ADAPTATION TO THE PULLET-REARING ENVIRONMENT BY PROVIDING LIGHTING DURING EMBRYO DEVELOPMENT

by

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Submitted in partial fulfillment of the requirements
for the degree of Master of Science
at
Dalhousie University
Halifax, Nova Scotia
January 2019

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ABSTRACT

In this study, it was hypothesized that providing a photoperiod during incubation will positively affect the production performance of laying hens. In the current study 640 eggs from each of two laying hen lines, (Lohmann Lite and Lohmann Brown) were incubated in 8 incubators as eighty eggs from each hen line/incubator. Blue, red and white LED lights were installed in 2 incubators each and 2 were left in the dark. In each lighting treatment the photoperiod used was 12L:12D. Hatched female chicks (n=384) were housed in 64 cages at 6 birds/cage and were provided with two post-hatch light regimes (long-days and short-days). During the study, production performance was monitored. Out of the four lighting treatments, red light reduced the total hatching time, improved chick quality, post-hatch growth rate and egg production. In conclusion, introduction of red LED to hatching eggs during incubation was beneficial to increase the production performance.

Key words: layers, hatch time, chick quality, post-hatch performance, red LED

LIST OF ABBREVIATIONS

12L: 12D	12 hours of light: 12 hours of dark
CAM	Chorio-allantoic membrane
CE	Controlled environment
CFL	Compact fluorescence lamp
DOB	Day old birds
ED	Embryonic day
FC	Foot candles
FSH	Follicle-stimulating hormone
GH	Growth hormone
GnIH	Gonadotropin-inhibitory hormone
GnRH	Gonadotropin-releasing hormone
IGF – 1	Insuline like growth factor - 1
IR	Infra-Red
LB	Lohmann Brown
LED	Light Emitting Diodes
LH	Luteinizing hormone

LL	Lohmann Lite
R21	Eggs incubated under red LED for the entire incubation period with a photoperiod of 12L: 12D
RH	Relative humidity
R18	Eggs incubated under red LED for the first 18 days of incubation (with a photoperiod of 12L: 12D) followed by 3 days of dark period
UV	Ultraviolet
W21	Eggs incubated under white LED for the entire incubation period with a photoperiod of 12L: 12D

ACKNOWLEDGEMENTS

I must deliver my deepest gratefulness to my supervisor Dr. Bruce Rathgeber, for his encouraging supervision and inspirational guidance to make this research project a success.

I heartily appreciate Dr. Rathgeber's support and valuable advices during the hard times when I was working on my experiments. I must also be thankful to my supervisory committee, Dr. Nancy McLean and Dr. Miriam Gordon for their valuable suggestions and support to navigate this project in the right direction. I am also thankful for the support and advocacy provided by Dr. Karen Schwean-Lardner.

I must acknowledge Nova Scotia Agricultural department and Egg farmers of Canada for providing the funds to carry forward my projects. I also, sincerely appreciate the support, companionship of, Ms. Janice MacIsaac, Mr. Xujie Li, Ms. Kayla Graham, Ms. Janessa Henry, Ms. Cassendra Stupart, Ms. Valene Macneil and the staff of Atlantic Poultry Research Institute.

CHAPTER 1: INTRODUCTION

Global demand and increasing competition in the poultry industry require productive layers to meet market requirements. Increased production performance demands well-developed chicks that can easily cope with the rearing environment (Wolanski et al., 2004). In a commercial environment setting, it is challenging to maximize early survival and improve the uniformity of flock while increasing the production performance. In this context, variation in the parameters of incubation environment can have long-lasting effects on the bird's well-being (Archer et al., 2009). It has been well documented that during incubation, temperature (Nakege et al., 2003), humidity (Bruzual et al., 2000) and turning (Deeming, 1989) can impact the development of embryo. Deviation of incubation temperature from optimum range (37.5-37.7 °C) reduces hatchability (lower than 90%) (Taylor, 2000) and deviation of humidity from optimum range (50-60%) increases the amount of late dead birds at the hatch (Bruzual et al., 2000). Also, these incubation parameters affect the post-hatch performance of laying hen birds including performance during egg production. Incubation temperatures higher than normal levels (0.5–2 °C higher) will reduce average egg mass/hen/day (Glatz, 1997). Introduction of light during incubation of hatching eggs is another factor that has the potential to affect embryo development and post-hatch performance (Archer and Mench, 2014).

