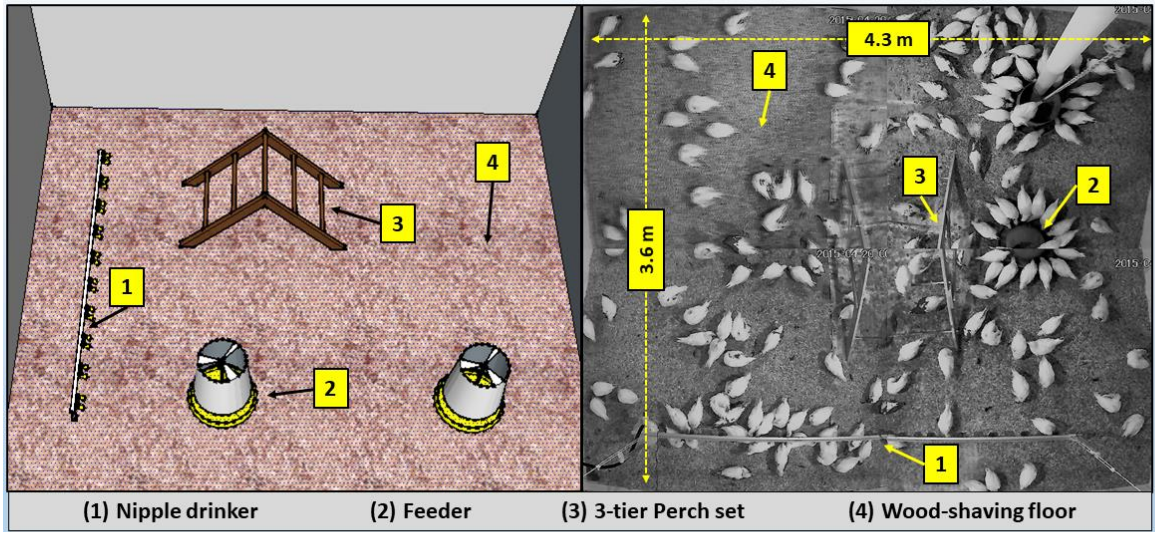

Figure 1. Schematic (left) and top photographic view (right) of the pullet-rearing room.

Lighting Regimens

Artificial light was the only light source in the rearing rooms. Two rooms used a commercial Dim-to-Blue® poultry-specific LED light (Agrishift MLB LED, 12W, dim-to-blue, controllable CCT from 4500K to 5300K, Once, Inc., Plymouth, MN, USA). "Dim-to-blue" is achieved by lowering power input to other color components, yielding higher proportion of blue light. The other two rooms used a typical CFL light (EcoSmart CFL, 9W, soft white, CCT = 2700K, Eco Smart Lighting Australia Pty Ltd, Sydney, Australia). Two light bulbs installed on the ceiling per room. The spectral profiles of both lights (Fig. 2a) were determined using a spectral meter (SpectraShift 2.0, Once, Inc.). Specifically, the LED light had a relatively even spectral profile as compared with the CFL light. The relatively elevated spectral peaks for the LED light occurred at 450 nm and 630 nm, whereas spectral spikes for the CFL light occurred at 545 nm and 610 nm. Light intensity and photoperiod (Table 1) used in the study, varying with bird age, followed the Hy-Line Commercial Layers Management Guideline (Hy-Line International, 2016). Actual light intensities (Table 1), in both lux and p-lux (poultry-perceived light intensity) (Prescott *et al.*, 2003), were measured using the spectral meter at the bird head level at five different spots within the rearing rooms (center and four quadrants below the cameras). Light intensities in p-lux for the LED and CFL lights were shown to be, respectively, 1.39 and 1.26 times the values measured in lux (Fig. 2b). Light intensities (lux) were comparable between the LED and CFL rooms at each intensity level.

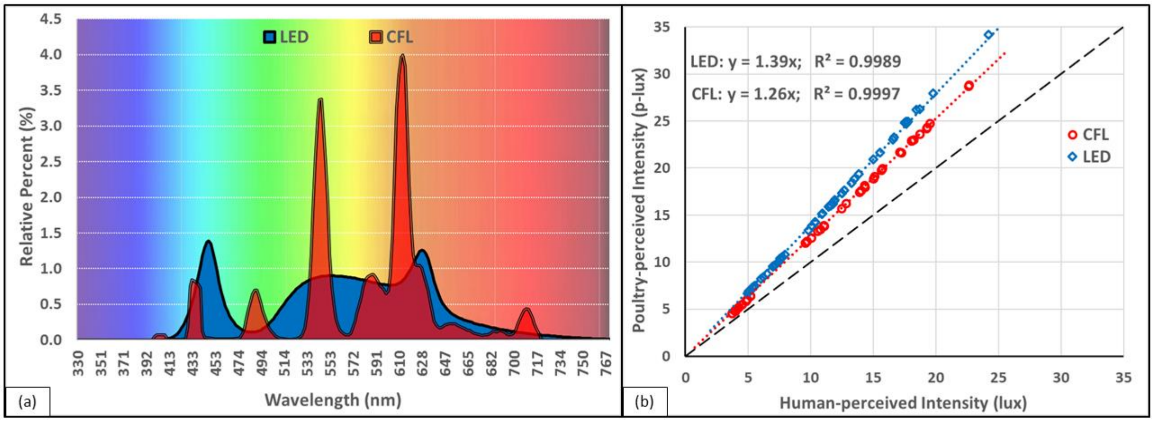

Figure 2. Spectral profiles (a) and relationship between poultry-perceived intensity and human-perceived intensity (b) for the light-emitting diode (LED) light (dim-to-blue, controllable correlated color temperature or CCT from 4500K to 5300K) and compact fluorescent (CFL) light (soft white, CCT = 2700K) lights used in this study.

Table 1. Lighting program and measured light intensities in the pullet-rearing rooms with the LED light (dim-to-blue, controllable correlated color temperature or CCT from 4500K to 5300K) and CFL light (soft white, CCT = 2700K)

Pullet age (wk)	Recommended intensity (lux)	Daily light period (hr)	CFL rooms		LED rooms	
			Lux[1]	p-lux[2]	lux	p-lux
1	30	20	21-30	26-37	20-29	27-40
2	25	18	17-25	21-31	17-26	23-36
3	20	17	13-18	16-23	12-18	16-25
4	15	16	10-14	13-18	10-15	14-21
5	10	15	7-10	9-13	6-10	8-14
6	7	14	5-7	6-9	5-8	7-11
7	7	13	5-7	6-9	5-8	7-11
8	7	12	5-7	6-9	5-8	7-11
9	7	11	5-7	6-9	5-8	7-11
10-13	7	10	5-7	6-9	5-8	7-11
14	15	10	10-14	13-18	10-15	14-21

[1] lux = human-perceived light intensity.
[2] p-lux = poultry-perceived light intensity.

Data Collection and Processing

Growing Performance

Individual BW of pullets was measured biweekly from day-old to 14 weeks of age (**WOA**) by the farm staff. Mortality was recorded daily and postmortem examination was conducted to determine the cause of death (e.g., injury, disease, etc.). Pullets with apparent injuries in each group were culled by the farm staff and were counted as mortality as well. BWU, BWG, and CMR were then calculated based on the farm records. BWU is expressed as the percent of individual weights that fall within 10% of the flock average (Hy-Line International, 2016). BWG is the difference between two successive BW values. CMR is measured as the percent of total dead and culled birds relative to the initial number of birds placed. Feed intake was not recorded in the study because all the rooms shared the same automated feeder conveyor which could not discern feed use for each individual room.

Activity Levels and Movement Index

Movement Index (**MI**) was used as the behavioral parameter for quantifying activity levels of the pullets in this study. MI was defined as the ratio of cumulative displacement area caused by moving pullets to the entire floor area at 1-s intervals. Although not identical definition, the principle and calculation procedure of MI was analogous to activity index described in two other studies (Aydin *et al.*, 2010; Costa *et al.*, 2014). During 5 to 14 WOA, locomotion behaviors of pullets in each rearing room were intermittently recorded (one day per WOA) using four digital cameras (720P HD, night vision, Backstreet Surveillance Inc., UT, USA) at 5 frames per second (missing video data due to system failure for the earlier part of the second batch, i.e., 5 to 8 WOA). Video analysis was implemented to calculate time-series MI of the pullets using automated image processing programs developed in

MATLAB (MATLAB R2014b, The MathWorks, Inc., Natick, MA, USA). Implementation of the image processing procedure is illustrated in Figure 3. *I(f)* and *I(f-1)* are two consecutive image frames captured at 0.2-s intervals. Subtracting the current frame *I(f)* (Fig. 3a) by the previous frame *I(f-1)* (Fig. 3b) yields the difference (Fig. 3c) between the two frames. The difference image is then converted to a binary image (Fig. 3d), where the white pixels correspond to movements of pullets. To minimize the noises and random errors derived from video recording procedures, MI values over 1-min interval was averaged to obtain mean MI (**MMI**). Three different parts of the day, i.e., early (the first hour of light-on), middle (1000-1100 h), and late part (the last hour of light-on), were chosen for comparing activity levels between the lighting regimens, covering 60 time-series MMI measures per part of the day.

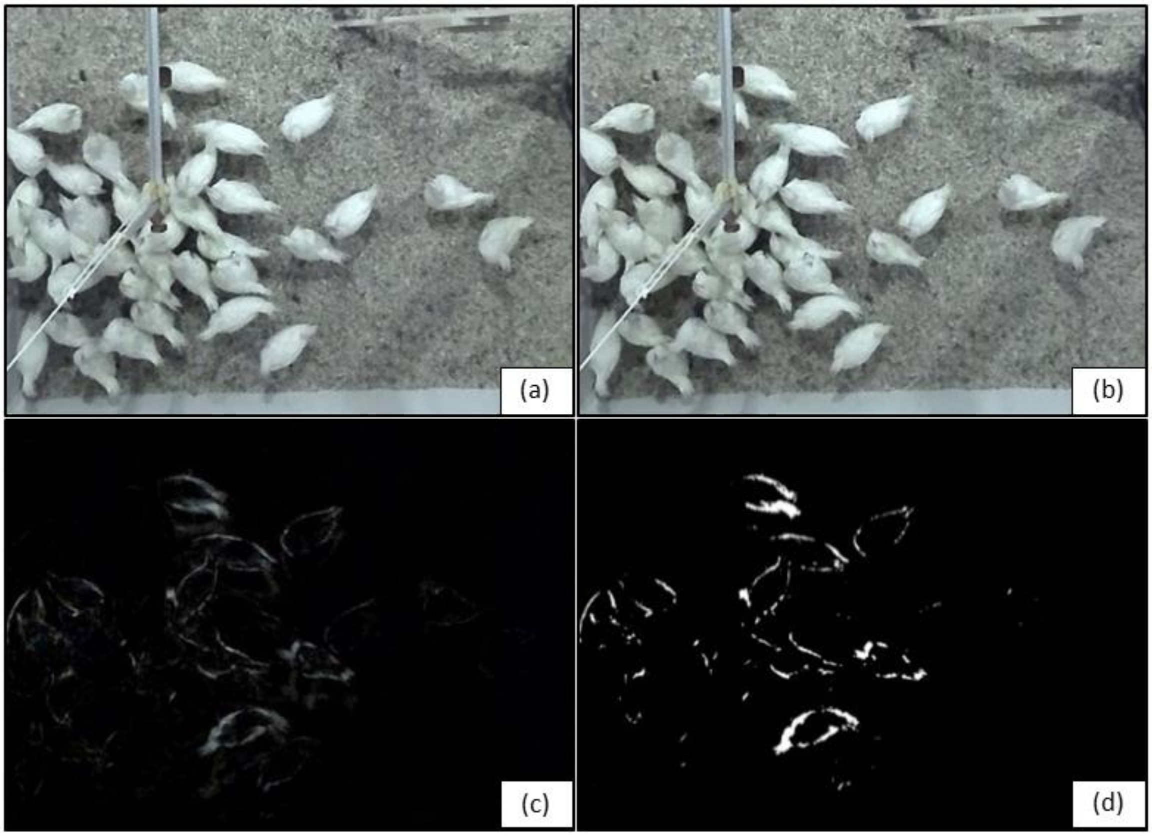

Figure 3. (a) Current image frame *I(t)*, (b) previous image frame *I(t-1)*, (c) grey-scale differential between *I(t)* and *I(t-1)*, (d) binary differential.

Feather and Comb Conditions

Feather and comb conditions of pullets were visually examined biweekly by the farm staff during the weighing procedures to observe any feather damage or comb wound. At the end of the rearing period (16 WOA), 60 pullets from each rearing room were randomly selected and transferred to our animal laboratory at Iowa State University (farm visit was restricted due to the high pathogenic avian influenza risk), where feather and comb conditions of the pullets were assessed according to the Welfare Quality Assessment Protocols (Welfare Quality, 2009). Per this protocol, feather conditions were scored independently on a 3-point scale (i.e., a = no or slight wear, b = moderate wear, featherless area < 5 cm in diameter at the largest extent; c = featherless area ≥ 5 cm) on three body parts, including neck/head, back/rump, and belly. An overall score (0, 1 or 2) for each pullet was then determined based on the scores of her three individual body parts (i.e., 0 = all body parts scored “a”; 1 = at least one part scored “b” but no “c” score; 2 = at least one part scored “c”). Comb conditions were scored on a 3-point scale as well (i.e., 0 = no evidence of pecking wounds; 1 = less than three pecking wounds; 2 = three or more pecking wounds).

Statistical Analysis

All statistical analyses were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, NC, USA) with the MIXED procedure. As the experiment followed the split-plot experimental design, the rearing room was treated as the experimental unit although some observations (i.e., BW and BWG) were made on individual pullets, thus leading to four replicates per light regimen. BW, BWU, BWG, and CMR were analyzed separately for each bird age (week 0, 2, 4, …, 14) using a linear mixed model expressed as:

$$Y_{ijk} = \mu + L_i + B_j + R(B)_{jk} + e_{ijk}$$

Where Y_{ijk} denotes the independent observation for light regiment i in room k of batch j; μ is the overall mean; L_i is the fixed light effect; B_j is the fixed batch effect; $R(B)_{jk}$ is the random effect of room within batch, $R(B)_{jk} \sim N(0, \sigma_R^2)$; and e_{ijk} is the random error, $e_{ijk} \sim N(0, \sigma^2)$. Likewise, MMI of pullets was also analyzed separately for each bird age (week 5, 6, 7, …, 14) using a linear mixed model expressed as:

$$Y_{ijkd} = \mu + L_i + B_j + R(B)_{jk} + P_d + (LP)_{id} + e_{ijkd}$$

Where Y_{ijkd} denotes the independent observation for light regiment i in room k of batch *j at part d of the day*; μ is the overall mean; L_i is the fixed light effect; B_j is the fixed batch effect; $R(B)_{jk}$ is the random effect of room within batch, $R(B)_{jk} \sim N(0, \sigma_R^2)$; P_d is the fixed effect of part of the day; $(LP)_{id}$ is the fixed interaction effect of light and part of the day; and e_{ijkd} is the random error, $e_{ijkd} \sim N(0, \sigma^2)$. For all models, Tukey-Kramer tests were used for pairwise comparisons if applicable. Normality and homogeneity of variance of data were examined by residual diagnostics. Effects were considered significant at $p < 0.05$. Unless otherwise specified, data are presented as least squares means along with SEM.

Results

Growing Performance of Pullets

As illustrated in Figures 4 and 5, all the growing performance parameters (BW, BWU, BWG, and CMR) were highly comparable between the two light regimens at any age throughout the 14-week rearing period ($p > 0.05$), with the exception that pullets under the LED light had higher BWG than pullets under the CFL light at 10 to 12 WOA (153 ± 1 g *vs*. 141 ± 1 g, $p < 0.001$). At 14 WOA, pullets under the LED light had BW of 1140 ± 5 g, BWU of 90.8 ± 1.0%, and CMR of 1.3 ± 0.6% compared with 1135 ± 5 g, 91.9 ± 1.0%, and 2.7 ± 0.6% for pullets under the CFL light, respectively (p = 0.41, 0.48, and 0.18 for BW, BWU, and CMR, respectively).

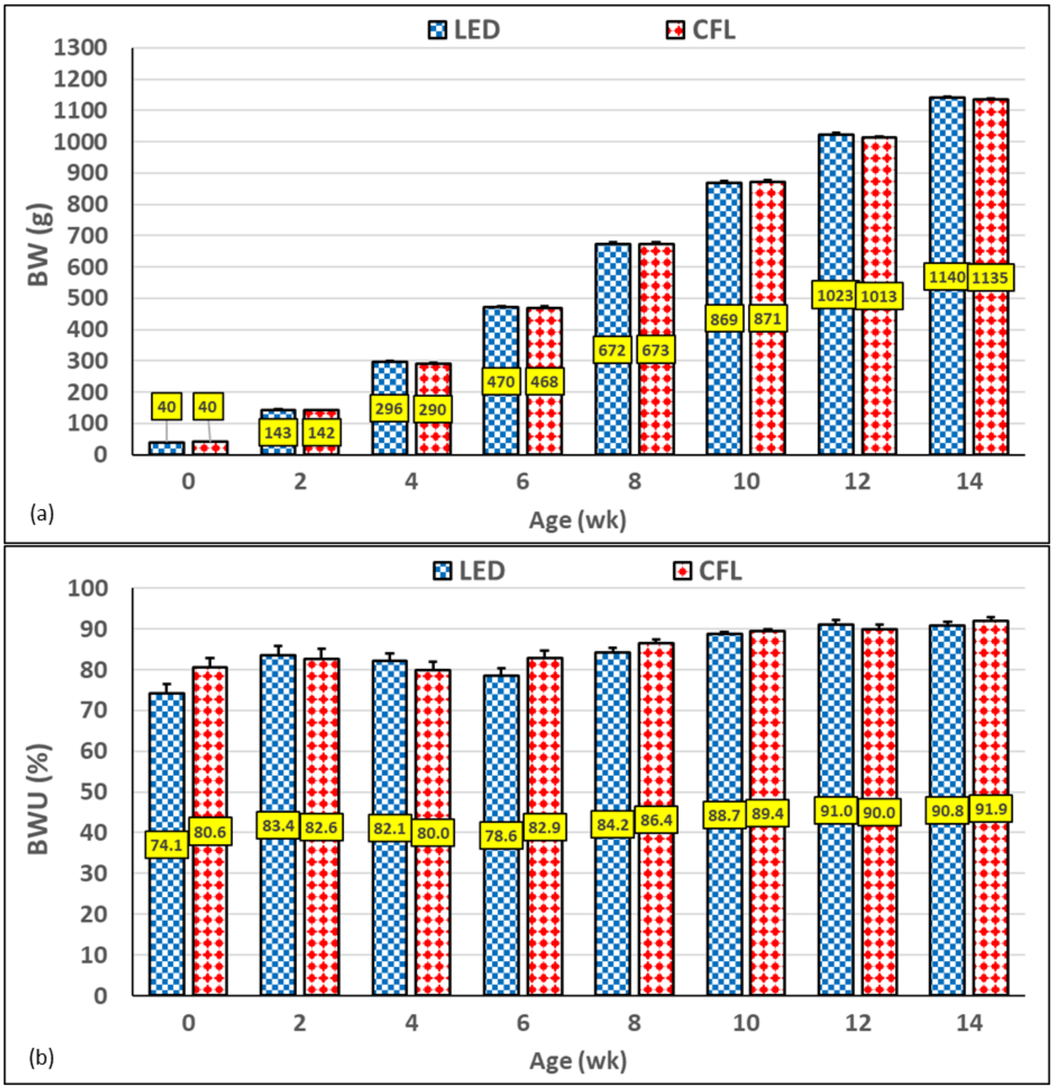

Figure 4. (a) BW and (b) BW uniformity (BWU) of W-36 pullets under the light-emitting diode (LED) light *vs.* the compact fluorescent (CFL) light. BWU is expressed as the percent of individual weights that fall within 10% of the flock average. Values are given as least squares means ± SEM; n = 4 per light regimen. At each age, values were significantly different between lights as indicated by *, **, and *** for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

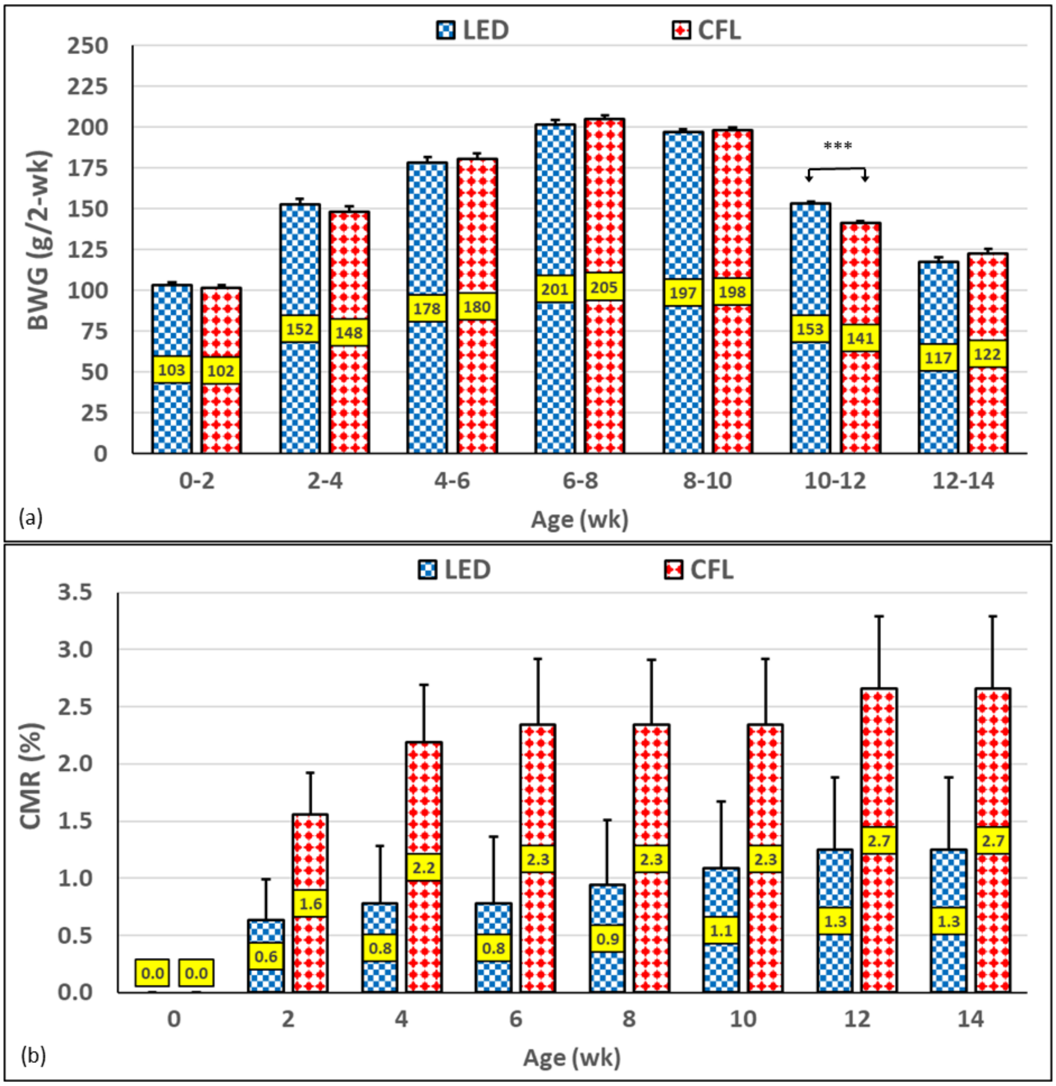

Figure 5. (a) BW gain (BWG) and (b) cumulative mortality rate (CMR) of W-36 pullets under the light-emitting diode (LED) light *vs.* the compact fluorescent (CFL) light. Values are given as least squares means ± SEM; n = 4 per light regimen. At each age, values were significantly different between lights as indicated by *, **, and *** for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Activity Levels of Pullets

In general, the light regimens had significant impacts on activity levels of the pullets (Table 2). Specifically, pullets had significantly larger MMI under the LED light than under the CFL light at 6 ($p < 0.01$), 7 ($p = 0.04$), 8 ($p = 0.05$), 9 ($p < 0.01$), 10 ($p = 0.02$), and 12 ($p < 0.01$) WOA. No significant difference was detected in MMI for pullets under the LED light *vs.* CFL light at any other age ($p > 0.05$). Part or time of the day showed consistently considerable influence on activity levels of pullets (Table 2), in that MMI was significantly greater during the early part of the day than during the middle and/or late parts of the day ($p < 0.05$). No interaction effect was detected between light regimen and the part of the day ($p > 0.05$).