Under natural incubation, when the mother hen turns the eggs and leaves the nest, the egg shell is temporally exposed to full spectrum daylight (Mrosovsky and Sherry 1980). The common practice in commercial hatcheries is to incubate in a completely dark environment (Archer and Mench, 2014). Recently, hatcheries using equipment designed to provide feed and water during the hatching phase provide light following transfer to the hatcher. This is typically the last three days of incubation (Hatch-Tech technical information, 2016).

Studies have reported that the chicken is sensitive to a light range from infrared wavelengths to ultra-violet (Prescott and Wathes, 1999). This is a wider visual spectrum than humans are sensitive to. During natural incubation, eggs experience a wide range of light spectrum. Light is an environmental factor that can affect physiological functions and behavioral processes that show circadian rhythms in living organisms (Archer and Mench, 2014). Light is absorbed by the chicken embryo mainly through the eyes which passes through to the photo-receptors rhodopsin and iodopsin. Light can also penetrate through the skull and be received directly by the pineal gland in the brain (Lewis and Morris, 2000).

Daily rhythms of light-dark cycle encourage melatonin synthesis in the pineal gland (Reed and Clark, 2011). Introduction of light during incubation establishes circadian rhythms in embryos through rhythmic melatonin production. Melatonin secretion from the pineal gland occurs during the dark period. Photoperiodic signals are converted into neuroendocrine signals through this hormone (Underwood et al., 2001). Studies have reported rhythmic production of melatonin in chicken embryos as early as the third day of incubation (Zeman and Herichova, 2011).

Early melatonin rhythms influence development of embryo and post-hatch growth (Reed and Clark, 2011). Archer and Mench (2014) showed that white LED light provided for 6 h per day during incubation improved hatchability by 3 percent compared to dark provided to the whole incubation (0L: 24D). In a recent study, Huth and Archer (2015a) noted providing white LED light (12L:12D) could improve the number of chicks hatched with no defects by 19 percent compared incubation in the dark (0L: 24D) and there was less vocalization in an isolation test (less fear to environmental stressors) of broiler birds incubated with light. The underlying mechanisms for these effects are thought to involve development of prenatal circadian rhythms that improve epigenetic adaptation which is a process that occurs during prenatal or early postnatal ontogeny.

During the early development stages, due to epigenetic adaptation the actual control levels at which the functional systems (ex: temperature regulation) are regulated, may determine the life-long response and are dependent on the environment that the embryos are exposed to (Tzschentke and Basta, 2002). This improves the ability of chicks to adjust to the post-hatch environment, providing a good start in life (Özkan et al., 2012a).

Studies have reported that the wavelength of light source used has various effects on physiological functions of birds. Rozenboim et al. (2003) reported that providing green LED light during incubation will increase the post-hatch breast muscle proliferation. Lewis and Morris, (2000) reported a variety of stimulatory effects from different wavelengths when measuring behavioral patterns and bird performance. Archer et al. (2017) showed that introduction of combination of white and red LED light (12L:12D) during incubation improved hatchability of broiler birds by 5 percent and lowered post-hatch fear response compared to birds hatched in the dark or provided green LED light.

All these studies used broiler hatching eggs as the test subject. Until now there have been no studies focused on providing light during incubation for hatching eggs from laying hen lines. Improving the ability of pullet's ability to adjust to their environment could benefit them with a good start during early life. Providing a photoperiod during embryo development could increase the ability of birds to cope with environmental stressors during the production cycle. This study will discuss incubation lighting as an environmental factor that has potential to affect the production performance of layers.

1.1 Hypothesis

Providing a photoperiod during incubation and different photoperiods in early post-hatch will positively affect the hatching, post-hatching and egg production performance of laying hens.

Specific hypothesis

1) Providing a photoperiod during incubation will narrow the group hatch time and increase the hatching performance of chicks.
2) Providing a photoperiod during incubation and different photoperiods in early post-hatch will positively affect the post-hatching performance of chicks, egg production and egg quality of laying hens.

1.2 Research objectives

To evaluate the mixed effect of providing light during incubation and different photoperiods during early post-hatch, on overall hatching, early post-hatching and egg production performance of laying hens.

Specific objectives

1) To narrow the hatch time and to increase the hatching and post-hatching performance of chicks that are provided a photoperiod during incubation.
2) To determine the mixed effect of providing lights of different wavelengths during incubation and different photoperiods in early post-hatch on the time to lay first egg, egg production and egg quality performance of laying hens.

CHAPTER 2: LITERATURE REVIEW

2.1 Incubation revolution

Successful laying hen egg production systems rely on many carefully organized and coordinated series of operations including hatching egg production, hatchery and incubation management, feed, water, vaccine, management of post-hatch environment. Out of these operations, significant attention is given to establish a proper environment during incubation as the environmental conditions have long-lasting effect on bird's post-hatch life (Archer et al., 2009).