Table 2. Mean Movement Index of W-36 pullets as affected by light regimen (light-emitting diode or LED light and compact fluorescent or CFL light) and part of the day

Age (wk)	Part of the day (P)				Light (L)				p-value		
	Early	Middle	Late	SEM	LED	CFL	SEM	RSD	P	L	P x L
5	13.2^{a}	10.3^{b}	12.7^{a}	0.3	12.2	12.1	0.3	0.5	<0.01	0.97	0.68
6	8.9^{a}	6.6^{b}	8.0^{a}	0.3	10.5^{a}	5.2^{b}	0.3	0.4	<0.01	<0.01	0.35
7	9.6^{a}	6.6^{b}	8.3^{ab}	0.5	10.0^{a}	6.3^{b}	0.6	0.7	<0.01	0.04	0.12
8	11.7^{a}	9.6^{b}	8.8^{b}	0.7	12.7^{a}	7.4^{b}	0.9	1.0	<0.01	0.05	0.45
9	9.0^{a}	6.3^{c}	8.0^{b}	0.5	9.6^{a}	6.0^{b}	0.7	1.2	<0.001	<0.01	0.14
10	10.2^{a}	7.7^{b}	8.6^{b}	0.4	9.8^{a}	7.8^{b}	0.4	0.9	<0.001	0.02	0.31
11	9.3^{a}	7.8^{b}	8.0^{b}	0.5	9.1	7.6	0.6	1.1	<0.01	0.13	0.26
12	10.5^{a}	9.3^{ab}	8.8^{b}	0.4	11.0^{a}	8.0^{b}	0.3	1.0	0.04	<0.01	0.66
13	9.7^{a}	8.9^{ab}	8.5^{b}	0.4	9.7	8.3	0.5	1.1	0.04	0.12	0.94
14	11.8^{a}	9.6^{b}	10.1^{b}	0.9	12.2	8.8	1.3	2.1	<0.01	0.12	0.46

Values are given as least squares means; n=2 for 5-8 weeks of age (WOA), n = 4 for 9-14 WOA. Differences between lights or parts of the day were considered significantly at $p < 0.05$. Row means among three parts of the day or between two lights with different superscript letters are significantly different at $p < 0.05$.

Feather and Comb Conditions of Pullets

Very limited detectable feather damages or comb wounds were observed among the pullets during the weighing process (reported by the farm staff). The exceptions were the eight pullets that were culled due to apparent pecking injuries on the rump or back. Among these eight culled pullets, three pullets were culled from the LED rooms and the reaming five were from the CFL rooms. For the randomly selected pullets at 16 WOA (60 pullets per room, 480 pullets in total), both feather and comb conditions were scored 0 for all pullets according to the previously described protocol. Therefore, feather and comb conditions were not further compared between the light regimens.

Discussion

To the best of our knowledge, this is the first study to compare the effects of a poultry-specific dim-to-blue LED light with a typical CFL light on growing performance, activity levels, and feather and comb conditions of non-beak-trimmed W-36 pullets. The primary interest was to investigate if the dim-to-blue LED light could improve growing performance, calm the birds, and/or enhance feather and comb conditions of pullets as compared to the typical CFL light.

Effects of Light Sources on Growing Performance of Pullets

The dim-to-blue LED and the CFL lights used in the study had distinctly different spectral characteristics. However, pullets under these two light regimens had comparable BW and BWU throughout the rearing period. These results, to some extent, implied that the impact of spectral characteristics of the light sources might be secondary or negligible on the growth performance of pullets. This inference seems to be supported by results of earlier studies. Schumaier *et al.*, (1968) found that pullets reared under red, green, and white fluorescent lights had comparable BW at 20 WOA, regardless of their beak conditions (debeaked or intact beak). Pyrzak *et al.*, (1986) reported that pullets reared under cool white fluorescent light, sunlight-simulating fluorescent light, and narrow-band blue, green, and red fluorescent lights had comparable BW at 16 and 20 WOA. Likewise, Baxter *et al.* (2014) reported that pullets reared under red, green, or white LED light had comparable BW until the sexual maturity at 23 WOA. Coincidently, consistent results have also been reported from lighting studies on broilers. Huth and Archer (2015) reported no effects of light sources on broiler growth in a study comparing broiler performance among a dim-to-blue LED light (same LED light as in the current study), a “NextGen” poultry specific LED light (3500K), and a dimmable CFL light (2700K). Olanrewaju *et al.* (2016) assessed effects of a cool poultry specific filtered LED light (5000K), a neutral LED light (3500K), a typical CFL light (2700K), and an incandescent light (2010K) on broiler growth and reported no light effects either. In addition, Yang *et al.* (2016) investigated the effects of monochromatic LED lights (e.g., white, yellow, green, red, and blue LED lights) on broiler growth and found broilers under yellow, green, and blue LED lights had similar growth performance. In contrast, a couple of studies reported opposite results that blue lights were found to improve growth of broilers as compared with white and red lights (Rozenboim *et al.*, 2004; Cao *et al.*, 2008). Although the authors attributed this difference in growth to the difference in perceived light intensities by broilers, the underlying mechanisms were not clearly delineated in these studies. It should be noted that broilers have been genetically selected for faster growth, whereas pullets are selected for lighter BW and improved skeleton integrity (Bessei, 2006). As such, pullets and broilers may have different growth responses to light regimens.

Pullets under the LED and CFL lights had comparable CMR throughout the rearing period in the current study (culled pullets were counted as mortality). Similar finding was reported by an earlier study in that mortality of pullets till 20 WOA was not affected by light treatments when reared under red, green, or white fluorescent light, regardless of their beak conditions (intact beak or debeaked) (Schumaier *et al.*, 1968). A long-term field study with commercial aviary hen houses revealed no difference in mortality rate of DeKalb white hens between a commercial LED light and a CFL light (Long *et al.*, 2016). Mortality of broilers was also not influenced by white incandescent, blue, green, yellow, or red fluorescent light

(Wabeck and Skoglund, 1974). However, mortality of both laying hens and broilers were greatly influenced by photoperiod (Lewis *et al.*, 1996). As a result, it is reasonable to infer that light sources would have slight or unnoticeable impact on the mortality of pullets. It should be cautioned that the current study involved rather small flock size (160 pullets per flock), and as such the outcome may change in large commercial flocks.

Effects of Light Sources on Activity Levels of Pullets

No existing literature was found regarding the activity levels of pullets under different light sources. As a result, activity levels of pullets in the present study were mainly discussed and compared with research findings from broilers. Prayitno *et al.* (1997) investigated the effects of red, blue, green, and white lights on the behavior of broilers and found that broilers in red light spent more time in aggressive interaction, pecking at the floor, and wing stretching as compared to birds in green and blue lights. Broilers were also found to have the greatest walking activity in white light but the least walking activity in green light (Prayitno *et al.*, 1997). Sultana *et al.* (2013) found that broilers decreased movement and increased sitting under short-wavelength light (e.g., blue, green-blue) and performed more physical movement and fear responses under long-wavelength light (e.g., red). In addition, broilers were found to be more active when exposed to fluorescent light and red LED light than exposed to blue LED light (Santana *et al.*, 2016). For all those cited studies, the underlying mechanisms were not clearly delineated, except that the authors once again attributed the differences in the bird behaviors or activity levels to differences in perceived light intensities. Activity levels of birds are known to be positively correlated to light intensity (Boshouwers and Nicaise, 1993; Deep *et al.*, 2012). Birds have been demonstrated to have much higher spectral sensitivity for long-wavelength light (e.g., yellow, red-yellow) than for short-wavelength light (e.g., blue, green-blue) (Prescott *et al.*, 2003; Saunders *et al.*, 2008). Thus the light intensity perceived by broilers under the pure red lights or white lights would be higher than those under the pure blue or green lights in these cited studies. However, results from the current study did not parallel the findings of the cited studies on broilers. In the current study, pullets under the dim-to-blue LED light had significantly higher activity levels compared to their counterparts under the CFL light. Light intensities for both LED and CFL rooms in the study were set according to Hy-Line Commercial Layers Management Guideline, adjusted based on human-perceived light intensity (lux). Although both the dim-to-blue LED light and the CFL light had full-spectral wavelength outputs, the LED light and the CFL light had distinct spectral profiles as described earlier. Consequently, the light intensities perceived by the pullets (p-lux) presumably differed between the LED and CFL regimens (8-14 *vs.* 7-13 p-lux at 5 WOA, 7-11 *vs.* 6-9 p-lux at 6-13 WOA, and 14-21 *vs.* 13-18 p-lux at 14 WOA). Albeit being considerably low in magnitude, the difference (1-3 p-lux) in light intensities between the two light regimens might have been enough to cause behavioral difference (e.g., higher activity levels under the LED) as found in those broiler studies. This different result, as compared to those with broilers, might also have arisen from physiological differences (e.g., BW, skeleton development, and bone strength) between pullets and broilers (Bessei, 2006) in that broilers have a high incidence of skeletal disorders due to the selection for fast early growth rate and consequently a low locomotor activity.

Effects of Lights Sources on Feather and Comb Conditions of Pullets

Schumaier *et al.* (1968) found that pullets reared under green and white lights lost most of their tail feathers during the rearing period, whereas pullets reared under red lights showed no apparent signs of feather damage. The authors reported that feather picking occurred spontaneously among the pullets reared under green and white lights at 12 WOA without apparent causes. de Haas *et al.* (2014) assessed risk factors for feather damage during laying period and found that the prevalence of severe feather pecking during the rearing period averaged 60% (between 37% and 66%) in commercial flocks. In the current study, very limited detectable feather damages or comb wounds were observed among the pullets under both light regimens, even though the pullets were not beak-trimmed. This result was in agreement with the conclusion from a recently published review on the development of feather pecking in commercial systems (Nicol *et al.*, 2013), namely, feather damage does not usually occur during the rearing period although gentle feather pecking is commonly observed and could start from as early as day-old. However, Nicol *et al.* (2013) also pointed out that low rates of feather pecking or slight feather damage during rearing present a significant risk for late feather pecking during laying period. In the current study, eight pullets were culled from the rearing rooms due to apparent pecking injures, indicating potential risk of severe feather pecking among the pullets. In addition, all the injuries on the culled pullets occurred at the rump or back, which is consistent with the finding by de Haas *et al.* (2014) who reported that the feather damage during rearing was limited to damage to the back of pullets.

During feather assessment in the current study, slight feather wears or damages were observed among the pullets. However, feather condition was scored 0 for all pullets per the protocol (Welfare Quality, 2009), as it has limitation in assessing slight feather damages (established for assessing laying hens). This limitation made it impossible to further compare feather conditions of pullets between the two light regimens. de Haas *et al.* (2014) improved the compatibility of this protocol by including cuts in the wings and tails as an indication of early feather damage (ab score), thus successfully quantified slight feather damages for pullets at 5, 10, and 15 WOA. Advanced sensing technologies are increasingly developed and adopted in modern animal production systems. New techniques, such as infrared thermography (Zhao *et al.*, 2013), can help improving the sensitivity of feather condition assessment because surface temperature and distribution of birds are closely related to their feather thickness and feather coverage.

Conclusions

Effects of a commercial poultry-specific dim-to-blue LED light *vs.* a typical CFL light on non-beak-trimmed W-36 pullets were evaluated with regards to growing performance (BW, BW uniformity or BWU, BW gain or BWG, and cumulative mortality rate or CMR), activity levels, and feather and comb conditions. Both the LED and CFL lights led to comparable pullet performance of BW, BWU and CMR by the end of 14-week rearing period, although varying BWG occurred during the intermediate period. Overall, the LED light showed an effect of stimulating locomotion activities of the pullets as compared to the CFL light, which might have stemmed from differences in spectrum and/or intensity between the two lights. In general, both lights had similar effects on feather and comb conditions of the pullets during the rearing period.

Acknowledgements

Funding for the study was provided in part by the Center for Industrial Research and Service (CIRAS) at Iowa State University and Hy-Line International and is acknowledged. Our special gratitude goes to Hy-Line International Research Farm staff for the immense support and superb collaboration throughout the study. We also wish to thank Once Innovation Inc. for providing the LED lights and the controller used in the study, and the

Agriculture Experiment Station (AES) Consulting Group at Iowa State University for the consistent assistance in statistical consulting for the study. Author Kai Liu also wishes to thank China Scholarship Council for providing part of the financial support for his PhD study at Iowa State University.

References

Aydin, A., Cangar, O., Ozcan, S. E., Bahr, C., & Berckmans, D. (2010). Application of a fully automatic analysis tool to assess the activity of broiler chickens with different gait scores. *Computers and Electronics in Agriculture*, *73*(2), 194–199. https://doi.org/10.1016/j.compag.2010.05.004

Baxter, M., Joseph, N., Osborne, V. R., & Bedecarrats, G. Y. (2014). Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poultry Science*, *93*(5), 1289–1297. https://doi.org/10.3382/ps.2013-03799

BESSEI, W. (2006). Welfare of broilers: a review. *World's Poultry Science Journal*, *62*(3), 455–466. https://doi.org/10.1079/WPS2005108

Boshouwers, F. M. G., & Nicaise, E. (1993). Artificial light sources and their influence on physical activity and energy expenditure of laying hens. *British Poultry Science*, *34*(1), 11–19. https://doi.org/10.1080/00071669308417558

Cao, J., Liu, W., Wang, Z., Xie, D., Jia, L., & Chen, Y. (2008). Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. *The Journal of Applied Poultry Research*, *17*(2), 211–218. https://doi.org/10.3382/japr.2007-00043

Costa, A., Ismayilova, G., Borgonovo, F., Viazzi, S., Berckmans, D., & Guarino, M. (2014). Image-processing technique to measure pig activity in response to climatic variation in a pig barn. *Animal Production Science*, *54*(8), 1075. https://doi.org/10.1071/AN13031 de Haas, E. N., Bolhuis, J. E., de Jong, I. C., Kemp, B., Janczak, A. M., & Rodenburg, T. B. (2014). Predicting feather damage in laying hens during the laying period. Is it the past or is it the present? *Applied Animal Behaviour Science*, *160*(1), 75–85. https://doi.org/10.1016/j.applanim.2014.08.009

Deep, A., Schwean-Lardner, K., Crowe, T. G., Fancher, B. I., & Classen, H. L. (2012). Effect of light intensity on broiler behaviour and diurnal rhythms. *Applied Animal Behaviour Science*, *136*(1), 50–56. https://doi.org/10.1016/j.applanim.2011.11.002

Eich, M. R. de S., Garcia, R. G. G., Naas;, I. de alencar, Caldara;, F. R., Royer;, A. flavia B., & Sgavioli, S. (2016). Behavior of broilers reared under monochromatic and fluorescent light sources. *International Journal of Poultry Science*, *15*(3), 96–102. https://doi.org/10.3923/ijps.2016.96.102

Huth, J. C., & Archer, G. S. (2015). Comparison of two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress, and fear. *Poultry Science*, *94*(9), 2027–2036. https://doi.org/10.3382/ps/pev215

Hy-Line International. (2016). W-36 Commercial Layers Management Guide 2016.

Lanson, R. K., & Sturkie, P. D. (1961). The influence of light and darkness upon the reproductive performance of the fowl: 1. effect of continuous, intermittent, and flashing light on egg production, feed consumption, and body weight. *Poultry Science*, *40*(6), 1751–1756. https://doi.org/10.3382/ps.0401751

Lewis, P. D., & Morris, T. R. (1998). Responses of domestic poultry to various light sources. *World's Poultry Science Journal*, *54*(1), 7–25. https://doi.org/10.1079/WPS19980002

Lewis, P. D., & Morris, T. R. (2000). Poultry and coloured light. *World's Poultry Science Journal*, *56*(3), 189–207. https://doi.org/10.1079/WPS20000015

Lewis, P. D., Morris, T. R., & Perry, G. C. (1996). Lighting and mortality rates in domestic fowl. *British Poultry Science*, *37*(2), 295–300. https://doi.org/10.1080/00071669608417860

Long, H., Zhao, Y., Wang, T., Ning, Z., & Xin, H. (2016). Effect of light-emitting diode vs. fluorescent lighting on laying hens in aviary hen houses: Part 1 – Operational characteristics of lights and production traits of hens. *Poultry Science*, *95*(1), 1–11. https://doi.org/10.3382/ps/pev121

Ma, H., Xin, H., Zhao, Y., Li, B., Shepherd, T. A., & Alvarez, I. (2016). Assessment of lighting needs by W-36 laying hens via preference test. *Animal*, *10*(4), 671–680. https://doi.org/10.1017/S1751731115002384

Min, J. K., Hossan, M. S., Nazma, A., Jae, C. N., Han, T. B., Hwan, K. K., Ok, S. S. (2012). Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biology Research*, *5*(2), 69–74. https://doi.org/10.3184/175815512X13350270679453

Mobarkey, N., Avital, N., Heiblum, R., & Rozenboim, I. (2010). The role of retinal and extra-retinal photostimulation in reproductive activity in broiler breeder hens. *Domestic Animal Endocrinology*, *38*(4), 235–243. https://doi.org/10.1016/j.domaniend.2009.11.002

Morris, T. R., & Fox, S. (1960). The use of lights to delay sexual maturity in pullets. *British Poultry Science*, *1*(1–3), 25–36. https://doi.org/10.1080/00071666009382375

Nicol, C. J., Bestman, M., Gilani, A.-M., De Haas, E. N., De Jong, I. C., Lambton, S.,Rodenburg, T. B. (2013). The prevention and control of feather pecking: application to commercial systems. *World's Poultry Science Journal*, *69*(4), 775–788. https://doi.org/10.1017/S0043933913000809

Olanrewaju, H. A., Miller, W. W., Maslin, W. R., Collier, S. D., Purswell, J. L., & Branton, S. L. (2016). Effects of light sources and intensity on broilers grown to heavy weights. Part 1: Growth performance, carcass characteristics, and welfare indices. *Poultry Science*, *95*(4), 727–735. https://doi.org/10.3382/ps/pev360

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. https://doi.org/10.1016/j.visres.2008.06.018

Parvin, R., Mushtaq, M. M. H., Kim, M. J., & Choi, H. C. (2014). Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. *World's Poultry Science Journal*, *70*(3), 543–556. https://doi.org/10.1017/S0043933914000592

Prayitno, D. S., Phillips, C. J., & Omed, H. (1997). The effects of color of lighting on the behavior and production of meat chickens. *Poultry Science*, *76*(3), 452–7.

Prescott, N. B., & Wathes, C. M. (2002). Preference and motivation of laying hens to eat under different illuminances and the effect of illuminance on eating behaviour. *British Poultry Science*, *43*(2), 190–195. https://doi.org/10.1080/00071660120121382

Prescott, N. B., Wathes, C. M., & Jarvis, J. R. (2003). Light, vision and the welfare of poultry. *Animal Welfare*, *12*(2), 269–288.

Pyrzak, R., Snapir, N., Goodman, G., Arnon, E., & Perek, M. (1986). The influence of light quality on initiation of egg laying by hens. *Poultry Science*, *65*(1), 190–193. https://doi.org/10.3382/ps.0650190

Rozenboim, I., Biran, I., Chaiseha, Y., Yahav, S., Rosenstrauch, A., Sklan, D., & Halevy, O. (2004). The effect of a green and blue monochromatic light combination on broiler growth and development. *Poultry Science*, *83*(5), 842–845. https://doi.org/10.1093/ps/83.5.842

Saunders, J. E., Jarvis, J. R., & Wathes, C. M. (2008). Calculating luminous flux and lighting levels for domesticated mammals and birds. *Animal*, *2*(6), 921–932. https://doi.org/10.1017/S1751731108002012

Schumaier, G., Harrison, P. C., & McGinnis, J. (1968). Effect of colored fluorescent light on growth, cannibalism, and subsequent egg production of single comb white leghorn pullets. *Poultry Science*, *47*(5), 1599–1602. https://doi.org/10.3382/ps.0471599

Smith, R. E., & Noles, R. K. (1963). Effects of varying daylengths on laying hen production rates and annual eggs. *Poultry Science*, *42*(4), 973–982. https://doi.org/10.3382/ps.0420973

Sultana, S., Hassan, M. R., Choe, H. S., & Ryu, K. S. (2013). The effect of monochromatic and mixed LED light colour on the behaviour and fear responses of broiler chicken. *Avian Biology Research*, *6*(3), 207–214. https://doi.org/10.3184/175815513X13739879772128

Wabeck, C. J., & Skoglund, W. C. (1974). Influence of radiant energy from fluorescent light sources on growth, mortality, and feed conversion of broilers. *Poultry Science*, *53*(6), 2055–2059. https://doi.org/10.3382/ps.0532055

Welfare Quality. (2009). Welfare Quality® assessment protocol for poultry (broilers, laying hens). *Welfare Quality® Consortium, Lelystad, Netherlands*, 21–59. Retrieved from www.welfarequality.net

Xie, D., Wang, Z. X., Dong, Y. L., Cao, J., Wang, J. F., Chen, J. L., & Chen, Y. X. (2008). Effects of monochromatic light on immune response of broilers. *Poultry Science*, *87*(8), 1535–1539. https://doi.org/10.3382/ps.2007-00317

Yang, Y., Jiang, J., Wang, Y., Liu, K., Yu, Y., Pan, J., & Ying, Y. (2016). Light-emitting diode spectral sensitivity relationship with growth, feed intake, meat, and manure characteristics in broilers. *Transactions of the ASABE*, *59*(5), 1361–1370. https://doi.org/10.13031/trans.59.11325

Zappia, J. V., & Rogers, L. J. (1983). Light experience during development affects asymmetry of forebrain function in chickens. *Developmental Brain Research*, *11*(1), 93–106. https://doi.org/10.1016/0165-3806(83)90204-3

Zhao, Y., Xin, H., & Dong, B. (2013). Use of infrared thermography to assess laying-hen feather coverage. *Poultry Science*, *92*(2), 295–302. https://doi.org/10.3382/ps.2012-02629

CHAPTER 5

CHOICE BETWEEN FLUORESCENT AND POULTRY-SPECIFIC LED LIGHTS BY PULLETS AND LAYING HENS

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A paper published in *Transactions of the ASABE* 60(6):in press

Abstract

Light plays an important role in poultry development, production performance, health, and well-being. Light technology continues to advance and accordingly new light products are finding applications in poultry operations. However, research concerning responses of young and adult laying hens to light sources is relatively lacking. This study assessed the choice between a Dim-to-Red® poultry-specific light emitting diode (LED) light (PS-LED, correlated color temperature or CCT = 2000K) and a warm-white fluorescent light (FL, CCT = 2700K) by pullets and laying hens (W-36 breed) via preference test. Birds with different prior lighting experiences were evaluated for the light choice, including a) pullets (14-16 weeks of age or WOA) reared under incandescent light (designated as P_{INC}), b) layers (44-50 WOA) under PS-LED (L_{LED}) throughout pullet and laying phases, and c) layers under FL (L_{FL}) throughout pullet and laying phases. Each bird category consisted of 12 replicates, three birds per replicate. Each replicate involved a 6-day preference test, during which the birds could move freely between two inter-connected compartments that contained PS-LED and FL, respectively. Time spent and feed intake by the birds under each light were measured and then analyzed with generalized linear mixed models. Results showed that regardless of prior lighting experience, birds in all cases showed stronger choice for FL (p = 0.001-0.030), as evidenced by higher proportions of time spent under it. Specifically, the proportion of time spent (mean ± SEM) under FL *vs.* PS-LED was 58.0 ± 2.9% *vs.* 42.0 ± 2.9% for P_{INC}, 53.7 ± 1.6% *vs.* 46.3 ± 1.6% for L_{LED}, and 54.2 ± 1.2% *vs.* 45.8 ± 1.2% for L_{FL}. However, the proportions of daily feed intake occurring under FL and PS-LED were comparable in all cases (p = 0.419-0.749). The study thus reveals that prior lighting experience of the pullets or layers did not affect their choice of the FL *vs.* PS-LED. While the birds exhibit a somewhat stronger choice for the FL, this tendency did not translate to differences in the proportion of feed use under each light type.

Keywords: Preference assessment, Computer vision, Behavior and welfare, Poultry Lighting

Nomenclature	
LED	Light emitting diode
PS-LED	Poultry-specific LED light
CCT	Correlated color temperature
FL	Fluorescent light
WOA	Week(s) of age
P_{INC}	Pullets reared under incandescent light
L_{LED}	Layers under PS-LED throughout pullet and laying phases
L_{FL}	Layers under FL throughout pullet and laying phases
UV	Ultraviolet
HPS	High pressure sodium
CFL	Compact fluorescent light
CCFL	Cold cathode fluorescent light
CV	Coefficient of variation
LPTC	Light preference test compartments
p-lux	Poultry-perceived light intensity; lux
RH	Relative humidity; %
FPS	Frame per second
PDFI	Proportion of daily feed intake under the PS-LED or the FL; %
LMF	Light-period moving frequency of birds between lights; times $bird^{-1}$ h^{-1}
PLTS	Proportion of light-period time spent under the PS-LED or the FL; %
L3F0	Proportion of the light period with all three birds under the PS-LED; %
L2F1	Proportion of the light period with two birds under the PS-LED and one bird under the FL; %
L1F2	Proportion of the light period with one bird under the PS-LED and two birds under the FL; %
L0F3	Proportion of the light period with all three birds under the FL; %
SEM	Standard error of the mean

Introduction

Light plays an important role in behavior, development, production performance, health, and well-being of poultry (Manser, 1996; Lewis and Morris, 2000; Olanrewaju *et al.*, 2006; Rajchard, 2009; Lewis, 2010). As such, extensive research on poultry lighting has been conducted over the past eight decades, leading to establishment of general guidelines on photoperiod and light intensity for improved animal performance and energy efficiency (ASABE Standards, 2014). As light technology continues to advance, new light products (animal- or production stage-specific lights) constantly emerge and some are increasingly finding applications in animal operations. However, controlled comparative research is relatively limited regarding the behavioral and performance responses of animals, especially pullets (young hens before lay) and laying hens, to the emerging lights.

Poultry have a different light spectral sensitivity compared to humans (Prescott and Wathes, 1999; Prescott *et al.*, 2003; Saunders *et al.*, 2008). In particular, poultry have five types of retinal cone photoreceptors that are sensitive to ultraviolet (UV), short-, medium-, and long-wavelength radiation (Osorio and Vorobyev, 2008), and can perceive light not only through their retinal cone photoreceptors in the eyes, but via extra-retinal photoreceptors in the brain (e.g., pineal gland and hypothalamic gland) (Mobarkey *et al.*, 2010). It has been demonstrated that retinal cone photoreceptors produce the perception of light colors by receiving lights at the peak sensitivities of approximately 415, 450, 550, and 700 nm; and that they are more related to poultry activities (e.g., feeding, drinking, and locomotion) and growth. However, the extra-retinal photoreceptors can only be activated by long-wavelength radiation (e.g., yellow-red and red) that can penetrate the skull and deep tissue of poultry, and impacts the sexual development and maturity (Lewis and Morris, 2000). Because different lighting sources (e.g., incandescent, high pressure sodium or HPS, fluorescent, and light emitting diode or LED lights) have different spectral characteristics, retinal and extra-retinal photoreceptors of birds may be stimulated differently when exposed to different lighting sources, thus causing different impacts on the animals. For example, research found that red light was associated with sexual development and maturity of pullets (Harrison *et al.*, 1969; Gongruttananun, 2011; Min *et al.*, 2012; Baxter and Joseph, 2014; Li *et al.*, 2014), while blue light was associated with improving broiler growth, calming the birds (albeit no delineation of the underlying mechanism), and enhancing the immune response (Prayitno *et al.*, 1997; Rozenboim *et al.*, 2004; Cao *et al.*, 2008; Xie *et al.*, 2008; Sultana *et al.*, 2013).

A lighting study investigating broilers reported that a Dim-to-Blue® poultry-specific LED light (correlated color temperature or CCT = 5000K) and a NextGen® poultry-specific LED light (CCT = 3500K) resulted in better well-being (better plumage, hock, and/or footpad conditions) and improved production (better feed conversion) when compared to a daylight compact fluorescent light (CFL, CCT = 5000K) (Huth and Archer, 2015). No explanation was provided regarding the underlying mechanism for the improvement. In contrast, another study reported no differences in growth, feed intake, feed conversion, mortality, ocular development or immune response of broilers reared under the same two types of LED lights, an incandescent light (CCT = 2010K), and a warm-white CFL (CCT = 2700K) (Olanrewaju *et al.*, 2016). Another recent lighting study revealed that the Dim-to-Blue® poultry-specific LED light and the warm-white CFL led to comparable W-36 pullet performance of body weight, body weight uniformity, and mortality (Liu *et al.*, 2017). Similarly, when applying a Nodark® poultry-specific LED light (CCT = 4100K) and the warm-white fluorescent lights in commercial aviary hen houses, no differences were detected in egg weight, egg production, feed use, mortality rate or egg quality parameters of DeKalb white hens between the two types of light (Long *et al.*, 2016a; 2016b). In addition, a study found that the effects of LED lights on broiler growth were age-related (Yang *et al.*, 2016). These inconsistent results, along with the increasing number of novel lights intended for poultry production, and the increasing focus on animal well-being, make it necessary to further investigate the responses of poultry to lighting conditions. Performance-based studies, such as those reported in the literature, although important and necessary, can be subject to the influence of other factors, such as thermal conditions, nutrition, feeding practices, space allowance, and indoor air quality. On the other hand, behavior-based assessment of the animal responses to light conditions under otherwise uniform environment may provide insights into lighting preference of the animal.

Preference tests investigate instantaneous behavioral responses of animals to various environmental stimuli rather than the long-term physiological impacts, thus they can offer an efficient assessment of animal preferences (Ma *et al.*, 2016). As a result, preference tests have been used extensively in poultry studies assessing different environmental conditions, including floor type (Hughes, 1976), nest box (Appleby *et al.*, 1984; Millam, 1987), perch height and shape (Struelens *et al.*, 2008; Lambe and Scott, 1998), ammonia level (Green, 2008; Kashiha *et al.*, 2014), and various light regimens as cited below. Broilers (Cobb breed) at 1-6 week(s) of age (WOA) were shown to have no preference for white or yellow LED lights at a light intensity of 5 lux (Mendes *et al.*, 2013). Turkeys (BIG6 breed) at 6-13 WOA preferred fluorescent light with supplementary UV radiation at a light intensity of 15 lux (Moinard and Sherwin, 1999). Turkeys (BUT8 breed) at 6-19 WOA were found to spend significantly longer time under a light intensity of 25 lux when given free choice among less than 1, 5, 10, and 25 lux (Sherwin, 1998). Laying hens (Shaver 288 breed) at 24 WOA preferred CFL lighting over incandescent lamps at a light intensity of 12 lux because they spent on average 73.2% of the time under CFL and only 26.8% under incandescent light (Widowski *et al.*, 1992); but did not have a preference for high (≥20,000 Hz) or low (120 Hz) flicker frequency of CFL at 19 WOA (Widowski and Duncan, 1996). Laying hens (Leghorn breed) at 20-23 WOA also had no preference for HPS or incandescent light (Vandenbert and Widowski, 2000). In addition, preference studies on pullets (LSL breed) reared under incandescent light or natural daylight revealed that the early lighting experience of pullets affects their later preference for lights: birds reared under incandescent light showed a preference for incandescent light as compared to birds reared under natural daylight at 14 WOA (Gunnarsson *et al.*, 2008; 2009). Nowadays more energy-efficient, readily-dimmable and long-lasting LED lights are increasingly finding applications in poultry operations. There is anecdotal evidence of some commercial poultry-specific LED lights being advantageous on performance and behavior of poultry over traditional fluorescent lights; however, concrete research data are lacking. Thus it is of socio-economic as well as scientific value to evaluate behavioral responses of poultry to various lighting sources through preference testing.

The objectives of this study were: a) to assess light preference of pullets and layers between a Dim-to-Red® poultry-specific LED light (PS-LED) and a warm-white fluorescent light (FL), and b) to evaluate the potential influence of prior lighting experience on the subsequent preference for light. The results are expected to contribute to improvement of current lighting guidelines on light source for pullet rearing and laying-hen production.

Materials and methods

The study was conducted in an environment-controlled animal research laboratory located at Iowa State University, Ames, Iowa, USA. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC # 3-15-7982-G).

Experiment Birds, Bird Husbandry, and Testing apparatus

Hy-Line W-36 commercial layers were used in this study. A total of 36 pullets and 72 layers were tested for their light preferences. All the birds were non-beak-trimmed, individually identified with wing-bands. The same lighting program based on the Hy-Line Commercial Layer Management Guideline (Hy-Line International, 2016) was followed while the birds were reared or kept under the respective light environments/sources prior to commencement of the preference test. Specifically, the pullets were reared in litter-floor rooms that only used incandescent light, and were randomly selected for the preference test at 14-16 WOA. The layers, transferred from litter-floor rooms as pullets at 16 WOA, were kept in conventional cages that used a Dim-to-Red® PS-LED (AgriShift, JLL, LED, 8 Watt, Once, Inc., Plymouth, MN, USA[14]) or a warm-white FL (MicroBrite MB-801D, cold cathode fluorescent light or CCFL, 8W, Litetronics, Alsip, IL, USA). The layers were randomly selected for the preference test at 44-50 WOA. Half of the layers (36) had been reared under a Dim-to-Blue® PS-LED (Agrishift MLB, LED, 12W, Once, Inc.) in the pullet phase, and the other half had been reared under a warm-white FL (EcoSmart, CFL, 9 W, Eco Smart Lighting Australia Pty Ltd, Sydney, Australia). The characteristics of light sources used in the study

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[14] Mention of product or company name is for presentation clarity and does not imply endorsement by the authors or Iowa State University, nor exclusion of other suitable products.

and their spectral distributions are described in Table 1 and Figure 1, respectively. Therefore, the birds were divided into three categories based on age or production stage and prior-lighting experience, i.e., pullets reared under incandescent light (P_{INC}), layers under PS-LED throughout pullet and laying phases (L_{LED}), and layers under FL throughout pullet and laying phases (L_{FL}). Each category consisted of 12 groups or replicates (experimental units), with three birds per group.

Table 1. Characteristics of the incandescent light, warm-white fluorescent light, Dim-to-Blue® PS-LED[1], and Dim-to-Red® PS-LED used in this study.

Light Type	Power at Full Intensity (W)	Light Output Equivalence to Incandescent (W)	CCT[2] (K)	Flicker Frequency (Hz)	Spectral Distribution
Incandescent light[3]	40	40	2550	None	Continuous spectrum, with increasing contributions at longer wavelengths
Warm-white fluorescent light[4]	8 or 9	40	2700	120	Discrete spectrum, main spectral spikes occur at 545 and 610 nm
Dim-to-Blue® PS-LED	12	100	4550	120	Continuous spectrum, spectral spikes occur at 450 and 630 nm, with a predominant spectral output at 430-460 nm
Dim-to-Red® PS-LED	8	40	2000	120	Continuous spectrum, spectral spikes occur at 450 and 630 nm, with a predominant spectral output at 610-640 nm

[1] PS-LED = poultry-specific LED light. [2] CCT = correlated color temperature. [3] Measures to ban incandescent lamps have been implemented in the European Union, the United States, and many other countries. [4] Fluorescent light refers to both compact fluorescent light (CFL) and cold-cathode fluorescent light (CCFL); CFL (9W) and CCFL (8W) have essentially identical spectral characteristics.

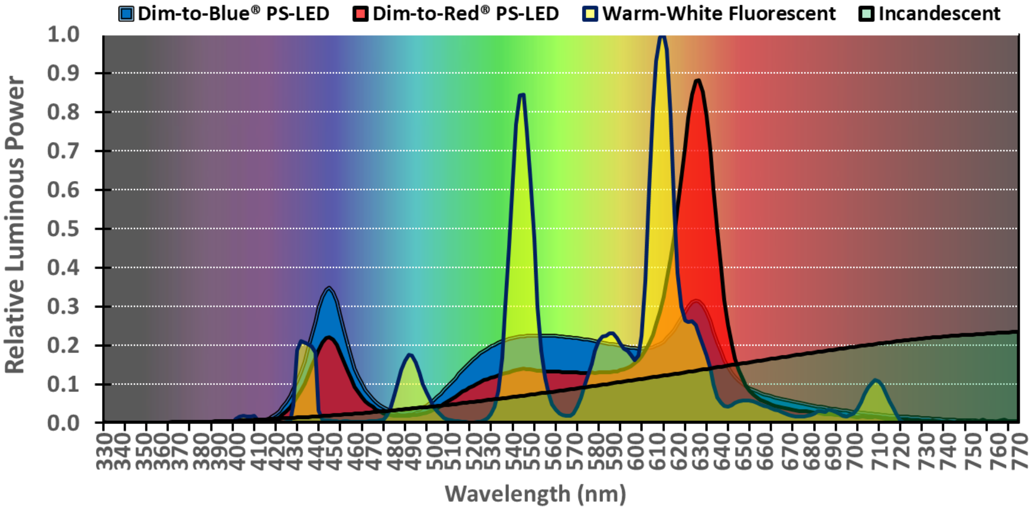


Figure 1. Spectral characteristics of the incandescent light, warm-white fluorescent light, Dim-to-Blue® PS-LED, and Dim-to-Red® PS-LED used in this study. PS-LED = poultry-specific LED light. Fluorescent light refers to both compact fluorescent light (CFL) and cold-cathode fluorescent light (CCFL); CFL and CCFL have essentially identical spectral characteristics.

A light preference test tunnel and an acclimation chamber were used for the study (Fig. 2). The preference test tunnel was modified from an existing system. It consisted of five identical compartments, each measuring 61 × 91 × 198 cm (W×D×H) and containing a 60 × 60 × 90 cm cage and an 18-cm plenum space (35 cm above the cage top). The test tunnel was equipped with mechanical (push-pull) ventilation so that all the compartments were maintained at essentially identical constant temperature of 21ºC throughout the experiment. All inner walls and ceiling of the compartments were covered by white plastic sheets. Each compartment had a rectangular feeder (50 × 15 × 10 cm) outside the front wall and two nipple drinkers (35 cm high) on the back wall of the cage. It also had an access door on the front side of the compartment that allowed the caretakers to refill feeder and collect eggs with minimum disturbance to the birds. The false ceiling of the plenum was made of perforated plastic panel (1.27 cm dia. holes and 48% open area). A light bulb under study was situated on the false ceiling panel of the plenum, pointing upwards. The coefficient of variation (CV) for the light distribution uniformity within the cage was < 8% for all cases based on 16-spot floor-level measurements. The acclimation chamber, measuring 216 × 91 × 150 cm, was used to house two inter-connected cages, each measuring 74 × 64 × 46 cm. The purpose of the acclimation chamber was to train the birds to use the passageway and expose them to the lights under study. Detailed specifications of the test tunnel and the acclimation chamber were given in a previously published article (Ma *et al.*, 2016), including their construction, ventilation system (air duct, inlet and exhaust fans), and egg and manure collection systems.

For the modified test tunnel, two pairs of light preference test compartments (LPTC) were formed by grouping the two adjacent compartments from both ends of the tunnel, with the middle compartment used as a separation space between the two pairs. A rectangular passageway, measuring 20 × 25 cm (W×H), was located at the lower portion (floor to 20 cm high) of the partition wall for each pair of LPTC, allowing birds to move freely between the two inter-connected cages (one bird at a time). As such, two groups of birds could be tested simultaneously in the test tunnel. Feed and water were available ad libitum in all cages. The same amount of feed was added to each feeder before assigning the birds, and refilled daily during the dark period. Eggs were also collected daily during the dark period. At the end of each trial, euthanasia procedures were performed on the test birds according to the IACUC protocol, and manure inside the compartments was removed. The test and acclimation systems were disinfected before the next trial.

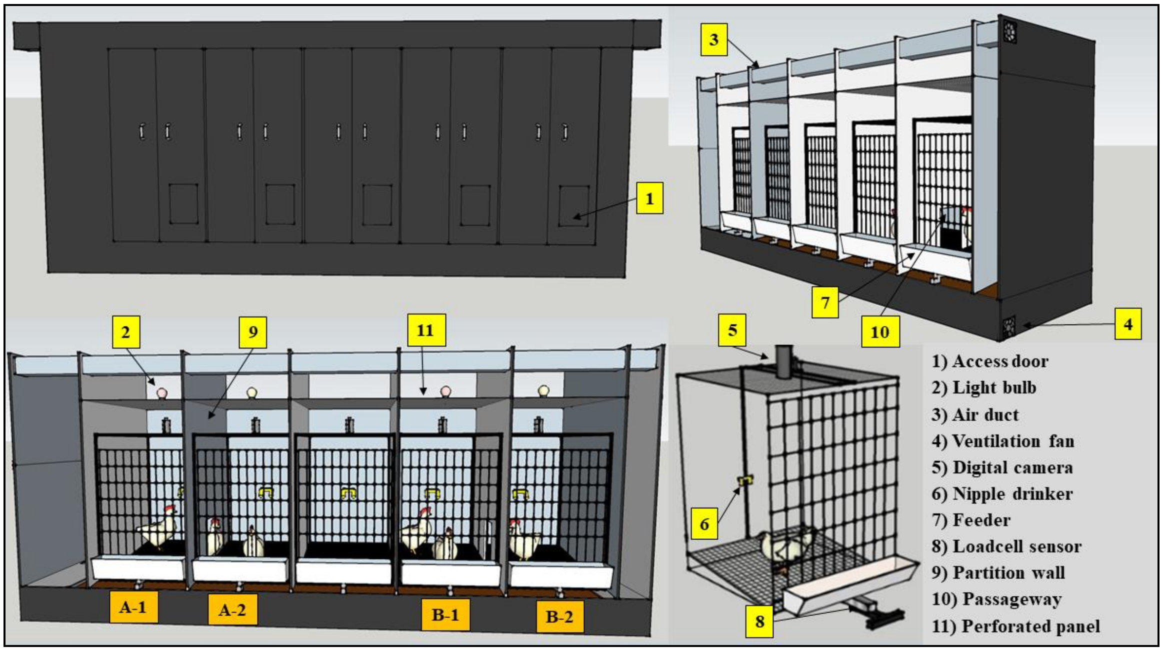


Figure 2. A schematic representation of the light preference test system.

Lighting Regimens

The preference or choice of light was tested between the Dim-to-Red® PS-LED and the warm-white FL (Fig.1). Light intensity was determined using a spectrometer (GL SPECTIS 1.0 Touch, JUST Normlicht Inc., Langhorne, PA, USA) coupled with a software (SpectraShift 2.0, Once, Inc.) for measuring poultry-perceived light intensity in p-lux (Saunders *et al.*, 2008; Liu *et al.*, 2017). Arrangement of the lights was made according to the experimental design as described below. In the acclimation chamber, light intensity varied from 18 to 30 p-lux, depending on the distance from the floor to the lights. In the LPTC, light intensities were adjusted to similar levels (i.e., 25 p-lux on the floor and 20 p-lux at the feeder) and maintained constant throughout the testing period. Constant photoperiods for pullets and layers were used, i.e., a 10-hr light and 14-hr dark or 10L:14D for pullets at 14-16 WOA and 16L:8D for layers at 44-50 WOA.

Experimental Procedures

A total of 36 groups of birds (12 groups for each bird category) were tested in 18 trials to evaluate light preference or choice by the birds. For each trial, six birds in two groups of the same category were tested simultaneously. The six test birds first underwent a 7-day acclimation period in the acclimation chamber (1578 cm^2 $bird^{-1}$ space allowance), during which they became used to passing through the passageway between the inter-connected cages. The acclimation chamber was alternately lit by the PS-LED and the FL from one day to the next, thus allowing birds to experience both test lights before being assigned to LPTC. After the acclimation period, these two groups of birds were randomly assigned to the two pairs of LPTC (2400 cm^2 $bird^{-1}$) for a 6-day test period. During the test period, the PS-LED and the FL were randomly assigned to the compartments, and alternated daily (during the dark period) to avoid potential compartment effect (e.g., location preference). The first two days in LPTC were used as acclimation period for the birds and the cooresponding data were excluded from the analysis. Thus, the results were analyzed based on data collected during the last four days.