Scientific knowledge gained over the years show that the transformation of technology of incubation has been helpful for increasing the production efficiency of the poultry sector (Boleli et al., 2016). Artificial incubation systems had gone through drastic technological changes over the past few decades. These changes include a shift from labor-intensive manual control incubation to large incubation machines allowing incubating more eggs. With advancement of the incubation sector, producers have focused more on providing better quality high yielding chicks and reducing the overall operational cost (energy and labour) (Boleli et al., 2016).

Centuries ago, techniques for artificial incubation were established taking the environmental conditions of heat, moisture, ventilation, and egg turning into consideration (Paniago, 2005). During ancient Egyptian times, temperature was controlled through radiant heat generated from straw, camel manure and charcoal that were burnt on the upper part of small rooms which were considered as incubators. The eggs were set in the lower part of the rooms. These rooms were located at each side of a central passageway in a large mud brick building. The vents located in the roof of these rooms facilitated ventilation. Moistened jute placed on eggs controlled the relative humidity (RH) of eggs which were manually turned twice per day (Paniago, 2005).

Reamur in Paris, France, invented mechanical incubation in 1749. Hearson manufactured the first commercial incubator in 1881 (Boleli et al., 2016). Invention of mechanical incubation

was improved with electronic control panels that regulated temperature within narrower temperature ranges of 0.1 ºC in variation automatic ventilators, humidifiers and egg turners. With the technological evolution, modern incubators were equipped with alarm systems, digital displays located outside the incubators and computerized systems to monitor and control the whole incubation process (Paniago, 2005). This sophisticated technology helped to improve the uniformity of the environment during incubation and subsequently increasing hatchling quality.

2.1.1 Effect of incubation conditions on hatchling quality

Optimizing incubation factors such as temperature, relative humidity, ventilation and turning by manipulation according to the embryonic stage has increased chick quality and resulted in better performing laying hens (Ar and Rahn, 1980; Glatz, 1997; Meijerhof, 2003).

Optimum temperature provided during incubation is the most critical parameter of incubation for hatching and has proved to be affective environmental factor determining broiler, turkey and laying hen performance. During the development stage, the optimum temperature should be 37.5-37.7 °C. (Decuypere et al., 2001; Meijerhof, 2009). Providing the optimum temperatures help to absorb yolk and improve navel seal (reduces chick infections due to open navels), thus producing good quality chicks (Meijerhof, 2003). Deviation of eggshell temperature from the optimum results in change of embryonic temperature, reduced hatchability, organ development, and chick growth (Michels et al., 1974; Decuypere, 1979; Geers et al., 1983; Taylor, 2000; Shafey, 2004; Molenaar et al., 2010). Deviation of eggshell temperature from the optimum will also reduce the growth of chicks, post-hatch growth performance, slaughter yield, and increase economic losses (Wilson, 1991; Lourens and Van Middelkoop, 2000).

Optimum relative humidity (RH) or the percentage amount of water vapor available in air of the amount of water needed to saturate at the same temperature for healthy embryo development

is 50-60% (Lundy, 1969). Optimum RH facilitates proper egg weight loss during incubation, which is approximately 12-14% (Ar and Rahn, 1980). Egg weight loss ensures adequate air cell size essential for lung respiration at the internal pip stage (Molenaar et al., 2010). Relative humidity levels lower than the optimum produces small and dehydrated chicks (Deeming, 2000). Higher levels of RH results in uncovered navels leading to yolk sac infections that increase the early post-hatch mortality (Sozcu and Ipek, 2013).

Ventilation is required to provide oxygen to the incubators and remove carbon dioxide. Studies have shown that an average egg with a weight of 60 g takes in around 6 L of oxygen and removes 4.5 L of carbon dioxide during the entire incubation (Taylor, 2000). When adequate ventilation is not provided oxygen and carbon dioxide levels become unbalanced. Higher levels of carbon dioxide during the hatching period may cause organ malformation (e.g. heart and lung), resulting in low quality chicks (Coleman and Coleman, 1991). However, providing a carbon dioxide concentration of 0.4% compared to 0.2% during embryonic day (ED) 14-19 has shown to increase the hatch weight of chicks in 3 g compared to providing 0.2% throughout the whole incubation (Buys et al., 1998). Additionally, lower rates of ventilation (< 0.2 m/s) throughout the whole incubation period reduces the rate of heat transfer from eggs and embryos increasing embryo mortality (Lourens et al., 2011).