Data Collection

A real-time sensor-based monitoring system was built by incorporating four load-cell scales (RL1040-N5, Rice Lake Weighing Systems, Rice Lake, WI, USA), four thermocouples (Type-T, OMEGA Engineering Inc., Stamford, CT, USA), and a relative humidity (RH) sensor (HMT100, Vaisala, Inc., Woburn, MA, USA) with a LabVIEW-based data acquisition system (version 7.1, National Instrument Corporation, Austin, TX, USA). The system consisted of a compact FieldPoint controller (NI cFP-2020, National Instrument Corporation) and multiple thermocouple input modules (NI cFP-TC-120, National

Instrument Corporation). The data were collected at 1-s intervals. Air temperature in each compartment, RH in the air duct near the exhaust fan (10 cm in front), and each feeder weight were monitored continuously. Air temperature was used for adjusting the ventilation rate to maintain consistent temperature in the compartments. Feeder weight was used for determining daily feed use in each compartment by calculating the feeder weight difference between the beginning and the end of the day.

A real-time vision system was built and used by incorporting four infrared video cameras (GS831SM/B, Gadspot Inc. Corp., Tainan city, Taiwan, China) and a PC-based video capture card (GV-600B-16-X, Geovision Inc., Taipei, Taiwan, China) with a surveillance system software (Version 8.5, GeoVision Inc.). One camera was installed atop each cage and recording top-view images. This vision system could record images from all four cameras simultaneously at 1 frame per second (FPS). Distribution of the birds in the LPTC was analyzed using an automated image processing program in MATLAB (R2014b, MathWorks Inc., Torrance, CA, USA) and VBA programs in Excel (Microsoft Office 2016, Redmond, WA, USA).

Determination of Time-Series Distribution of the Birds

Images were recorded at 1 FPS. Thus each individual image recoded represented a momentary state of the birds in the LPTCs. The algorithm for determining the dristribution of the birds in the LPTCs consisted of four main procedures: 1) extracting pixels representing the birds in each image (Fig. 3a-e), 2) counting number of bird blobs detected in each image (Fig. 3e), 3) determining area of each blob (Fig. 3f), and 4) determining the number of birds in each cage (Table 2 and Fig. 4). The two simultaneous images from each pair of LPTC were analyzed separately for each cage. As such, if a bird is passing through or staying at the passageway, one bird would be detected as two blobs, one per image (Fig. 4), as depicted in scenarios (8), (9), and (10). A blob could also be a single bird, as in scenarios (5) and (6), or multiple contacting birds, as in scenarios (1), (2), and (4). In the current study, contacting birds were not individually segmented during the image processing. Instead of implementing a computation-intensive segmention procedure, a simple enumeration method was applied. Specifically, with only three birds in LPTC, there were a maximum of four total detected blobs and 10 possible scenarios for distributions of the birds (Fig. 4). Namely, the possibilities are one blob for scenario (1), two blobs for scenarios (2)-(4), three blobs for scenarios (5)-(8), and four blobs for scenarios (9) and (10). The detailed criteria for scenario classfication for the distributions of the birds are described in Table 2.

With the knowledge of number of blobs in each cage and area of each blob, the number of birds in each cage was determined using an automated VBA program in Excel. Specifically, the VBA program first checked the number of detected blobs in each cage. When there was an empty cage (no detected blob), all three birds had to be in the other cage, i.e., scenarios (1), (2), or (5). Then, a threshold for blob area, 6000 pixels for pullet and 8000 pixels for layer was applied to the blob(s) because a blob consisting of a single bird had approximately 12000 pixels for a pullet and approximately 16000 pixels for a layer. If both cages had only one blob and each blob area was larger than the threshold, the cage with the larger blob was considered to have two birds, i.e., scenario (3) or in certain cases, scenario (4). If one cage had two blobs and the other cage had only one blob, and all the blobs were larger than the threshold, the cage with two blobs was considered to have two birds. i.e., scenario (6) or in certain cases, scenario (7). If four total blobs were detected in two cages or if any blob was smaller than the threshold (6000 or 8000 pixels), there was a bird passing through or staying at the passageway, i.e., scenarios (8), (9) and (10), or in certain cases, scenarios (4) and (7). For those scenarios that had a bird passing through or staying at the passageway, the blob smaller than the threshold could be excluded. Thus these scenarios would be analyzed similarly to others, i.e., scenario (4) similar to (1) or (3); scenario (7) similar to (3) or (6); scenario (8) similar to (2) or (3); scenario (9) similar to (5) or (6); and scenario (10) similar to (6). Consequently, for every recorded frame, the number of birds in the corresponding cage could be determined. The algorithm applied in the analysis was validated by human observation of the time-series images, with an accuracy of 98% or better. The false determinations of bird number were mainly attributed to the infrequent wing-flapping of the birds or sudden frame loss from the cameras.

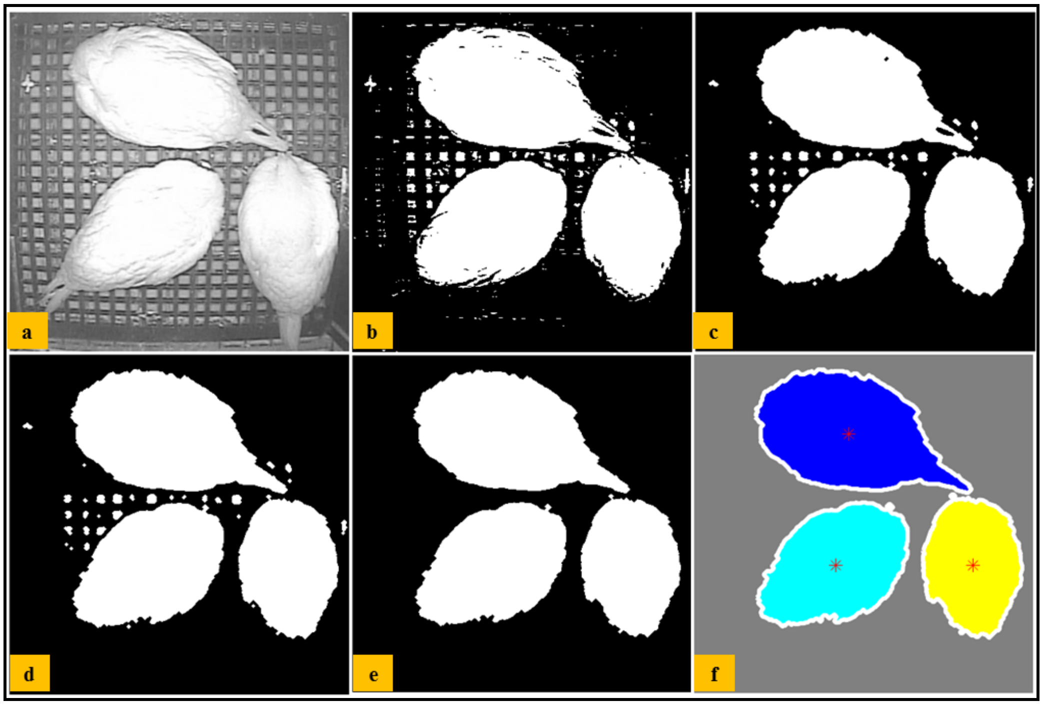
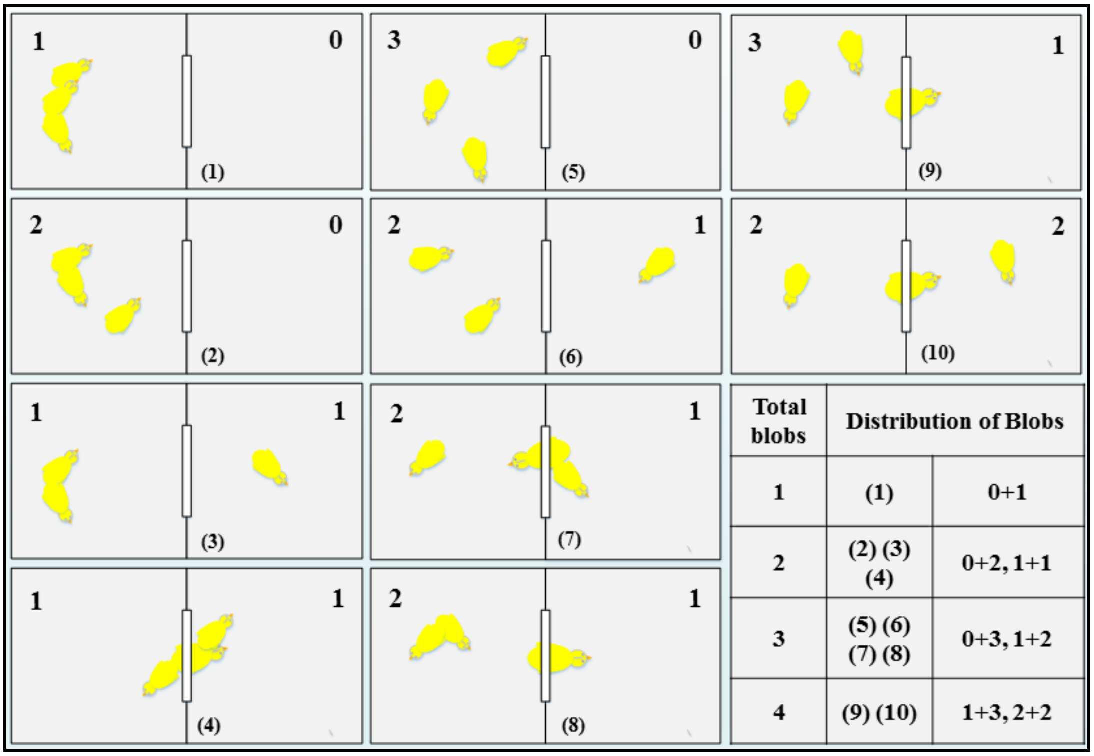


Figure 3. Image processing procedures. (a) RGB image of birds, (b) binary image of birds without enhancement, (c) binary image of birds with morphological opening operation, (d) binary image of birds with morphological closing operation, (e) binary image of birds with small objects removed, and (f) detected blobs in the binary image.

Table 2. Criteria for scenario classification of bird distribution in the light preference test compartments.

Scenario	Criteria for Scenario Classification[1]
(1)	All three birds were in one cage, having body contact with at least one of the other two birds.
(2)	All three birds were in one cage, with one bird apart from the other two that were in contact with each other.
(3)	One bird was in one cage alone and the other two contacting birds in the other cage.
(4)	One bird was passing through or staying at the passageway, with at least one contact among the birds.
(5)	All three birds were in one cage and apart from one another.
(6)	One bird was in one cage alone and the other two birds were in the other cage without body contact.
(7)	One bird was passing through or staying at the passageway and in contact with one bird. The third bird was by herself.
(8)	One bird was passing through or staying at the passageway, while the other two were away and in contact with each other.
(9)	One bird was passing through or staying at the passageway; the other two were away in one cage without body contact.
(10)	One bird was passing through or staying at the passageway; the other two were in separate cages and no contact with the passing bird.

[1] Distribution of the birds in the light preference test compartments was classified as a certain scenario based on the total number of detected blobs, the number of blobs detected in each cage, and the number of birds with body contacts to each other.



Total blobs	Distribution of Blobs	
1	(1)	0+1
2	(2) (3) (4)	0+2, 1+1
3	(5) (6) (7) (8)	0+3, 1+2
4	(9) (10)	1+3, 2+2

Figure 4. Representative distributions of birds in the light preference test compartments. Numbers in parentheses are scenario ID's. For each scenario, three birds were present in two adjoining compartments. The small rectangular in the center represents the passageway between the compartments. The number in each corner of the compartment box represents the number of blobs detected in that compartment.

Calculation of the behavior variables

With the knowledge of the time-series distributions of the birds in the LPTC, time budgets and moving frequency of the birds were calculated and summarized using a separate VBA program in Excel. The proportion of daily feed intake of birds under the PS-LED or the FL (PDFI, %) was also calculated. All the behavior variables analyzed in the study are described in Table 3. The amount of time spent under the PS-LED or the FL was calculated by dividing the time the birds spent under the PS-LED or the FL by the length of the photoperiod on a per-bird basis (min bird^{-1}). The amount of time with no bird, one bird, two birds, or three birds under the PS-LED or the FL was calculated by dividing the respective durations by the length of the photoperiod. In this study, birds were not individually identified with the vision and the sensor systems, thus all behavior variables were presented as group averages.

Table 3. Behavior variables of birds measured during the preference test.

Abbreviation	Description
LMF	Light-period moving frequency of birds between lights; times bird^{-1} h^{-1}
PLTS	Proportion of light-period time spent under the PS-LED or the FL; %
L3F0	Proportion of the light period with all three birds under the PS-LED; %
L2F1	Proportion of the light period with two birds under the PS-LED and one bird under the FL; %
L1F2	Proportion of the light period with one bird under the PS-LED and two birds under the FL; %
L0F3	Proportion of the light period with all three birds under the FL; %
PDFI	Proportion of daily feed intake under the PS-LED or the FL; %

Statistical Analysis

Statistical analyses were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, NC, USA). The behavior variables shown in Table 3 were analyzed to determine light preference/choice and to compare differences among the three categories of birds (P_{INC}, L_{LED}, and L_{FL}). Behavior variables (Table 3), i.e., LMF, PDFI, PLTS, L3F0, L2F1, L1F2 and L0F3, were analyzed with generalized linear mixed models by implementing PROC GLIMMIX procedure. A Gaussian distribution was specified for the analysis of LMF; whereas a beta distribution was specified for the analysis of PDFI, PLTS, L3F0, L2F1, L1F2, and L0F3. All the statistical models were of the folowing form:

$$Y_{ijkd} = \mu + B_i + P_j + (BP)_{ij} + G(BP)_{ijk} + D(BPG)_{ijkd} + e_{ijkd}$$

Where Y_{ijkd} denotes the independent observation on day d for group k in $LPTC_j$ of bird category i; μ is the overall mean; B_i is the bird category effect (fixed); P_j is the LPTC effect (fixed); $(BP)_{ij}$ is the interaction effect (fixed) of bird category and LPTC; $G(BP)_{ijk}$ is the group effect (random) tested within each LPTC for each bird category, $D(BPG)_{ijkd}$ is the day effect (random) for each group, adjusted with first-order autoregressive or AR (1) covariance structure; and e_{ijkd} is the random error with a normal distribution with mean μ and variance σ^2 $[N \sim (\mu, \sigma^2)]$.

Evaluation of the light preference was accomplished by testing the null hypothesis that the proportion of time spent duirng light period (PLTS) or the proportion of daily feed intake (PDFI) under each light equals 0.5. As the beta distribution used a logit link function, the evalaution was actually testing if the intercept equals zero [logit(0.5) = 0]. In addition, Tukey-Kramer tests were used for pairwise comparisons among bird catogries for all the behavior variables. Differences were considered significant at $p < 0.05$. Normality and homogeneity of variance of data were examined by residual diagnostics. Unless otherwise specified, data are presented as least squares means along with the standard error of the mean (SEM).

Results and Discussion

Time Spent by the Birds Under Different Lights

As shown in Figure 5, all three categories of birds performed a stronger choice for the FL than for the PS-LED in terms of light-period time spent (p = 0.011, 0.030, and 0.001 for P_{INC}, L_{LED}, and L_{FL}, respectively), and the tendency of this choice was not affected by the prior lighting experience (p = 0.422). Specifically, PLTS under the FL was 58.0 ± 2.9%, 53.7 ± 1.6%, and 54.2 ± 1.2% for P_{INC}, L_{LED}, and L_{FL}, respectively. Correspondingly, PLTS under the PS-LED was 42.0 ± 2.9%, 46.3 ± 1.6%, and 45.8 ± 1.2% for P_{INC}, L_{LED}, and L_{FL}, respectively. The results of the current study were similar to the findings of an earlier study that reported laying hen’s preference of CFL over incandescent light at a light intensity of 12 lux by spending on average 73.2% of time under CFL and only 26.8% of time under incandescent light (Widowski *et al.*, 1992). However, there was no explanation as to why birds preferred CFL over the other light in the cited study. Laying hens were reported to show no preference for HPS or incandescent light (Vandenbert and Widowski, 2000). Broilers were reported to show no behavioral sign of preference between white and yellow LED lights at a light intensity of 5 lux (Mendes *et al.*, 2013). However, turkeys were found to prefer fluorescent light with supplementary UV radiation compared to without UV radiation at a light intensity of 15 lux (Moinard and Sherwin, 1999). Research has demonstrated that poultry have a fourth retinal cone photoreceptor that allows them to see in the UVA wavelength (315-400 nm) (Prescott and Wathes, 1999; Cuthill *et al.*, 2000). As a result they may use UVA perception to modify various behavioral functions such as feeding, peer recognition, mate selection, and social encounters (Lewis and Gous, 2009). With UVA radiation forming 3-4% of fluorescent light, but almost none in incandescent light and most of the newly emerging LED lights (Lewis and Gous, 2009), attraction of the birds to the FL as observed in the current study may be a reflection of the UVA light effect. Further investigation of bird preference for UVA light seems warranted.

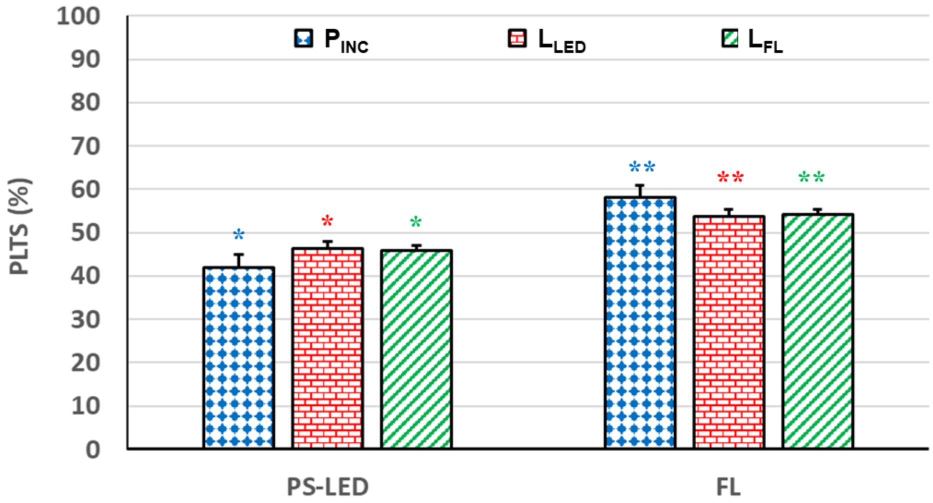


Figure 5. Proportions of light-period time spent (PLTS) under the poultry-specific LED light (PS-LED) and the fluorescent light (FL). P_{INC} = pullets reared under incandescent light; L_{LED} = layers under PS-LED throughout pullet and laying phases; L_{FL} = layers under FL throughout pullet and laying phases. Data bars with single asterisk (*) are significantly lower than 50% at $p < 0.05$; data bars with double asterisks (**) are significantly higher than 50% at $p < 0.05$. For PS-LED or FL, no distinct difference was detected among the three categories of birds at $p < 0.05$.

Light-Period Distributions of Birds

Light-period distributions of the birds between the two light types provide more detailed illustration on their choices (Fig. 6). In general, birds in all three categories spent significantly more time splitting into the two cages than staying together in one cage, with a tendency of choosing the FL when more birds stayed together. Specifically, L1F2 (40.7 ± 2.4%) and L2F1 (33.6 ± 2.5%) for P_{INC} were significantly higher than L0F3 (18.9 ± 2.6%, $p = 0.001$ and 0.021, respectively) or L3F0 (6.8 ± 0.8%, $p < 0.001$ and $P < 0.001$, respectively). L1F2 (31.6 ± 1.4%) for L_{LED} was significantly higher than L0F3 (22.6 ± 1.7%, $p = 0.031$) or L3F0 (15.3 ± 1.5%, $p < 0.001$), and L2F1 (30.5 ± 1.6%) for L_{LED} was also significantly higher than L3C0 (p < 0.001). Likewise, L1F2 (33.6 ± 1.2%) and L2F1 (31.6 ± 1.4%) for L_{FL} were significantly higher than L0F3 (20.6 ± 1.7%, p = 0.005 and p <0.001, respectively) or L3F0 (14.2 ± 1.2%, p < 0.001 and p < 0.001, respectively). These distribution patterns differed from those found in a previous study in which laying hens spent about 60% of time during the light period with 3-4 hens in the same cage when four hens were housed in five inter-connected cages (Ma *et al.*, 2016).

As mentioned earlier, laying hens were reported to spend on average 73.2% of time under CFL and only 26.8% of time under incandescent light (Widowski *et al.*, 1992). By comparison, the degree of the preference was not as strong in the current study, as reflected by the time spent of the birds (55% *vs.* 45%). The lower degree of preference in the current study might have arisen from a dominant-subordinate relationship among the birds which tends to exist in small groups. The establishment of dominance hierarchies in pullets and laying hens housed in small groups usually starts as early as the first encounter and maintains relatively consistent during subsequent production stages. Where dominance hierarchies exist, the subordinate birds usually benefit from avoiding encounters with the dominant ones (Pagel and Dawkins, 1997; D’Eath and Keeling, 2003). In the current study, floor space, feeder space, and nipple drinkers provided in each cage were considered sufficient for all birds, which might have weakened the significance of hierarchy. However, aggressive pecking was observed among the test pullets and layers during the early rearing period and the behavior seemed to continue after assignment to the test environments.

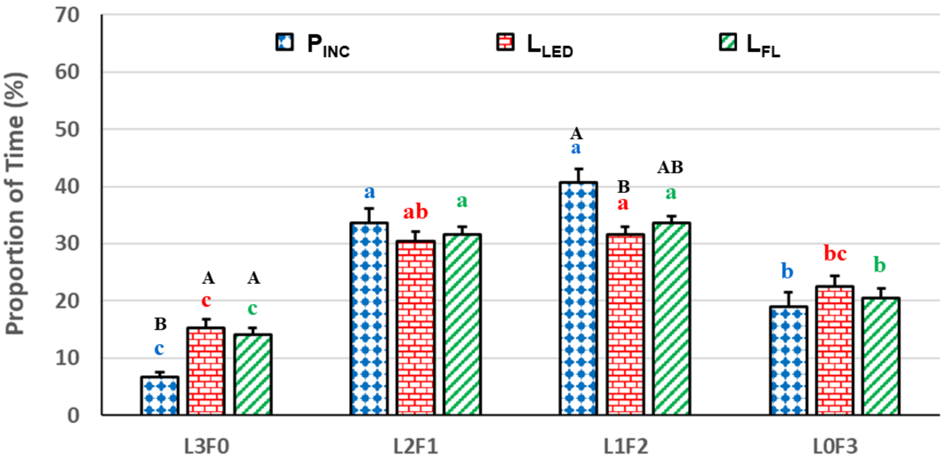


Figure 6. Light-period bird distributions under the poultry-specific LED light (PS-LED) and the fluorescent light (FL). P_{INC} = pullets reared under incandescent light; L_{LED} = layers under PS-LED throughout pullet and laying phases; L_{FL} = layers under FL throughout pullet and laying phases; LxFy = proportion of the light period with x birds under the PS-LED and y birds under the FL. Within a distribution pattern (LxFy), bars with different uppercase letters differ significantly at $p < 0.05$. For each of the three bird categories (P_{INC}, L_{LED}, or L_{FL}), bars with different lowercase letters differ significantly at $p < 0.05$.

Light-Period Moving Frequency of Birds

Birds were observed to move frequently between the inter-connected cages for feeding, drinking, resting, foraging, and nest-seeking during the light period. LMF of P_{INC}, L_{LED}, and L_{FL} averaged 19.8 ± 1.0, 31.9 ± 2.4, and 29.9 ± 1.9 times bird^{-1} h^{-1}, respectively (Fig. 7). L_{LED} and L_{FL} had significantly higher LMF than P_{INC} ($p < 0.001$), while LMF of L_{LED} and L_{FL} was highly comparable ($p = 0.804$). The higher LMF of layers than that of pullets probably stemmed from the intensive nest-seeking behavior of the hens because nest boxes were not provided during the current study. Hens were highly motivated to gain access to nest boxes prior to oviposition and displayed frustration when nests were not available (Cooper and Appleby, 1996). They tended to aggressively compete to lay eggs in the curtained nest area when housed in small cages (Hunniford *et al.*, 2014). But this was not a behavioral characteristic for the 14-16 WOA pullets. In an earlier study, a significant negative correlation was found between the degree of bird's preference for a particular light and its movement between lights (Widowski *et al.*, 1992); namely, birds having a stronger preference for a particular light moved less frequently between lights. However, this relationship was not apparent in the current study, as birds in all the three categories showed similar degrees of preference for the FL light during the light period.

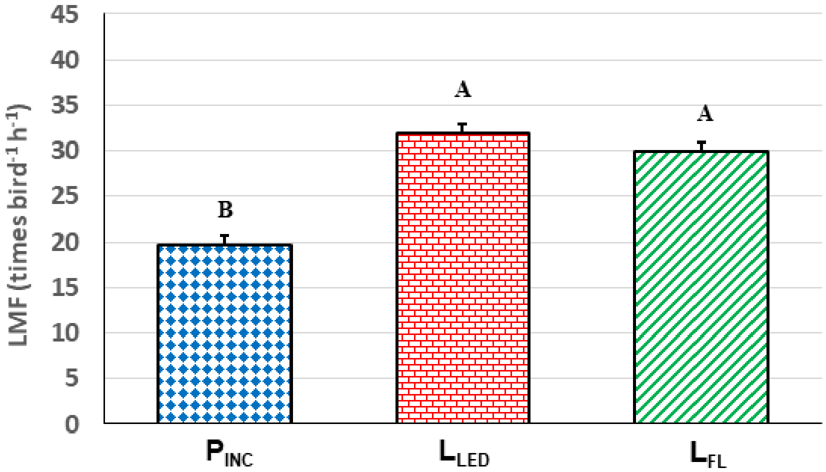


Figure 7. Light-period moving frequency (LMF) between the poultry-specific LED light (PS-LED) and the fluorescent light (FL). P_{INC} = pullets reared under incandescent light; L_{LED} = layers under PS-LED throughout pullet and laying phases; L_{FL} = layers under FL throughout pullet and laying phases. Bars with different letters differ significantly at $p < 0.05$.

Daily Feed Intake

Birds in all the three categories showed no light preference for feeding, as reflected by PDFI (p = 0.419, 0.566, and 0.749 for P_{INC}, L_{LED}, and L_{FL}, respectively, Fig. 8). Specifically, 51.8 ± 2.3%, 51.2 ± 2.0%, and 49.6 ± 1.4% of the daily feed intake occurred under the PS-LED for P_{INC}, L_{LED}, and L_{FL}, respectively. Correspondingly, 48.2 ± 2.3%, 48.8 ± 2.0%, and 50.4 ± 1.4% of daily feed intake happened under the FL for P_{INC}, L_{LED}, and L_{FL}, respectively. The result of no light preference for feeding did not parallel the findings of some earlier studies. Shaver hens under fluorescent light were found to perform more ingestion behaviors (feeding, drinking, and ground pecking) than under incandescent light (Widowski *et al.*, 1992). Broilers were found to eat substantially more feed in chambers equipped with white LED light than with yellow LED light (Mendes *et al.*, 2013). However, the preference for light types was confounded by light intensities in these earlier studies as the bird-perceived light intensities were not equal when lights applied to the cages or chambers were adjusted using human light meters (Prescott and Wathes, 1999; Prescott *et al.*, 2003; Saunders *et al.*, 2008). Indeed, feed intake of birds seemed to be more associated with light intensity than with light type or spectrum. Broilers reared in high light intensity (2.5-35 lux) were found to have significantly higher feed consumption than broilers under low light intensity (2.5 lux) (Purswell and Olanrewaju, 2017). ISA Brown hens were observed to eat for the longest time under the brightest (200 lux) and the shortest amount of time under the dimmest (less than1 lux) light intensity when given free choice of a light intensity of less than 1, 6, 20 or 200 lux (Prescott and Wathes, 2002). In contrast, Hy-Line W-36 commercial layers were found to have the highest feed intake at 5 lux (32.5%) and lowest at 100 lux (6.7%) when given free choice of a light intensity of less than 1, 5, 15, 30 or 100 lux (Ma *et al.*, 2016).

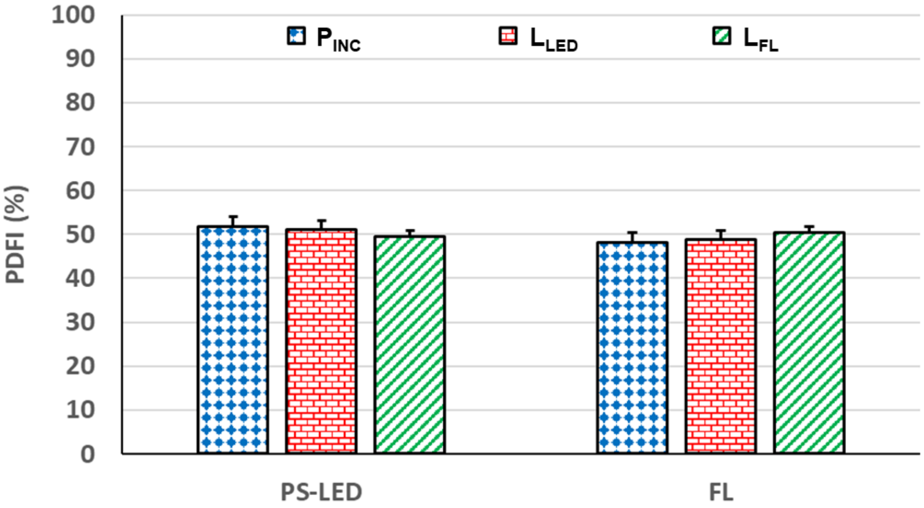


Figure 8. Proportions of daily feed intake (PDFI) under the poultry-specific LED light (PS-LED) and the fluorescent light (FL). P_{INC} = pullets reared under incandescent light; L_{LED} = layers under PS-LED throughout pullet and laying phases; L_{FL} = layers under FL throughout pullet and laying phases. For all bird categories, PDFI was not significantly different from 50%. Within PS-LED or FL, no distinct difference was detected among the three bird categories.

Conclusions

In this study, light preference of Hy-Line W-36 pullets and laying hens between a Dim-to-Red® poultry-specific LED light (PS-LED) and a warm-white fluorescent light (FL) was assessed in free-choice light preference test compartments. Three categories of birds each with different prior lighting experience were tested, including pullets reared under incandescent light (P_{INC}), layers under PS-LED throughout pullet and laying phases (L_{LED}), and layers under FL throughout pullet and laying phases (L_{FL}). Each category consisted of 12 groups (replicates), three birds per group. The following observations and conclusions were made.

- The pullets and layers showed a moderate degree of preference for the FL *vs.* the PS-LED during the light period (53-58% *vs.* 47-42%), although the proportions of time spent under the respective light type were statistically different.

- The pullets and layers had comparable proportions of daily feed intake for the FL and the PS-LED conditions.
- Prior lighting experience of the pullets and layers did not influence their choice for the LF or the PS-LED or proportions of daily feed intake under each during subsequent exposure to the lights.

Acknowledgements

Funding for the study was provided in part by the Center for Industrial Research and Service (CIRAS) at Iowa State University and Hy-Line International and is acknowledged. We also wish to thank Once Innovation Inc. for providing the LED lights and the controller used in the study, and the Agriculture Experiment Station (AES) Consulting Group at Iowa State University for the consistent assistance in statistical consulting for the study. Author Kai Liu also wishes to thank China Scholarship Council for providing part of the financial support toward his PhD study at Iowa State University.

References

Appleby, M. C., McRae, H. E., & Peitz, B. E. (1984). The effect of light on the choice of nests by domestic hens. *Applied Animal Ethology, 11*(3), 249–254. https://doi.org/10.1016/0304-3762(84)90031-2

ASABE Sandards. (2014). EP344.4: Lighting systems for agricultrual facilities. St. Joseph, MI: ASABE.

Baxter, M., Joseph, N., Osborne, V. R., & Bedecarrats, G. Y. (2014). Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poultry Science, 93*(5), 1289–1297. https://doi.org/10.3382/ps.2013-03799

Cao, J., Liu, W., Wang, Z., Xie, D., Jia, L., & Chen, Y. (2008). Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. *The Journal of Applied Poultry Research, 17*(2), 211–218. https://doi.org/10.3382/japr.2007-00043

Cooper, J. J., & Appleby, M. C. (1996). Demand for nest boxes in laying hens. *Behavioural Processes, 36*(2), 171–182. https://doi.org/10.1016/0376-6357(95)00027-5

Cuthill, I. C., Partridge, J. C., Bennett, A. T. D., Church, S. C., Hart, N. S., & Hunt, S. (2000). Ultraviolet vision in birds. *In Advances in the Study of Behavior* (Vol. 29, pp. 159–214). https://doi.org/10.1016/S0065-3454(08)60105-9

D'Eath, R. B., & Keeling, L. J. (2003). Social discrimination and aggression by laying hens in large groups: from peck orders to social tolerance. *Applied Animal Behaviour Science, 84*(3), 197–212. https://doi.org/10.1016/j.applanim.2003.08.010

Gongruttananun, N. (2011). Influence of red light on reproductive performance, eggshell ultrastructure, and eye morphology in Thai-native hens. *Poultry Science, 90*(12), 2855–2863. https://doi.org/10.3382/ps.2011-01652

Green, A. R. (2008). A systematic evaluation of laying hen housing for improved hen welfare. A PhD dissertation, Iowa State University Parks Library, Ames, Iowa 50011, USA

Gunnarsson, S., Heikkilä, M., Hultgren, J., & Valros, A. (2008). A note on light preference in layer pullets reared in incandescent or natural light. *Applied Animal Behaviour Science, 112*(3–4), 395–399. https://doi.org/10.1016/j.applanim.2007.09.004

Gunnarsson, S., Valros, A., Briese, A., Clauss, M., Springorum, A., & Hartung, J. (2009). Effect of enrichment, day length and natural versus artificial light on behaviour and light preference in layer chicks. In Proceedings of the 14th International Congress of the International Society for Animal Hygiene (Vol. 1, pp. 345–348).

Harrison, P., McGinnis, J., Schumaier, G., & Lauber, J. (1969). Sexual maturity and subsequent reproductive performance of white leghorn chickens subjected to different parts of the light spectrum. *Poultry Science, 48*(3), 878–83.

Hughes, B. O. (1976). Preference decisions of domestic hens for wire or litter floors. *Applied Animal Ethology, 2*(2), 155–165. https://doi.org/10.1016/0304-3762(76)90043-2

Hunniford, M. E., Torrey, S., Bédécarrats, G., Duncan, I. J. H., & Widowski, T. M. (2014). Evidence of competition for nest sites by laying hens in large furnished cages. *Applied Animal Behaviour Science, 161*(1), 95–104. https://doi.org/10.1016/j.applanim.2014.08.005

Huth, J. C., & Archer, G. S. (2015). Comparison of Two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress, and fear. *Poultry Science, 94*(9), 2027–2036. https://doi.org/10.3382/ps/pev215

Hy-Line International. (2016). W-36 commercial layers management guide 2016. Retrieved on 2 December 2016, from http://www.hyline/com/userdocs/pages/36_Com_ENG.pdf

Kashiha, M. A., Green, A. R., Sales, T. G., Bahr, C., Berckmans, D., & Gates, R. S. (2014). Performance of an image analysis processing system for hen tracking in an environmental preference chamber. *Poultry Science, 93*(10), 2439–2448. https://doi.org/10.3382/ps.2014-04078

Lambe, N. R., & Scott, G. B. (1998). Perching behaviour and preferences for different perch designs among laying hens. *Animal Welfare*, *7*(2), 203–216.

Lewis, P. D. (2010). Lighting, ventilation and temperature. *British Poultry Science*, *51*(S1), 35-43.

http://dx.doi.org/10.1080/00071668.2010.497298

Lewis, P. D., & Gous, R. M. (2009). Responses of poultry to ultraviolet radiation. *World's Poultry Science Journal*, *65*(3), 499–510. https://doi.org/10.1017/S0043933909000361

Lewis, P. D., & Morris, T. R. (2000). Poultry and coloured light. *World's Poultry Science Journal*, *56*(3), 189–207. https://doi.org/10.1079/WPS20000015

Li, D., Zhang, L., Yang, M., Yin, H., Xu, H., Trask, J. S., Zhu, Q. (2014). The effect of monochromatic light-emitting diode light on reproductive traits of laying hens. *The Journal of Applied Poultry Research*, *23*(3), 367–375. https://doi.org/10.3382/japr.2013-00746

Liu, K., Xin, H., & Settar, P. (2017). Effects of light-emitting diode light v. fluorescent light on growing performance, activity levels and well-being of non-beak-trimmed W-36 pullets. *Animal*, 1–10. https://doi.org/10.1017/S1751731117001240

Long, H., Zhao, Y., Wang, T., Ning, Z., & Xin, H. (2016). Effect of light-emitting diode vs. fluorescent lighting on laying hens in aviary hen houses: Part 1 – Operational characteristics of lights and production traits of hens. *Poultry Science*, *95*(1), 1–11. https://doi.org/10.3382/ps/pev121

Long, H., Zhao, Y., Xin, H., Hansen, H., Ning, Z., & Wang, T. (2016). Effect of light-emitting diode (LED) vs. fluorescent (FL) lighting on laying hens in aviary hen houses: Part 2 – Egg quality, shelf-life and lipid composition. *Poultry Science, 95*(1), 115–124. https://doi.org/10.3382/ps/pev306

Ma, H., Xin, H., Zhao, Y., Li, B., Shepherd, T. A., & Alvarez, I. (2016). Assessment of lighting needs by W-36 laying hens via preference test. *Animal, 10*(4), 671–680. https://doi.org/10.1017/S1751731115002384

Manser, C. E. (1996). Effects of lighting on the welfare of domestic poultry: A review. *Animal Welfare, 5*(4), 341–360.

Mendes, A. S., Paixao, S. J., Restelatto, R., Morello, G. M., Jorge de Moura, D., & Possenti, J. C. (2013). Performance and preference of broiler chickens exposed to different lighting sources. *The Journal of Applied Poultry Research, 22*(1), 62–70. https://doi.org/10.3382/japr.2012-00580

Millam, J. R. (1987). Preference of turkey hens for nest-boxes of different levels of interior illumination. *Applied Animal Behaviour Science, 18*(3–4), 341–348. https://doi.org/10.1016/0168-1591(87)90227-9

Min, J. K., Hossan, M. S., Nazma, A., Jae, C. N., Han, T. B., Hwan, K. K., Ok, S. S. (2012). Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biology Research*, *5*(2), 69–74. https://doi.org/10.3184/175815512X13350270679453

Mobarkey, N., Avital, N., Heiblum, R., & Rozenboim, I. (2010). The role of retinal and extra-retinal photostimulation in reproductive activity in broiler breeder hens. *Domestic Animal Endocrinology*, *38*(4), 235–243. https://doi.org/10.1016/j.domaniend.2009.11.002

Moinard, C., & Sherwin, C. . (1999). Turkeys prefer fluorescent light with supplementary ultraviolet radiation. *Applied Animal Behaviour Science*, *64*(4), 261–267. https://doi.org/10.1016/S0168-1591(99)00043-X

Olanrewaju, H. A., Miller, W. W., Maslin, W. R., Collier, S. D., Purswell, J. L., & Branton, S. L. (2016). Effects of light sources and intensity on broilers grown to heavy weights. Part 1: Growth performance, carcass characteristics, and welfare indices. *Poultry Science*, *95*(4), 727–735. https://doi.org/10.3382/ps/pev360

Olanrewaju, H. A., Thaxton, J. P., III, W. A. D., Purswell, J., Roush, W. B., & Branton, S. L. (2006). A review of lighting programs for broiler production. *International Journal of Poultry Science*, *5*(4), 301–308. https://doi.org/10.3923/ijps.2006.301.308

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. https://doi.org/10.1016/j.visres.2008.06.018

Pagel, M., & Dawkins, M. . (1997). Peck orders and group size in laying hens: `futures contracts' for non-aggression. *Behavioural Processes*, *40*(1), 13–25. https://doi.org/10.1016/S0376-6357(96)00761-9

Prayitno, D. S., Phillips, C. J., & Omed, H. (1997). The effects of color of lighting on the behavior and production of meat chickens. *Poultry Science*, *76*(3), 452–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9068043

Prescott, N. B., & Wathes, C. M. (1999). Spectral sensitivity of the domestic fowl (Gallus g. domesticus). *British Poultry Science, 40*(3), 332–339. https://doi.org/10.1080/00071669987412

Prescott, N. B., & Wathes, C. M. (2002). Preference and motivation of laying hens to eat under different illuminances and the effect of illuminance on eating behaviour. *British Poultry Science, 43*(2), 190–195. https://doi.org/10.1080/00071660120121382

Prescott, N. B., Wathes, C. M., & Jarvis, J. R. (2003). Light, vision and the welfare of poultry. *Animal Welfare, 12*(2), 269–288.

Purswell, J. L., & Olanrewaju, H. A. (2017). Effect of fan induced photoperiod on live performance and yield of male broiler chickens. *The Journal of Applied Poultry Research*, pfw067. https://doi.org/10.3382/japr/pfw067

Rajchard, J. (2009). Ultraviolet (UV) light perception by birds: a review. *Veterinarni Medicina, 54*(8), 351-359.

Rozenboim, I., Biran, I., Chaiseha, Y., Yahav, S., Rosenstrauch, A., Sklan, D., & Halevy, O. (2004). The effect of a green and blue monochromatic light combination on broiler growth and development. *Poultry Science, 83*(5), 842–845. https://doi.org/10.1093/ps/83.5.842

Saunders, J. E., Jarvis, J. R., & Wathes, C. M. (2008). Calculating luminous flux and lighting levels for domesticated mammals and birds. *Animal, 2*(6), 921–932. https://doi.org/10.1017/S1751731108002012

Sherwin, C. . (1998). Light intensity preferences of domestic male turkeys. *Applied Animal Behaviour Science, 58*(1–2), 121–130. https://doi.org/10.1016/S0168-1591(97)00138-X

Struelens, E., Tuyttens, F. A. M., Duchateau, L., Leroy, T., Cox, M., Vranken, E., Sonck, B. (2008). Perching behaviour and perch height preference of laying hens in furnished cages varying in height. *British Poultry Science, 49*(4), 381–389. https://doi.org/10.1080/00071660802158332

Sultana, S., Hassan, M. R., Choe, H. S., & Ryu, K. S. (2013). The effect of monochromatic and mixed LED light colour on the behaviour and fear responses of broiler chicken. *Avian Biology Research, 6*(3), 207–214. https://doi.org/10.3184/175815513X13739879772128

Vandenbert, C., & Widowski, T. M. (2000). Hens' preferences for high-intensity high-pressure sodium or low-intensity incandescent lighting. *The Journal of Applied Poultry Research, 9*(2), 172–178. https://doi.org/10.1093/japr/9.2.172

Widowski, T. M., & Duncan, I. J. H. (1996). Laying hens do not have a preference for high-frequency versus low-frequency compact fluorescent light sources. *Canadian Journal of Animal Science, 76*(2), 177–181.

Widowski, T. M., Keeling, L. J., & Duncan, I. J. H. (1992). The preferences of hens for compact fluorescent over incandescent lighting. *Canadian Journal of Animal Science, 72*(2), 203–211. https://doi.org/10.4141/cjas92-027

Xie, D., Wang, Z. X., Dong, Y. L., Cao, J., Wang, J. F., Chen, J. L., & Chen, Y. X. (2008). Effects of monochromatic light on immune response of broilers. *Poultry Science, 87*(8), 1535–1539. https://doi.org/10.3382/ps.2007-00317

Yang, Y., Jiang, J., Wang, Y., Liu, K., Yu, Y., Pan, J., & Ying, Y. (2016). Light-emitting diode spectral sensitivity relationship with growth, feed intake, meat, and manure characteristics in broilers. *Transactions of the ASABE, 59*(5), 1361–1370.

CHAPTER 6

EFFECT OF FLUORESCENT VS. POULTRY-SPECIFIC LIGHT-EMITTING DIODE LIGHTS ON PRODUCTION PERFORMANCE AND EGG QUALITY OF W-36 LAYING HENS

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A manuscript accepted by *Poultry Science*

Abstract

More energy-efficient, durable, affordable, and dimmable light-emitting diode (LED) lights are finding applications in poultry production. However, data are lacking on controlled comparative studies concerning the impact of such lights during pullet rearing and subsequent laying phase. This study evaluated two types of poultry-specific LED light (PS-LED) *vs.* fluorescent light (FL) with regards to their effects on hen laying performance. A total of 432 W-36 laying hens were tested in two batches using four environmental chambers (nine cages per chamber and 6 birds per cage) from 17 to 41 weeks of age (WOA). A Dim-to-Red® PS-LED or a warm-white FL was used in the laying phase. The hens had been reared under a Dim-to-Blue® PS-LED or a warm-white FL from 1 to 16 WOA. The measured performance variables included a) timing of sexual maturity (age and body weight at sexual maturity), b) egg production performance (hen-day egg production, eggs per hen housed, egg weight, daily feed intake, and feed conversion), c) egg quality (egg weight, albumen weight, albumen height, Haugh unit, shell thickness, shell strength, yolk weight, yolk percentage, and yolk color factor), and d) egg yolk cholesterol (cholesterol concentration and total yolk cholesterol). Results showed that the two types of light used during the laying phase had comparable performance responses for all the aspects ($p > 0.05$) with a few exceptions during the 17-41 WOA. Specifically, eggs in the PS-LED regimen had lower shell thickness (mean ± SE of 0.42 ± 0.00 *vs*. 0.44 ± 0.00 mm, $p = 0.01$) and strength (37.5 ± 0.22 *vs*. 38.8 ± 0.22 N, $p = 0.03$) than those in the FL regimen at 41 WOA. The two types of light used during the rearing phase did not influence the 17-41 WOA laying performance, except that hens reared under the PS-LED laid eggs with lower shell thickness (0.43 ± 0.00 *vs*. 0.44 ± 0.00 mm, $p = 0.02$) at 32 WOA as compared to hens reared under the FL. The study demonstrates that the emerging poultry-specific LED lights yield comparable production performance and egg quality of W-36 laying hens to the traditional fluorescent lights.

Key words: Poultry lighting, Light characteristic, Egg production, Egg quality, Yolk cholesterol

Nomenclature

LED	Light emitting diode
PS-LED	Poultry-specific LED light
FL	Fluorescent light
WOA	Weeks of age
CCT	Correlated color temperature, K
GnRH	Gonadotrophin receptor hormone
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
CFL	Compact fluorescent light
CCFL	Cold cathode fluorescent light
RH	Relative humidity, %
P_{LED}	Hen with pullet phase under PS-LED
P_{FL}	Hen with pullet phase under FL
L_{LED}	Hen with layer phase under PS-LED
L_{FL}	Hen with layer phase under FL
L_{LED}-P_{LED}	Hen with both layer and pullet phases under PS-LED
L_{LED}-P_{FL}	Hen with layer phase under PS-LED and pullet phase under FL
L_{FL}-P_{LED}	Hen with layer phase under FL and pullet phase under PS-LED
L_{FL}-P_{FL}	Hen with both layer and pullet phases under FL
CV	Coefficient of variation
ASM	Age at sexual maturity, day
BWSM	Body weight at sexual maturity, kg
HDEP	Hen-day egg production, %
EHH	Eggs per hen housed
EW	Egg weight, g
DFI	Daily feed intake, g/bird-day
FCR	Feed conversion ratio, kg feed/kg egg
AW	Albumen weight, g
AH	Albumen height, mm
HU	Haugh unit
ST	Shell thickness, mm
SS	Shell strength, N
YW	Yolk weight, g
YP	Yolk percentage, %
YCF	Yolk color factor
YCC	Yolk cholesterol concentration, mg/g yolk
TCC	Total cholesterol content, mg/egg yolk
SEM	Standard error of the mean

Introduction

Research on poultry lighting dates back to the early 1930s. Since then, extensive research has led to a broad understanding of lighting effects on poultry. The early studies focused on photoperiod and light intensity, leading to the establishment of general lighting guidelines (e.g., ASABE EP344.4 – Lighting systems for agricultural facilities) for improved animal performance and energy efficiency (ASABE Standard, 2014). Nowadays, more energy-efficient, durable, affordable, and dimmable light-emitting diode (**LED**) lights are increasingly finding applications in poultry production. As light is a crucial environmental factor that affects bird behavior, development, production performance, health and well-being (Lewis and Morris, 1998; Parvin *et al.*, 2014), the emerging LED lighting in poultry housing has drawn increasing attention from both scientific and industrial communities.

Poultry has five types of retinal cone photoreceptors in the eyes. These photoreceptors produce the perception of light colors by receiving lights at the peak sensitivities of approximately 415, 450, 550, and 700 nm, and are directly related to poultry activities and growth (Osorio and Vorobyev, 2008). Besides the retinal cone photoreceptors in the eyes, poultry can also perceive light via extra-retinal photoreceptors in the brain (e.g., pineal gland and hypothalamic gland) (Mobarkey *et al.*, 2010). Light stimuli perceived by the extra-retinal photoreceptors can impact sexual development and reproductive traits of poultry (Harrison, 1972; Lewis and Morris, 2000). However, the extra-retinal photoreceptors can only be activated by long-wavelength radiation that can penetrate the skull and deep tissue of head (Harrison, 1972; Lewis and Morris, 2000). It has been demonstrated that red lights can pass through hypothalamic extra-retinal photoreceptors and stimulate reproductive axis by controlling the secretion of gonadotrophin receptor hormone (**GnRH**) and stimulating the release of luteinizing hormone (**LH**) and follicle-stimulating hormone (**FSH**) (Lewis and Morris, 2000; Mobarkey *et al.*, 2010). With the knowledge of the spectral sensitivity of poultry and their responses to light stimulus, it seems feasible to impact poultry (e.g., growth, reproduction, and behavior) by manipulating light stimulations to their retinal and extra-retinal photoreceptors.

The emphasis of poultry lighting has been placed on various light colors (e.g., blue, green, red, and white) and lighting sources (e.g., incandescent, fluorescent, and LED lights) in more recent decades (Lewis and Morris, 2000; Parvin *et al.*, 2014). Research has demonstrated that red lights have an accelerating effect on sexual development and maturity of poultry (Woodard *et al.*, 1969; Harrison *et al.*, 1969; Gongruttananun, 2011; Min *et al.*, 2012; Huber-Eicher *et al.*, 2013; Baxter *et al.*, 2014; Yang *et al.*, 2016). In contrast, blue lights were found to be more associated with improving growth, calming the birds, and enhancing the immune response, although the underlying mechanisms have not been clearly delineated (Prayitno *et al.*, 1997; Rozenboim *et al.*, 2004; Cao *et al.*, 2008; Xie *et al.*, 2008; Sultana *et al.*, 2013). Based on these earlier research findings, many lighting manufacturers have designed LED lights specifically for poultry production by integrating some light traits that have been shown to be beneficial to certain poultry production aspect (e.g., growth, reproduction, or well-being). Recently there have been anecdotal claims about advantages of some commercial poultry-specific LED lights over traditional incandescent or fluorescent lights with regards to their effects on poultry performance and behavior. However, a thorough literature review revealed that most of the existing studies involving LED lights only investigated monochromatic LED lights. Data from controlled comparative studies are lacking concerning the impact of the emerging poultry-specific LED lights.

A few studies recently compared the emerging LED lights with traditional incandescent or fluorescent lights in pullet and laying hen houses. Hy-Line W-36 (white) pullet reared under a Dim-to-Blue® poultry-specific LED light (correlated color temperature or **CCT** of 4500K) had comparable performance of body weight, body weight uniformity, and mortality as compared to the counterparts reared under a warm-white fluorescent light (CCT of 2700K), but pullets under the LED light maintained higher circadian activity levels (Liu *et al.*, 2017). ATAK-S commercial laying hens under incandescent, fluorescent, and cool-daylight LED (CCT of 6200K) lights had no difference in body weight at sexual maturity, feed intake, feed conversion, livability, egg production, or egg quality parameters at 16-52 weeks of age (**WOA**) (Kamanli *et al.*, 2015). When comparing a Nodark® poultry-specific LED light (CCT of 4100K) with a warm-white fluorescent light in commercial aviary hen houses, no differences were detected in egg weight, hen-day egg production, feed use, or mortality of DeKalb white hens for 20-70 WOA (Long *et al.*, 2016a). However, hens under the fluorescent light had higher number of eggs per hen housed and better feed conversion than those under the LED light (Long *et al.*, 2016a). This study also revealed that hens under the LED light laid eggs with higher egg weight, albumen height, and albumen weight at 27 WOA, thicker eggshells at 40 WOA, but lower egg weight at 60 WOA (Long *et al.*, 2016). Considering these limited and inconsistent results, along with the increasing adoption of the poultry-specific LED lights, it seems justifiable to further investigate the responses of poultry to the emerging LED lighting.

The objectives of this study were: a) to assess the effects of a Dim-to-Red® poultry-specific LED light (**PS-LED**) *vs.* a warm-white fluorescent light (**FL**) on timing of sexual maturity, egg production performance, egg quality, and egg yolk cholesterol content of W-36 laying hens during laying phase at 17-41 WOA, and b) to evaluate the earlier exposure to a Dim-to-Blue® PS-LED *vs.* a warm-white FL during pullet-rearing phase (1-16 WOA) on the above-mentioned parameters. The results are expected to contribute to supplementing the existing lighting guidelines or decision-making about light source for egg production.

Materials and Methods

This study was conducted in the Livestock Environment and Animal Production Laboratory at Iowa State University, Ames, Iowa, USA. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC Log # 3-15-7982-G).

Experimental Light, Birds, and Facility

Experimental Light

A Dim-to-Red® PS-LED (AgriShift, JLL, LED, 8 W, Once, Inc., Plymouth, MN, USA[15]) and a warm-white FL (MicroBrite MB-801D, CCFL, 8W, Litetronics, Alsip, IL, USA) were used for the laying phase; whereas a Dim-to-Blue® PS-LED (AgriShift, MLB, LED, 12 W, Once, Inc.) and a warm-white FL (EcoSmart, CFL, 9 W, Eco Smart Lighting Australia Pty Ltd, Sydney, Australia) were used for pullet-rearing. The characteristics and the spectral distributions of these light sources are described in Table 1 and Figure 1, respectively.

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[15] Mention of product or company name is for presentation clarity and does not imply endorsement by the authors or Iowa State University, nor exclusion of other suitable products.

Table 1. Characteristics of the warm-white fluorescent light, Dim-to-Blue® PS-LED [1], and Dim-to-Red® PS-LED involved in this study

Light Type	CCT [2] (K)	Flicker Frequency (Hz)	Spectral Distribution
Warm-white fluorescent light [3]	2700	120	Discrete spectrum, main spectral spikes at 545 and 610 nm
Dim-to-Blue® PS-LED	4550	120	Continuous spectrum, spectral spikes at 450 and 630 nm, with a predominant spectral output at 430-460 nm
Dim-to-Red® PS-LED	2000	120	Continuous spectrum, spectral spikes at 450 and 630 nm, with a predominant spectral output at 610-640 nm

[1] PS-LED = poultry-specific LED light

[2] CCT = correlated color temperature

[3] Fluorescent light refers to both compact fluorescent light (CFL) and cold-cathode fluorescent light (CCFL). CFL and CCFL have essentially identical spectral characteristics.

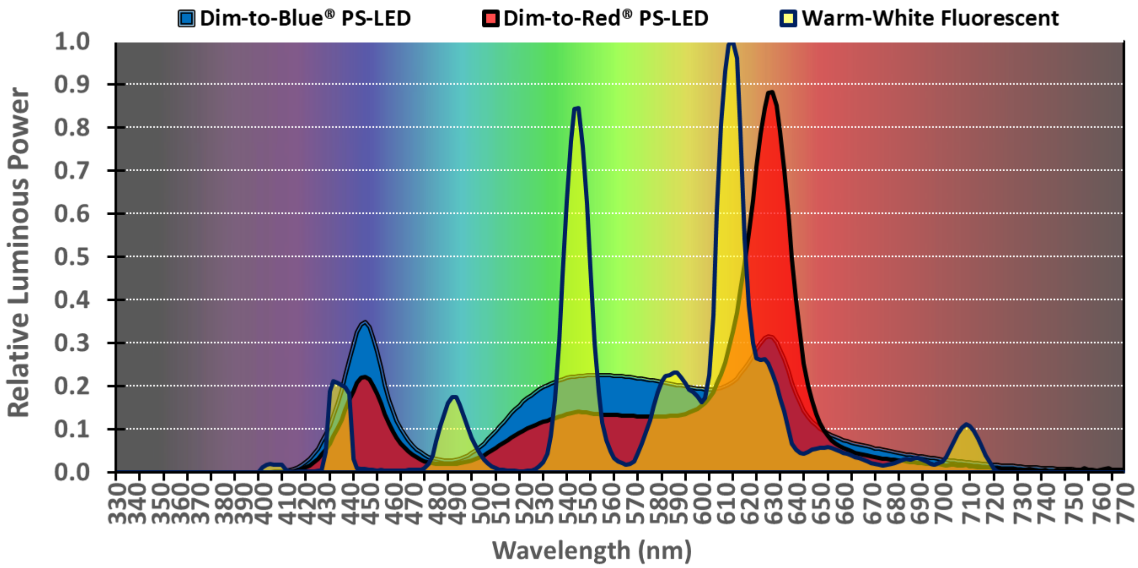

Figure 1. Spectral characteristics of the warm-white fluorescent light, Dim-to-Blue® PS-LED, and Dim-to-Red® PS-LED involved in this study. PS-LED = poultry-specific LED light. Fluorescent light refers to compact fluorescent light (CFL) and cold-cathode fluorescent light (CCFL) which have essentially identical spectral characteristics.

Experimental Birds

Hy-Line W-36 layers were used in the study. A total of 432 birds in two successive batches (216 birds per batch) were procured from Hy-Line Research Farm Facility at Dallas Center, Iowa, USA. The birds were hatched at Hy-Line hatchery on Mar 19, 2015 and Oct 9, 2015, respectively. All the birds were reared in litter floor rooms until onset of the experiment at 17 WOA. The birds were not beak-trimmed and identified individually with wing bands. Detailed information regarding the rearing conditions (housing, lighting, feeding management, etc.) of the birds and their growing performance (body weight, body weight uniformity, and mortality) during the rearing phase have been presented in a separated paper (Liu *et al.*, 2017). Of the 216 birds of each batch, half (108) had been reared under the Dim-to-Blue® PS-LED and the other half under the warm-white FL. Consequently, the birds were separated into two categories according to their light exposure during the rearing phase, namely, hens with pullet phase under PS-LED (**$\mathbf{P_{LED}}$**) and hens with pullet phase under FL (**$\mathbf{P_{FL}}$**). All the birds had similar physiological and welfare conditions at the experiment onset, including comparable body weight, skeleton and feet health, and feather coverage. Birds from each category were then randomly assigned to 18 groups, with 6 birds per group.

Experimental Facility

Four identical environmental chambers, each measuring 1.8 × 1.5 × 2.4 m (L×W×H), were used in the laying phase. Two chambers used the Dim-to-Red® PS-LED and the other two used the warm-white FL. Each chamber contained nine cages (3 cages per tier × three tiers), with each measuring 50 × 56 × 40 cm and holding up to six hens with a space allowance of 467 cm^2/bird. Each cage had a 48 × 15 × 10 cm rectangular feeder outside the front wall, two nipple drinkers on the back wall (36 cm above floor), and a 48 × 60 × 5 cm manure collection pen underneath the wire-mesh floor. The thermal environment conditions in the chambers were controlled using an air handling unit with an air flow rate of 0.24 m^3/s (Parameter Generation & Control, Black Mountain, NC, USA). The indoor temperature and relative humidity (**RH**) were essentially identical in all four chambers, maintained at 20-26°C and 45-65% RH. The actual indoor temperature and RH during the laying phase in this study are shown in Figure 2.

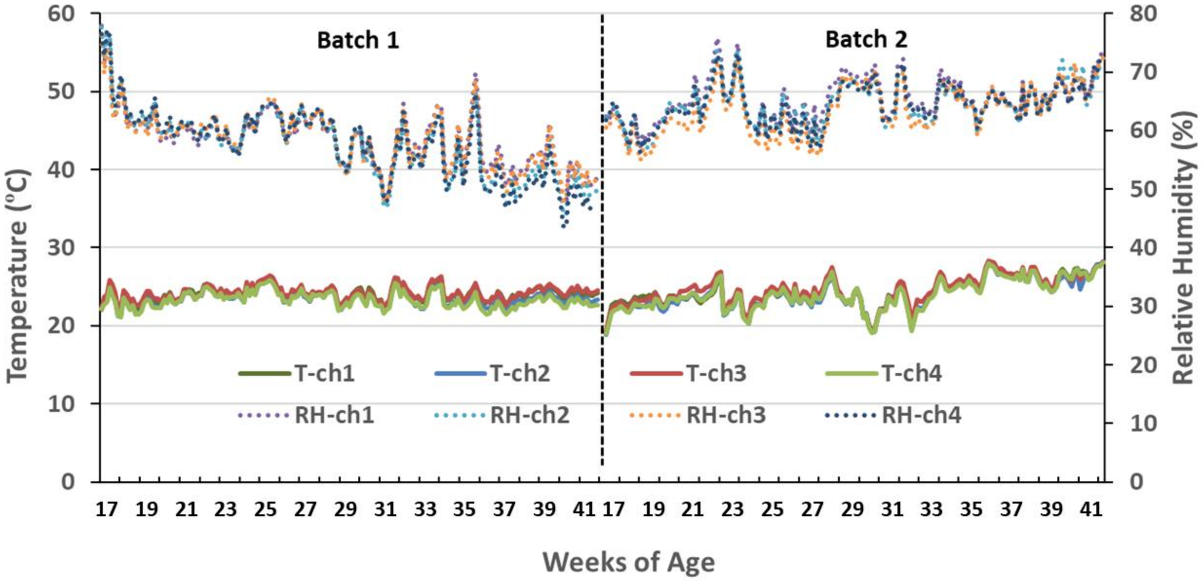

Figure 2. Daily mean indoor temperature (T) and relative humidity (RH) throughout the experiment. Legends "T-ch1" and "RH-ch1" stand for T and RH in chamber 1, respectively.

Birds Assignment, Light Program, and Birds Husbandry

Birds Assignment

For each test batch, eighteen 6-bird groups of each bird category (P_{LED} or P_{FL}) were randomly assigned to the four environmental chambers (Fig. 3). Specifically, nine groups of P_{LED} or P_{FL} were randomly assigned to nine cages in two chambers equipped with PS-LED and the other nine groups were randomly assigned to nine cages in the other two chambers equipped with FL, with four or five groups per chamber. Birds were then separated into two categories according to the light conditions for the laying phase, namely, hens with layer phase under PS-LED (**L_{LED}**) and hens with layer phase under FL (**L_{FL}**). Consequently, birds were designated by their light exposure during laying and rearing phases, i.e., L_{LED}-P_{LED}, L_{LED}-P_{FL}, L_{FL}-P_{LED}, and L_{FL}-P_{FL}.

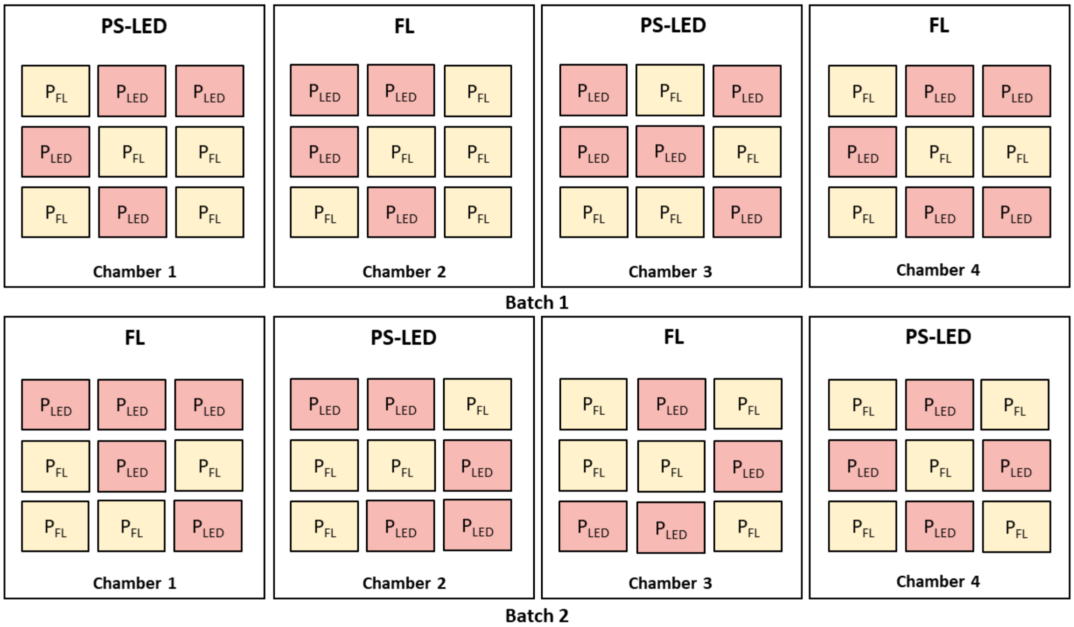

Figure 3. Treatment arrangements in the study. PS-LED = poultry-specific LED light; FL = fluorescent light; P_{FL} = hens with pullet phase under FL; P_{LED} = hens with pullet phase under PS-LED. “PS-LED” and “FL” stand for light type used in the environmental chamber.

Light Program

Daily photoperiod used in the study, varying with bird age, followed the Hy-Line W-36 Commercial Layers Management Guideline (Hy-Line International, 2016), i.e., 11-h light at 17 WOA; increased by 0.5 h per week till 23 WOA; then increased by 0.25 h per week until reaching a 16-h light at 31 WOA; 16-h light afterwards. Light intensity was determined using a spectrometer (GL SPECTIS 1.0 Touch, JUST Normlicht Inc., Langhorne, PA, USA) coupled with a software (SpectraShift 2.0, Once, Inc.) specifically designed for measuring poultry-perceived light intensity in p-lux (Prescott *et al.*, 2003). Inside each environmental chamber, two light bulbs were installed on the side wall (same side as the feeders). The light bulbs were partially covered by lightproof film strips to provide a relatively uniform light distribution among the cages. Light intensities were 25 p-lux at the feeder level for all the cages at the beginning of the experiment and then lowered to 15 p-lux at 21 WOA due to observed aggression among some birds. The coefficient of variation (**CV**) of the light intensity distributions at the feeders in each chamber was < 10%.

Birds Husbandry

All the layers were housed in the environmental chambers for a 25-week test period (17-41 WOA). Commercial corn and soy diets were formulated to meet the nutritional recommendations for layers based on their production rate and egg size (Hy-Line International, 2016), i.e., pre-lay diet [16.50% CP, 2911-2955 kcal/kg ME], peaking diet [16.00% CP, 2844-2955 kcal/kg ME], and layer diet [15.50% CP, 2844-2944 kcal/kg ME]. Feed and water were available *ad-libitum* throughout the test period. A daily quantify of feed was manually added to each feed trough in the morning (07:00 h-08:00 h) to prevent spillage. The remaining feed was weighed at the end of each week to determine weekly feed use. Eggs were collected daily from each cage in the afternoon (15:00 h -16:00 h). The number of eggs and total weight for each cage were recorded. Birds were visually inspected daily. Birds with apparent injury (bleeding, open wounds, etc.) were removed from the cage according to the IACUC protocol. Manure pens were cleaned twice a week. Hens were weighed at 17 (placement), 21 (sexual maturity), 25, 29, 33, and 41 WOA on a cage basis.

Data Collection and Measurements

Timing of Sexual Maturity

Age at sexual maturity (**ASM**, d) was determined for each bird group by determining the age of each group when their egg production rate reached 50%. Hens were then weighted to determine the body weight at sexual maturity (**BWSM**, kg) on a cage basis.

Egg Production Performance

The test period was divided into six sub-periods (**SP**), i.e., SP 1 at 17-21 WOA, SP 2 at 22-25 WOA, SP 3 at 26-29 WOA, SP 4 at 30-33 WOA, SP 5 at 34-37 WOA, and SP 6 at 38-41 WOA. Hen-day egg production (**HDEP**, %), egg weight (**EW**, g), daily feed intake (**DFI**, g/bird-day), and feed conversion ratio (**FCR**, kg feed/kg egg) during each SP and over the entire test period (17-41 WOA) were calculated for each cage based on the experiment records (daily egg number, daily egg mass, and weekly feed use). Eggs per hen-housed (**EHH**) by the end of the test period (41 WOA) was also calculated.

Egg Quality

Egg quality parameters were analyzed at 23, 32, and 41 WOA, with 12 fresh eggs per cage measured at each age. All the eggs were collected in two or three consecutive days and were tested within 24 h after collection. Egg weight (**EW**, g), albumen height (**AH**, mm), Haugh unit (**HU**), yolk color factor (**YCF**), shell strength (**SS**, N), and shell thickness (**ST**, mm) were measured using a Digital Egg Tester (NABEL DET 6000, NABEL Co., Ltd., Kyoto, Japan). Yolk was separated from the albumen to determine yolk weight (**YW**, g) and yolk percentage (**YP**, %). Albumen weight (**AW**, g) was calculated by subtracting yolk and shell weights from egg weight. Mean values of the 12 eggs of each cage were then calculated for the subsequent statistical analyses. The separated yolks were mixed homogenously for each cage for the subsequent cholesterol determination.

Egg Yolk Cholesterol

Yolk cholesterol concentration (**YCC**, mg/g yolk) and total cholesterol content (**TCC**, mg/egg yolk) were analyzed at 23, 32, and 41 WOA following the analysis of egg quality. The yolk samples of the four or five cages from the same category of birds (P_{LED} or P_{FL}) in each chamber were randomly combined into two samples for the subsequent cholesterol determination, thus forming four samples per chamber. The concentration and total cholesterol in yolk samples were determined using a colorimetric method by applying a Wako commercial cholesterol kit (Cholesterol E, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Yolk samples were dried using a freeze dryer (Virtis Genesis 25LE, SP Scientific Company, NY, USA) and ground with a mortar and pestle. Each freeze-dried yolk sample was separated into two subsamples for analysis. All the operations followed the standard procedures stated in the cholesterol kit manual. Specifically, a small quantity of freeze-dried yolk sample (2 mg) was well mixed with 2 mL of buffer and color reagent from the kit. For the blank and standard samples, deionized water and standard cholesterol regent provided in the kit was used, respectively. The mixtures were incubated for 75 min at 37ºC for color development and then filtered with 0.45 µm polytetrafluoroethylene filter (Thermo fisher Scientific Inc., MA, USA). All the samples were then tested at 600 nm using a Multi-Mode Microplate Reader (Synergy H4 Hybrid, BioTek Instruments, Inc., Winooski, VT, USA). Cholesterol concentration was calculated using the equation derived from the curve developed using the standard samples.

Statistical Analysis

Statistical analyses were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, NC, USA). All variables were analyzed with linear mixed models by implementing PROC MIXED procedure. As the experiment followed a split-plot design, the environmental chambers (whole plots) and the individual cages (split-plots) were treated as the experimental units for light treatments during the laying phase (laying-light) and the rearing phase (rearing-light), respectively. All the variables were analyzed separately for each age or period. All the statistical models were of the following form:

$$Y_{ijklm} = \mu + B_i + L_j + R_k + (BL)_{ij} + (BR)_{ik} + (LR)_{jk} + (BLR)_{ijk} + (CB)_{li} + (SCB)_{mli} + E_{ijklm}$$

Where Y_{ijklm} denotes the independent observation; μ is the overall mean; B_i is the batch effect (fixed); L_j is the laying-light effect (fixed); R_k is the rearing-light effect (fixed); $(BL)_{ij}$ is the interaction effect of batch and laying-light (fixed); $(BR)_{ik}$ is the interaction effect of batch and rearing-light (fixed); $(LR)_{jk}$ is the interaction effect of laying-light and rearing-light (fixed); $(BLR)_{ijk}$ is the interaction effect of batch, laying-light, and rearing-light (fixed); $(CF)_{li}$ is the chamber effect within each batch (random); $(SCB)_{mli}$ is the sample or cage effect within each chamber for each batch (random); and E_{ijklm} is the random error with a normal distribution with mean μ and variance σ^2 $[N \sim (\mu, \sigma^2)]$. For all models, Tukey-Kramer tests were used for pairwise comparisons, if applicable. Normality and homogeneity of variance of data were examined by residual diagnostics. Effects were considered significant when $p < 0.05$. Unless otherwise specified, data are presented as least squares means with the standard error of the mean (**SEM**).

Results

Overall, light sources of PS-LED and FL during the laying phase of 17-41 WOA or during the rearing phase of 1-16 WOA had no effect on timing of sexual maturity (Table 2), egg production performance (Table 3), egg quality parameters (except for ST and SS) (Table 4), or yolk cholesterol of laying hens (Table 5). However, interaction between light exposure during the laying and rearing phases were found on EW, SS, and ST. Detailed results for each performance aspect are presented in the following sections.

Timing of Sexual Maturity

L_{LED} and L_{FL}, or P_{LED} and P_{FL} had comparable ASM and BWSM (Table 2).

Table 2. Age and body weight at sexual maturity (50% rate of lay) as affected by light during rearing and laying phases [1]

Parameter	Light during Laying (L)			Light during Rearing (P)			p-value		
	L_{LED} [2]	L_{FL} [3]	SEM	P_{LED} [4]	P_{FL} [5]	SEM	L	P	L×P
ASM [6] (d)	143.4	141.7	0.67	142.9	142.2	0.55	0.14	0.23	0.21
BWSM [7] (kg)	1.45	1.46	0.01	1.46	1.45	0.01	0.77	0.57	0.72

[1] Data are least square means ± SEM. For each category, data with different superscript letters are significantly different at $p < 0.05$. [2] L_{LED} = hens with layer phase under PS-LED. [3] L_{FL} = hens with layer phase under FL. [4] P_{LED} = hens with pullet phase under PS-LED. [5] P_{FL} = hens with pullet phase under FL. [6] ASM = age at sexual maturity (d). [7] BWSM = body weight at sexual maturity (kg)

Egg Production Performance

L_{LED} and L_{FL}, or P_{LED} and P_{FL} had comparable HDEP, EHH, EW, DFI, and FCR for the test period of 17-41 WOA (Table 3). However, L_{FL}-P_{FL} laid eggs with significantly lower EW than L_{FL}-P_{LED} (57.9 ± 0.36 g *vs.* 58.9 ± 0.36 g, $p = 0.01$). When comparing production performance of the laying hens for each SP, L_{LED} had significantly higher DFI at 34-37 WOA and tended to have higher DFI and HDEP at 38-41 WOA as compared to L_{FL}. P_{LED}

had significantly higher DFI at 30-33 WOA and 38-41 WOA, and tended to have higher HDEP at 30-33 WOA as compared to P_{FL}. In addition, L_{FL}-P_{FL} laid eggs with significantly lower EW than L_{FL}-P_{LED} (59.5 ± 0.32 g *vs.* 60.6 ± 0.32 g, p = 0.03) at 30-33 WOA.

Egg Quality

L_{LED} and L_{FL}, or P_{LED} and P_{FL} had comparable EW, AW, AH, HU, YW, YP, and YCF at 23, 32, and 41 WOA (Table 4). However, L_{LED} laid eggs with significantly lower ST and SS at 41 WOA as compared to L_{FL}. P_{LED} laid eggs with significantly lower ST at 32 WOA as compared to P_{FL}. In addition, L_{FL}-P_{LED} laid eggs with significantly higher EW than L_{LED}-P_{LED} (63.3 ± 0.41 g *vs.* 61.7 ± 0.41 g, p = 0.04) at 41 WOA. L_{FL}-P_{FL} laid eggs with significantly higher SS than L_{LED}-P_{FL} (38.9 ± 0.41 N *vs.* 37.4 N, p = 0.04) at 41 WOA. Besides, L_{FL}-P_{LED} laid eggs with the highest ST (0.44 ± 0.00 mm), while L_{LED}-P_{LED} laid eggs with the lowest ST (0.42 ± 0.00 mm) at 41 WOA.

Table 3. Egg production at 17-41 weeks of age (WOA) as affected by light during rearing and laying phases [1]

Parameter	Period (WOA)	Light during Laying (L)			Light during Rearing (P)			p-value		
		L_{LED} [2]	L_{FL} [3]	SEM	P_{LED} [4]	P_{FL} [5]	SEM	L	P	L×P
EHH [6]	17-41	125.0	124.7	1.50	125.6	124.1	2.56	0.87	0.75	0.86
HDEP [7] (%)	17-41	74.9	75.1	0.49	75.2	74.9	0.61	0.78	0.76	0.90
	17-21	11.7	13.7	1.06	12.0	13.4	0.91	0.25	0.17	0.28
	22-25	89.5	90.5	0.31	90.0	90.0	0.62	0.10	0.99	0.62
	26-29	95.0	94.8	0.92	95.1	94.7	0.85	0.92	0.71	0.48
	30-33	94.7	93.9	0.50	95.1	93.4	0.58	0.33	0.08	0.35
	34-37	92.2	90.7	0.97	91.3	91.6	0.99	0.35	0.83	0.22
	38-41	90.2	87.6	0.79	88.7	89.1	0.86	0.08	0.77	0.33
EW [8] (g)	17-41	58.3	58.4	0.31	58.4	58.3	0.25	0.80	0.54	0.01
	17-21	47.7	47.8	0.35	47.8	47.7	0.33	0.85	0.77	0.17
	22-25	53.7	53.9	0.33	53.8	53.8	0.26	0.80	0.81	0.29
	26-29	57.8	57.8	0.28	57.9	57.6	0.32	0.97	0.35	0.18
	30-33	59.9	60.0	0.25	60.0	59.9	0.23	0.73	0.63	0.05
	34-37	60.6	61.0	0.34	60.8	60.8	0.27	0.35	0.95	0.14
	38-41	61.8	62.0	0.32	61.9	61.9	0.28	0.57	0.96	0.22
DFI [9] (g/day-bird)	17-41	96.9	96.4	0.49	97.3	96.0	0.53	0.55	0.10	0.21
	17-21	71.2	72.0	0.95	71.6	71.7	0.75	0.56	0.88	0.41
	22-25	94.9	94.7	0.87	95.5	94.2	0.79	0.88	0.20	0.26
	26-29	103.9	104.4	0.83	104.8	103.4	0.78	0.69	0.18	0.95
	30-33	106.2	105.3	0.98	106.7^{a}	104.8^{b}	0.80	0.55	0.02	0.10
	34-37	106.1^{a}	103.8^{b}	0.49	105.3	104.6	0.74	0.04	0.57	0.26
	38-41	109.0	107.2	0.51	109.2^{a}	107.0^{b}	0.65	0.07	0.05	0.33
FCR [10] (kg feed/kg egg)	17-41	2.22	2.20	0.02	2.22	2.21	0.02	0.43	0.62	0.77
	17-21	19.68	13.52	3.22	17.82	15.38	2.58	0.25	0.32	0.41
	22-25	1.98	1.95	0.02	1.98	1.95	0.02	0.29	0.24	0.58
	26-29	1.90	1.91	0.02	1.91	1.90	0.02	0.72	0.66	0.87
	30-33	1.88	1.87	0.01	1.87	1.87	0.01	0.75	1.00	0.43
	34-37	1.90	1.88	0.02	1.90	1.88	0.02	0.39	0.47	0.16
	38-41	1.97	1.97	0.02	2.00	1.94	0.02	0.82	0.09	0.17

[1] Data are least square means ± SEM. For each category, data with different superscript letters are significantly different at $p < 0.05$. [2] L_{LED} = hens with layer phase under PS-LED. [3] L_{FL} = hens with layer phase under FL. [4] P_{LED} = hens with pullet phase under PS-LED. [5] P_{FL} = hens with pullet phase under FL. [6] EHH = eggs per hen housed. [7] HDEP = hen-day egg production (%). [8] EW = egg weight (g). [9] DFI = daily feed intake (g/bird-day). [10] FCR = feed conversion ratio (kg feed/kg egg).

Table 4. Egg quality at 23, 32, and 41 weeks of age (WOA) as affected by light during rearing and laying phases [1]

Parameters	Age (WOA)	Light during Laying (L)			Light during Rearing (P)			p-value		
		L_{LED} [2]	L_{FL} [3]	SEM	P_{LED} [4]	P_{FL} [5]	SEM	L	P	L×P
EW [6] (g)	23	53.7	53.6	0.24	53.7	53.6	0.25	0.84	0.71	0.41
	32	60.1	60.2	0.16	60.3	60.0	0.22	0.50	0.26	0.27
	41	62.0	62.7	0.33	62.5	62.2	0.29	0.25	0.31	0.05
AW [7] (g)	23	36.5	36.2	0.21	36.4	36.3	0.19	0.43	0.74	0.24
	32	39.1	39.2	0.14	39.3	39.0	0.17	0.80	0.29	0.16
	41	39.7	40.0	0.34	39.9	39.8	0.28	0.52	0.66	0.12
AH [8] (mm)	23	9.6	9.7	0.07	9.6	9.7	0.07	0.22	0.27	0.39
	32	9.1	9.1	0.06	9.1	9.1	0.07	0.90	0.64	0.97
	41	9.0	9.0	0.06	9.0	9.1	0.07	0.77	0.42	0.86
HU [9]	23	98.4	98.8	0.31	98.3	98.9	0.32	0.46	0.25	0.58
	32	95.1	95.0	0.31	94.9	95.2	0.32	0.91	0.56	0.92
	41	93.5	92.6	0.38	92.9	93.2	0.36	0.14	0.47	0.26
ST [10] (mm)	23	0.44	0.44	0.00	0.44	0.44	0.00	0.43	0.96	0.76
	32	0.43	0.43	0.00	0.43^{b}	0.44^{a}	0.00	0.89	0.02	0.15
	41	0.42^{b}	0.44^{a}	0.00	0.43	0.43	0.00	0.01	0.53	0.01
SS [11] (N)	23	42.4	42.1	0.30	42.0	42.5	0.34	0.55	0.43	0.77
	32	39.1	39.2	0.36	39.0	39.3	0.39	0.88	0.43	0.87
	41	37.5^{b}	38.8^{a}	0.22	38.2	38.1	0.38	0.03	0.99	0.01
YW [12] (g)	23	11.4	11.5	0.08	11.5	11.5	0.08	0.40	0.83	0.79
	32	14.8	14.9	0.05	14.9	14.8	0.07	0.26	0.34	0.41
	41	16.0	16.2	0.10	16.2	16.0	0.09	0.22	0.17	0.22
YP [13] (%)	23	21.3	21.6	0.11	21.4	21.4	0.10	0.15	0.96	0.16
	32	24.6	24.8	0.07	24.7	24.7	0.08	0.23	0.55	0.15
	41	25.8	25.9	0.08	25.9	25.8	0.09	0.53	0.54	0.16
YCF [14]	23	6.9	6.9	0.04	6.9	6.9	0.04	0.51	0.31	0.54
	32	6.7	6.7	0.04	6.7	6.7	0.04	0.64	0.77	0.91
	41	7.1	7.1	0.04	7.1	7.1	0.04	0.33	0.70	0.42

[1] Data are least square means ± SEM. For each category, data with different superscript letters are significantly different at $p < 0.05$. [2] L_{LED} = hens with layer phase under PS-LED. [3] L_{FL} = hens with layer phase under FL. [4] P_{LED} = hens with pullet phase under PS-LED. [5] P_{FL} = hens with pullet phase under FL. [6] EW = egg weight (g). [7] AW = albumen weight (g). [8] AH = albumen height (mm). [9] HU = Haugh Unit. [10] ST = shell thickness (mm). [11] SS = shell strength (N). [12] YW = yolk weight (g). [13] YP = yolk percentage (%). [14] YCF = yolk color factor

Egg Yolk Cholesterol

L_{LED} and L_{FL}, or P_{LED} and P_{FL} had comparable YCC and TCC at 23 and 32 WOA (Table 5). However, L_{LED} tended to lay eggs with lower YCC and TCC at 41 WOA than L_{FL} ($p = 0.06$ and 0.07, respectively).

Table 5. Egg cholesterol content at 23, 32, and 41 weeks of age (WOA) as affected by light during rearing and laying phases [1]

Parameters	Age (WOA)	Light during Laying (L)			Light during Rearing (P)			p-value		
		L_{LED} [2]	L_{FL} [3]	SEM	P_{LED} [4]	P_{FL} [5]	SEM	L	P	L×P
YCC [6] (mg/g yolk)	23	10.1	10.0	0.27	10.1	9.9	0.24	0.77	0.48	0.90
	32	8.5	8.8	0.31	8.7	8.6	0.26	0.48	0.82	0.33
	41	8.3	8.7	0.12	8.5	8.5	0.16	0.06	0.78	0.18
TCC [7] (mg/egg yolk)	23	115.0	115.2	3.34	116.4	113.8	3.18	0.97	0.54	0.95
	32	125.6	131.9	4.69	129.7	127.8	3.94	0.39	0.65	0.31
	41	132.6	141.4	2.76	137.0	137.1	2.88	0.07	0.98	0.23

[1] Data are least square means ± SEM. For each category, data with different superscript letters are significantly different at $p < 0.05$. [2] L_{LED} = hens with layer phase under PS-LED. [3] L_{FL} = hens with layer phase under FL. [4] P_{LED} = hens with pullet phase under PS-LED. [5] P_{FL} = hens with pullet phase under FL. [5] YCC = yolk cholesterol content (mg/g yolk). [6] TCC = total cholesterol content (mg/egg yolk).

Discussion

Our review of literature revealed limited data from comparative studies regarding the effects of poultry-specific LED lights on laying hen performance. The current study assessed timing of sexual maturity, egg production, egg quality, and egg yolk cholesterol of W-36 laying hens subjected to poultry-specific LED lights *vs.* fluorescent lights during rearing and laying phases, and showed that the light treatments during rearing or laying phase led to comparable laying hen performance.

Effect of Light on Timing of Sexual Maturity

Earlier studies demonstrated that exposure to long-wavelength lights (e.g., red light) could accelerate sexual development and maturity of poultry as compared to exposure to short-wavelength lights (e.g., blue and green) (Woodard *et al.*, 1969; Gongruttananun, 2011; Min *et al.*, 2012; Hassan *et al.*, 2013; Huber-Eicher *et al.*, 2013; Baxter *et al.*, 2014; Yang *et al.*, 2016). Based on this result, it seems reasonable to assume that a lighting source emitting relatively higher proportion of light at long-wavelength range would be more efficient in facilitating sexual development and advancing sexual maturity of juvenile hens than a lighting source emitting lower proportion of light at long-wavelength range, especially when all the other factors remain the same (e.g., photoperiod, light intensity, and nutrition). However, our results from the current study did not support this hypothesis. In this study, the Dim-to-Red® PS-LED (about 48% of light components are red lights) and the warm-white FL (about 19% of light component are red lights) led to comparable sexual development of the W-36 laying hens. These results might infer that advancement of sexual maturity of poultry is not proportional to the amount of stimulation (e.g., red light radiation) perceived by the birds. There may exist a threshold in poultry's response to long-wavelength radiation. When the amount of the long-wavelength radiation reaches the threshold, the reproductive axis of poultry may not be further stimulated. The typical lighting sources used in commercial poultry production systems, such as incandescent, fluorescent, and poultry-specific LED lights, emit considerable amounts of red light. Consequently, these lighting sources may provide sufficient exposure to the birds to yield comparable sexual maturity. This inference seems consistent with findings from several earlier studies. Pyrzak *et al.* (1986) found incandescent, cool-white fluorescent, and sunlight-simulating fluorescent lights had no effect on age at the first egg of juvenile hens. Kamanli *et al.* (2015) found the use of incandescent, fluorescent, or white LED light did not cause a significant difference in body weight at sexual maturation. On the contrary, Bobadilla-Mendez *et al.* (2016) found that white LED light was more efficient at activating the reproductive cycle, hastening the onset of sexual maturity, and increasing the development of reproductive organs after puberty of female Japanese quail as compared to incandescent and fluorescent lights. As quail and laying hen are very different in their physiology (e.g., quail reaches sexual maturity much earlier than laying hens), the different responses to lighting sources may be attributed to their physiological differences.

Effect of Light on Egg Production Performance

Some earlier studies also demonstrated that exposure to long-wavelength lights (e.g., red light) could facilitate egg production of poultry as compared to exposure to short-wavelength lights (Pyrzak *et al.*, 1987; Min *et al.*, 2012; Huber-Eicher *et al.*, 2013; Borille *et al.*, 2013; Hassan *et al.*, 2014; Baxter *et al.*, 2014; Wang *et al.*, 2015; Yang *et al.*, 2016). Thus, the initial hypothesis for the study was that the Dim-to-Red® PS-LED would lead to improved egg production performance as compared to the warm-white FL. However, the results from the current study did not support this hypothesis. Instead, the Dim-to-Red® PS-LED and the warm-white FL in this study led to comparable egg production performance of the hens at 17-41 WOA. Again, these results seem to provide evidence supporting the existence of a threshold in poultry response to long-wavelength radiation beyond which the reproductive axis (e.g., egg production) would not be further stimulated. The results of the current study agreed well with several earlier studies. Siopes (1984) found that there were no significant differences in feed intake and egg production of turkey breeder hens between incandescent and fluorescent lights during two 20-wk reproductive cycles. Gongruttananun (2011) found that Thai-native hens exposed to red light or natural daylight supplemented with fluorescent light had comparable egg production performance. Kamanli *et al.* (2015) found the use of incandescent, fluorescent, or LED light did not cause significant differences in daily feed intake, feed conversion efficiency, or egg production. Similar to the current study, Long *et al.* (2016a) reported comparable egg weight, hen-day egg production, and feed use of Dekalb white hens under a Nodark® poultry-specific LED *vs.* a warm-white fluorescent light in commercial aviary houses. However, hens under the fluorescent light had higher eggs per hen housed (321 *vs.* 308) and better feed conversion (1.99 *vs.* 2.03 kg feed/kg egg) than those under the LED light (Long *et al.*, 2016a). In terms of the light exposure during rearing period, Schumaier *et al.* (1968) found the rearing light color of red, green, or white had no effect on egg production or egg weight of White leghorn hens at 20-61 WOA. Wells (1971) found that red and white lights used during rearing had no effect on peak egg production, eggs per hen-housed, feed consumption, or feed conversion of Hybrid-3 laying hens at 20-52 WOA. The current study agreed with these earlier findings as the two light treatments during rearing did not cause any difference in production performance of hens during the subsequent laying phase.

Effect of Light on Egg Quality Parameters

Some earlier studies found that exposure to short-wavelength lights (e.g., green and blue lights) led to improved egg quality (e.g., increased egg weight, shell thickness, or shell strength) as compared to exposure to long-wavelength lights (e.g., red light) (Pyrzak *et al.*, 1987; Er *et al.*, 2007; Min *et al.*, 2012; Hassan *et al.*, 2014; Li *et al.*, 2014). Interestingly, the improved egg quality in these cited studies, to a certain extent, was associated with the relatively lower egg production rate of birds as reported in the studies. Among the many cited studies that reported no differences between or among lights in sexual maturity or egg production performance of birds (Wells, 1971; Gongruttananun, 2011; Borille *et al.*, 2013; Borille *et al.*, 2015; Kamanli *et al.*, 2015; Nunes *et al.*, 2016), the different lighting sources or spectra were also found to have no effect on egg quality. For example, Borille *et al.* (2013) found that the internal egg quality (albumen height, specific gravity, and Haugh units) of ISA Brown hens at 56-72 WOA were not influenced by lighting source of incandescent light, blue, yellow, green, red, or white LED light. Kamanli *et al.* (2015) found that the use of incandescent, fluorescent, or LED light did not cause significant differences in egg quality parameters. On the other hand, a few studies reported opposite results. Li *et al.* (2014) found that hens exposed to red light laid heavier eggs with a greater egg shape index than hens exposed to white, blue or green light. Min *et al.* (2012) found the birds reared under red light exhibited significantly increased egg shell thickness compared to birds reared under incandescent light and blue light. In general, the results from this study are consistent with the most findings from the earlier studies. Namely, the Dim-to-Red® PS-LED and the warm-white FL in the current study led to comparable egg quality parameters of laying hens in terms of the egg weight, albumen weight, Haugh unit, yolk weight, yolk percent, or yolk color factor at 23, 32 and 41 WOA. However, hens under the PS-LED light laid eggs with significantly lower shell thickness and shell strength than hens under the fluorescent light at 41 WOA in the current study. These results are opposite to an earlier study conducted by Long *et al.* (2016b) who reported that Dekalb white hens in commercial aviary houses under a poultry-specific LED laid eggs with significantly higher shell thickness at 40 WOA as compared to hens under a warm-white fluorescent light. One speculation is that Hy-Line W-

36 hens used in the current study may have different responses to the lights as compared to Dekalb white hens due to their genetic differences. These two breeds of hens have been found to have different responses to dietary energy (Harms *et al.*, 2000). However, the speculation of genetic differences regarding responses to the lights remains to be further examined.

Effect of Light on Egg Yolk Cholesterol

Our literature review revealed very limited information regarding the effect of lights on egg yolk cholesterol. In laying hens, cholesterol is primarily biosynthesized in the liver and ovary of birds, and the egg represents a major excretory route of cholesterol (Elkin 2006). Elkin (2006) reviewed common strategies for reducing egg cholesterol content and pointed out that cholesterol content in egg yolks are mainly affected by genetics of birds, dietary nutrients, and feed intakes. Obviously, light has not be considered as an influential factor for egg cholesterol content. A recent study conducted by Long *et al.* (2016b) showed that the light exposure affected the cholesterol content, although the influence seems to be limited as compared to the other factors. When applying a Nodark® poultry-specific LED light and a warm-white fluorescent light in commercial aviary hen houses, Long *et al.* (2016b) found that the total cholesterol of eggs laid by Dekalb white hens under the LED light was significantly lower than that under fluorescent light at 60 WOA, albeit no difference between the lights in total egg cholesterol at 27 or 40 WOA, or in yolk cholesterol concentration at 27, 40, or 60 WOA. Results of the current study also inferred that the light exposure may affect the cholesterol metabolism in laying hens, although the underlining mechanism was not understood. In this study, the Dim-to-Red® PS-LED and the warm-white FL led to comparable egg yolk cholesterol content at 23 and 32 WOA, but the hens under the PS-LED tended to lay eggs with lower cholesterol than hens under the fluorescent light at 41 WOA. As most earlier lighting studies had not investigated egg cholesterol and potential effects of lights on egg cholesterol metabolism, it would be prudent to include egg cholesterol as a measurement in future lighting studies and to further study the underlining principle.

Conclusions

A total of 432 W-36 laying hens (6 hens per group) at 17-41 WOA were tested in four environmental chambers to comparatively evaluate the effects of a Dim-to-Red® PS-LED (CCT of 2000K) *vs.* a warm-white FL (CCT of 2700K) on production performance and egg quality. Half of the experimental hens were reared under a Dim-to-Blue® PS-LED (CCT of 4500K) during the pullet phase (1-16 WOA) whereas the other half reared under a warm-white FL. Hence, both prior lighting experiences were included in the laying performance test. The following general observations and conclusions were made.

- The Dim-to-Red® PS-LED and the warm-white FL during the laying period of 17-41 WOA led to comparable laying performance in all the aspects except for eggshell thickness and strength. Hens under the PS-LED laid eggs with significantly lower shell thickness and strength as compared to hens under the FL at 41 WOA. In addition, eggs in the PS-LED tended to have lower yolk cholesterol content at 41 WOA.
- Light exposure to the Dim-to-Blue® PS-LED or the warm-white FL during pullet rearing (1-16 WOA) showed no effect on the subsequent laying performance at 17-41 WOA, with the exception that hens reared under the PS-LED laid eggs with significantly lower shell thickness at 32 WOA than hens reared under the FL.

- The poultry-specific LED lights provide a viable alternative to the traditional fluorescent lights for maintaining the laying hen production performance.

Acknowledgments

Funding for the study was provided in part by the Center for Industrial Research and Service (CIRAS) at Iowa State University and Hy-Line International and is acknowledged. We also wish to thank Once Innovation Inc. for providing the LED lights and the controller used in the study, and the Agriculture Experiment Station (AES) Consulting Group at Iowa State University for the assistance in statistical analysis for the study. Author Kai Liu also wishes to thank China Scholarship Council for providing part of the financial support toward his PhD study at Iowa State University.

References

ASABE Standard. (2014). ASAE EP344.4 JAN2014-Lighting Systems for Agricultural Facilities.

Baxter, M., Joseph, N., Osborne, V. R., & Bedecarrats, G. Y. (2014). Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poultry Science, 93*(5), 1289–1297. https://doi.org/10.3382/ps.2013-03799

Bobadilla-Mendez, M. F., Rojas-Granados, C. P., Andrade, E. F., Retes, P. L., Ferreira, L. G., Alvarenga, R. R., Zangeronimo, M. G. (2016). Effect of different light sources on reproductive anatomy and physiology of Japanese quail (Coturnix coturnix japonica). *Animal Reproduction Science, 168,* 50–56. https://doi.org/10.1016/j.anireprosci.2016.02.025

Borille, R., Garcia, R. G., Nääs, I. A., Caldara, F. R., & Santana, M. R. (2015). Monochromatic light-emitting diode (LED) source in layers hens during the second production cycle. *Rodrigo Borille et Al. R. Bras. Eng. Agríc. Ambiental*, *1919*(99), 877–881. https://doi.org/10.1590/1807-1929/agriambi.v19n9p877-881

Borille, R., Garcia, R., Royer, A., Santana, M., Colet, S., Naas, I., Castilho, V. (2013). The use of light-emitting diodes (LED) in commercial layer production. *Revista Brasileira de Ciência Avícola*, *15*(2), 135–140. https://doi.org/10.1590/S1516-635X2013000200009

Cao, J., Liu, W., Wang, Z., Xie, D., Jia, L., & Chen, Y. (2008). Green and blue monochromatic lights promote growth and development of broilers via stimulating testosterone secretion and myofiber growth. *The Journal of Applied Poultry Research*, *17*(2), 211–218. https://doi.org/10.3382/japr.2007-00043

Elkin, R. G. (2006). Reducing shell egg cholesterol content. I. Overview, genetic approaches, and nutritional strategies. *World's Poultry Science Journal*, *62*(4), 665–687. https://doi.org/10.1079/WPS2006120

Er, D., Wang, Z., Cao, J., & Chen, Y. (2007). Effect of monochromatic light on the egg quality of laying hens. *The Journal of Applied Poultry Research*, *16*(4), 605–612. https://doi.org/10.3382/japr.2006-00096

Gongruttananun, N. (2011). Influence of red light on reproductive performance, eggshell ultrastructure, and eye morphology in Thai-native hens. *Poultry Science*, *90*(12), 2855–2863. https://doi.org/10.3382/ps.2011-01652

Harms, R. H., Russell, G. B., & Sloan, D. R. (2000). Energy utilization of four strains of commercial layers and influence on suggested dietary methionine level. *Journal of Applied Animal Research*, *18*(1), 25–31. https://doi.org/10.1080/09712119.2000.9706320

Harrison, P. C. (1972). Extraretinal photocontrol of reproductive responses of Leghorn hens to photoperiods of different length and spectrum. *Poultry Science*, *51*(6), 2060–4.

Harrison, P., McGinnis, J., Schumaier, G., & Lauber, J. (1969). Sexual maturity and subsequent reproductive performance of white leghorn chickens subjected to different parts of the light spectrum. *Poultry Science*, *48*(3), 878–83.

Hassan, M. R., Sultana, S., Choe, H. S., & Ryu, K. S. (2013). Effect of monochromatic and combined light colour on performance, blood parameters, ovarian morphology and reproductive hormones in laying hens. *Italian Journal of Animal Science*, *12*(3), e56. https://doi.org/10.4081/ijas.2013.e56

Hassan, M. R., Sultana, S., S.Choe, H., & S. Ryu, K. (2014). Effect of combinations of monochromatic LED light color on the performance and behavior of laying hens. *The Journal of Poultry Science*, *51*(3), 321–326. https://doi.org/10.2141/jpsa.0130105

Huber-Eicher, B., Suter, A., & Spring-Stahli, P. (2013). Effects of colored light-emitting diode illumination on behavior and performance of laying hens. *Poultry Science*, *92*(4), 869–873. https://doi.org/10.3382/ps.2012-02679

Hy-Line International. (2016). W-36 commercial layers management cuide 2016.

Kamanli, S., Durmus, I., Demir, S., & Tarim, B. (2015). Effect of different light sources on performance and egg quality traits in laying hens. *Europ.Poult.Sci.*, *79*(August). https://doi.org/10.1399/eps.2015.109

Lewis, P. D., & Morris, T. R. (1998). Responses of domestic poultry to various light sources. *World's Poultry Science Journal*, *54*(1), 7–25. https://doi.org/10.1079/WPS19980002

Lewis, P. D., & Morris, T. R. (2000). Poultry and coloured light. *World's Poultry Science Journal*, *56*(3), 189–207. https://doi.org/10.1079/WPS20000015

Li, D., Zhang, L., Yang, M., Yin, H., Xu, H., Trask, J. S., Zhu, Q. (2014). The effect of monochromatic light-emitting diode light on reproductive traits of laying hens. *The Journal of Applied Poultry Research*, *23*(3), 367–375. https://doi.org/10.3382/japr.2013-00746

Liu, K., Xin, H., & Settar, P. (2017). Effects of light-emitting diode light v. fluorescent light on growing performance, activity levels and well-being of non-beak-trimmed W-36 pullets. *Animal*, 1–10. https://doi.org/10.1017/S1751731117001240

Long, H., Zhao, Y., Wang, T., Ning, Z., & Xin, H. (2016a). Effect of light-emitting diode vs. fluorescent lighting on laying hens in aviary hen houses: Part 1 – Operational characteristics of lights and production traits of hens. *Poultry Science*, *95*(1), 1–11. https://doi.org/10.3382/ps/pev121

Long, H., Zhao, Y., Xin, H., Hansen, H., Ning, Z., & Wang, T. (2016b). Effect of light-emitting diode (LED) vs. fluorescent (FL) lighting on laying hens in aviary hen houses: Part 2 – Egg quality, shelf-life and lipid composition. *Poultry Science*, *95*(1), 115–124. https://doi.org/10.3382/ps/pev306

Min, J. K., Hossan, M. S., Nazma, A., Jae, C. N., Han, T. B., Hwan, K. K., Ok, S. S. (2012). Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biology Research*, *5*(2), 69–74. https://doi.org/10.3184/175815512X13350270679453

Mobarkey, N., Avital, N., Heiblum, R., & Rozenboim, I. (2010). The role of retinal and extra-retinal photostimulation in reproductive activity in broiler breeder hens. *Domestic Animal Endocrinology*, *38*(4), 235–243. https://doi.org/10.1016/j.domaniend.2009.11.002

Nunes, K., Garcia, R., Nääs, I., Eyng, C., Caldara, F., Sgavioli, S., Ayala, C. (2016). Effect of LED Lighting Colors for Laying Japanese Quails. *Revista Brasileira de Ciência Avícola*, *18*(spe), 51–56. https://doi.org/10.1590/1806-9061-2015-0176

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. https://doi.org/10.1016/j.visres.2008.06.018

Parvin, R., Mushtaq, M. M. H., Kim, M. J., & Choi, H. C. (2014). Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. *World's Poultry Science Journal*, *70*(3), 543–556. https://doi.org/10.1017/S0043933914000592

Prayitno, D. S., Phillips, C. J., & Omed, H. (1997). The effects of color of lighting on the behavior and production of meat chickens. *Poultry Science*, *76*(3), 452–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9068043

Prescott, N. B., Wathes, C. M., & Jarvis, J. R. (2003). Light, vision and the welfare of poultry. *Animal Welfare*, *12*(2), 269–288.

Pyrzak, R., Snapir, N., Goodman, G., Arnon, E., & Perek, M. (1986). The influence of light quality on initiation of egg laying by hens. *Poultry Science*, *65*(1), 190–193. https://doi.org/10.3382/ps.0650190

Pyrzak, R., Snapir, N., Goodman, G., & Perek, M. (1987). The effect of light wavelength on the production and quality of eggs of the domestic hen. *Theriogenology*, *28*(6), 947–960. https://doi.org/10.1016/0093-691X(87)90045-8

Rozenboim, I., Biran, I., Chaiseha, Y., Yahav, S., Rosenstrauch, A., Sklan, D., & Halevy, O. (2004). The effect of a green and blue monochromatic light combination on broiler growth and development. *Poultry Science*, *83*(5), 842–845. https://doi.org/10.1093/ps/83.5.842

Schumaier, G., Harrison, P. C., & McGinnis, J. (1968). Effect of colored fluorescent light on growth, cannibalism, and subsequent egg production of single comb white leghorn pullets. *Poultry Science*, *47*(5), 1599–1602. https://doi.org/10.3382/ps.0471599

SIOPES, T. D. (1984). The effect of full-spectrum fluorescent lighting on reproductive traits of caged turkey hens. *Poultry Science*, *63*(6), 1122–1128. https://doi.org/10.3382/ps.0631122

Sultana, S., Hassan, M. R., Choe, H. S., & Ryu, K. S. (2013). The effect of monochromatic and mixed LED light colour on the behaviour and fear responses of broiler chicken. *Avian Biology Research*, *6*(3), 207–214. https://doi.org/10.3184/175815513X13739879772128

Wang, Y., Ding, J. T., Yang, H. M., Cao, W., & Li, Y. B. (2015). The effect of new monochromatic light regimes on egg production and expression of the circadian gene BMAL1 in pigeons. *Poultry Science*, *94*(5), 836–840. https://doi.org/10.3382/ps/pev057

Wells, R. G. (1971). A comparison of red and white light and high and low dietary protein regimes for growing pullets. *British Poultry Science*, *12*(3), 313–325. https://doi.org/10.1080/00071667108415887

Woodard, a E., Moore, J. a, & Wilson, W. O. (1969). Effect of wave length of light on growth and reproduction in Japanese quail (Coturnix coturnix japonica). *Poultry Science*, *48*(1), 118–123. https://doi.org/10.3382/ps.0480118

Xie, D., Wang, Z. X., Dong, Y. L., Cao, J., Wang, J. F., Chen, J. L., & Chen, Y. X. (2008). Effects of monochromatic light on immune response of broilers. *Poultry Science*, *87*(8), 1535–1539. https://doi.org/10.3382/ps.2007-00317

Yang, Y. F., Jiang, J. S., Pan, J. M., Ying, Y. B., Wang, X. S., Zhang, M. L., Chen, X. H. (2016). The relationship of spectral sensitivity with growth and reproductive response in avian breeders (Gallus gallus). *Scientific Reports*, *6*(January), 19291. https://doi.org/10.1038/srep19291

CHAPTER 7

GENERAL SUMMARY AND CONCLUSIONS, PRACTICAL IMPLICATIONS, AND RECOMMENDATIONS FOR FUTURE STUDY

As global demand for egg-source protein increases, so has the public concerns over laying hen welfare. There has been increasing adoption of alternative hen housing systems, as compared to the conventional cage housing, for egg production. This trend has been particularly strong in the European Union and it is now growing in the United States. In the meantime, certain scientific information is lacking for guiding the design and magement of the alternative housing systems. This dissertation had the overarching goal of generating the much-needed knowledge related to alternative laying hen housing design and management for improved laying hen welfare, efficiency of resource utilization, and production performance. Supporting the overarching goal were two primary research objectives that aimed to quantify behavioral and production responses of pullets and laying hens to perch design/configuration and light type/source. Toward that end, five experiments conducted in controlled environment were included in this dissertation. The purpose was to provide science-based data about the behavioral and production responses of pullets and laying hens to housing enrichment (i.e., perch design and placement) and lighting source (poultry-specific LED light *vs.* fluorescent light).

General Summary and Conclusions and Practical Implications

The following is a summary of the main findings and conclusions of the experiments covered in this dissertation and their practical implications.

- Lohmann White hens used in the study showed comparable choice for round *vs.* hexagon perches (p = 0.587-0.987). Specifically, 50.1 ± 4.3% *vs*. 49.9 ± 4.3% of daily perching time, 49.7 ± 1.0% *vs.* 50.3 ± 1.0% of daily perch visit, and 47.7 ± 4.1% *vs.* 52.3 ± 4.1% of dark-period perching birds were on round *vs.* hexagon perches. Upon transfer from a cage-rearing environment to an enrich colony housing, the novice young hens (no prior perching experience) showed increasing use of perches over time, taking them up to 5-6 weeks of perch exposure to approach stabilization of perching behaviors in the enrich colony setting. These findings imply that laying hens have no preference for perch shape of hexagon or round, thus provision of either type of perch could safeguard laying hen welfare from the standpoint of meeting the hen perching behavior needs. In addition, rearing pullets without access to perches would not significantly impact their subsequent perching behaviors. (Chapter 2)
- Reduction of horizontal distance (HD) between parallel perches to 25 cm did not restrain Lohman White hens' perching behaviors as hens perched interlacing with one another to maximize use of the perches during the dark period at the HD of 25 cm. However, HD of 20 or 15 cm restrained hens' perching to some extent. These findings imply that HD of 25 cm between parallel perches was shown to be the lower threshold to accommodate the hen's perching behaviors. As such, HD of 25 cm may be considered if reducing HD from 30 to 25 cm would allow placement of more perches to meet the perching needs of all hens. (Chapter 3)
- W-36 pullets under the poultry-specific LED light and the fluorescent light had comparable body weight (1140 ± 5 g *vs.* 1135 ± 5 g, p = 0.41), body weight uniformity (90.8 ± 1.0% *vs.* 91.9 ± 1.0%, p = 0.48), cumulative mortality rate (1.3 ±

0.6% *vs.* 2.7 ± 0.6%, p = 0.18), and comb and feather conditions at 14 weeks of age. The circadian activity levels of the pullets were higher under the poultry-specific LED light than under the fluorescent light during the rearing phase. These findings imply that the poultry-specific LED light may serves as a viable alternative lighting source for rearing pullets. As the poultry-specific LED light showed more stimulating effect on the pullet activity levels, the poultry-specific LED light may be desirable from the standpoint of developing a stronger bone in the birds for subsequent egg production. (Chapter 4)

- The pullets and layers in all cases showed stronger choice for fluorescent light (p = 0.001-0.030), regardless of prior lighting experience, as evidenced by higher proportions of time spent under the light. Specifically, the proportion of time spent under fluorescent light *vs.* poultry-specific LED light was 58.0 ± 2.9% *vs.* 42.0 ± 2.9% for P_{INC} (pullets reared under incandescent light), 53.7 ± 1.6% *vs.* 46.3 ± 1.6% for L_{LED} (layers reared and kept under LED light), and 54.2 ± 1.2% *vs.* 45.8 ± 1.2% for L_{FL} (layers reared and kept under fluorescent light). However, the proportions of daily feed intake occurring under the fluorescent light and the poultry-specific LED light were comparable in all cases (p = 0.419-0.749). These findings imply that prior lighting experience of pullets and layers would not influence their choice for the fluorescent light *vs.* the poultry-specific LED light. Although pullets and laying hens exhibited a somewhat stronger choice for the fluorescent light as compared to the poultry-specific LED light, this tendency did not translate to differences in the proportion of feed use under each light type. The findings indicate that the poultry-specific LED light may be used as an alternative lighting source without causing negative impacts on the production performance (e.g., feed use). (Chapter 5)

- The fluorescent and the poultry-specific LED lights used during the laying phase had comparable performance responses for all the aspects (i.e., age and body weight at sexual maturity, hen-day egg production, eggs per hen housed, egg weight, daily feed intake, feed conversion, albumen weight, albumen height, Haugh unit, yolk weight, yolk percentage, yolk color factor, and yolk cholesterol content) with a few exceptions during the 17-41 weeks of age (WOA). Specifically, eggs in the poultry-specific LED light regimen had lower shell thickness (0.42 ± 0.00 *vs*. 0.44 ± 0.00 mm, p = 0.01) and strength (37.5 ± 0.22 *vs.* 38.8 ± 0.22 N, p = 0.03) than those in the fluorescent light regimen at 41 WOA. The fluorescent and the poultry-specific LED lights used during the rearing phase did not influence the laying performance at 17-41 WOA, except that hens reared under the poultry-specific LED laid eggs with lower shell thickness (0.43 ± 0.00 *vs*. 0.44 ± 0.00 mm, p = 0.02) at 32 WOA as compared to hens reared under the fluorescent light. These findings imply that the poultry-specific LED lights provide a viable alternative to the traditional fluorescent lights for maintaining the laying hen production performance. (Chapter 6)

Recommendations for Future Research

Based on results of the experiments covered in this dissertation, the following studies are recommended as possible topics of future/further investigation.

- Although the laying hens showed no preference for the perch shape of hexagon *vs.* round in the study, the long-term effects of the perch shape on the hen production performance and welfare parameters (e.g., feet and keel bone conditions) warrant examination.

- The young novice hens transferred from a cage-rearing environment to enriched colony were found to take 5-6 weeks to become used to the perches. It would be worthwhile to comparatively quantify the temporal perching behaviors of young hens from other types of pullet rearing systems (e.g., litter-floor, enriched housing) where they have prior perching exposure or experience.
- The laboratory study revealed that a horizontal perch distance of 25 cm may be considered if reducing horizontal distance from 30 to 25 cm would allow placement of more perches to meet the perching needs of all hens. Verification of such a practice in commercial settings involving more hens in terms of its long-term impact would be very beneficial.
- Pullets reared under the poultry-specific LED light were shown to maintain a higher circadian activity level (locomotion activity) than pullets under the fluorescent light in the study. The impact of such higher activities on potential stronger bone development in the birds should be investigated.
- Pullets and laying hens showed stronger choice for fluorescent light as compared to the poultry-specific LED light, regardless of the prior lighting experience. One of the possible explanations is that birds prefer light sources that partially emit UVA radiation. Hence, it would be worthwhile to investigate responses of pullets and laying hens to various levels of UVA light.
- Laying hens under poultry-specific LED light were shown to have comparable production performance and egg quality as compared to those under fluorescent light.

However, the effects of these light sources on the hen physiological responses and welfare were not investigated in the study. This aspect may also be considered in future studies.