Turning of eggs during the early stages (before ED 18) of embryo development is necessary, because it prevents embryo adherence to the eggshell membrane, facilitates the development of the chorio-allantois sac which helps embryo gasses transfer, and minimizes malposition of embryos (Deeming, 1989). Egg turning can vary from a minimum of 24 times to a maximum of 96 times per day to ensures optimum hatchability. Turning deviates from this range will cause embryo mortality and lowers hatchability (Wilson, 1991).

Many studies have found that variation of incubation conditions affect the chick quality at hatch and post-hatch production performance. Industrially, efficient egg production and early sexual maturity of layers are important to meet with consumer demands for egg consumption. Therefore, incubation environment and optimal conditions during incubation are important tools to ensure high quality chicks with high post-hatch performance. Consequently, researchers are interested in finding the best incubation conditions to improve productivity of birds. So far, research has focused on incubation parameters such as, temperature, RH, ventilation and egg turning. However, to optimize incubation conditions, researchers are working on the introduction of light during the incubation process. The effects of providing light during incubation, including later life performance of layer birds, are yet to be discovered.

2.1.2 Natural incubation vs. current commercial practice

Industrial eggs are incubated in the dark and exposure light is intermittent typically limited to when the incubator door is opened (Archer, 2015b). Under natural incubation, when the mother hen turns the eggs and leaves the nest, egg surfaces are temporally exposed to radiation of the full spectrum of sunlight (Mrosovsky and Sherry, 1980). Thus, eggs experience light ranging from infrared (IR) wavelengths to ultra-violet (UV) during natural incubation.

As incubation evolves, the poultry industry has begun to provide light during incubation. Hatcheries have started using equipment that designed to provide feed and water during the hatching phase with lights provided (Hatch-Tech technical information, 2016).

2.2 Effect of light on poultry production performance

2.2.1 Bird sensitivity to the light spectrum

Visible light/ light spectrum are electromagnetic waves, which spans wavelengths of 380-780 nm. Birds can see within the visible spectrum as well as parts of UV and IR, which humans

are not able to see. Birds are sensitive below 400 nm (up to 100 nm) and above 700 nm of wavelengths (Lewis & Morris, 2006).

Birds sense light in two main ways. They sense light through eyes and extra-retinal receptors (Lewis & Morris, 2006). The retina ensures clear visibility of the surrounding environment (Wilson and Lindstrom, 2011). Rods and cones are the structures that are found in retina of the eye which are sensitive the light (King-Smith, 1971). Extra-retinal receptors are glands that secret hormones directly into the blood, in response to light. These extra retinal receptors are in endocrine glands such as the pineal gland and the hypothalamus (Siopes and Wilson, 1980). Light penetrates through the skull of the bird and stimulates these endocrine glands. The pineal gland is located in a triangular area behind the brain, between the hemisphere and cerebellum (Siopes and Wilson, 1980). The pineal gland is involved in controlling the circadian rhythms, mobility, body temperature and various endocrine functions through releasing melatonin hormone (Pelham et al., 1972; Baxter et al., 2014). The hypothalamus is located in the deep brain tissue in the preoptic section of the forebrain. The extra retinal receptors in the hypothalamus are involved in controlling gonadotropin-releasing hormone (GnRH), regulating bird metabolism and reproduction (Siopes and Wilson, 1980; Lewis and Morris, 2006).

2.2.2 Characteristics of light

Artificial light sources are commonly used in the poultry industry. Light has three main characteristics, which are brightness (intensity), duration (photoperiod) and color (wavelength) (Blatchford et al., 2009). Intensity of light is measured in lux (standard metric unit) or foot-candles (fc) (1 lux = 0.09). Light intensity provided to the poultry during production, should be strong enough to penetrate skull and cranial tissues to stimulate the pineal gland and hypothalamus. Minimum light intensities for starting chicks are 20-55 lux, older broilers 1 lux, laying hens 5-10

lux, and broiler breeders 17-45 lux (Lewis and Morris, 2006). The day and night cycle that is provided for birds is known to be the photoperiod of light. The daily light/ dark cycles strongly affect the behavioral pattern of birds strongly. As an example, to stop laying hen birds reaching the sexual maturity reducing the day length is necessary during the grow out and to reach the sexual maturity increase of day length is needed (Lohmann LSL lite layer management guide, 2005). Thus, behaviors can be manipulated through adjusting the photoperiodic cycle (Prescott et al., 2003).

Researchers have shown that birds are sensitive to a range of wavelengths through retinal and extra-retinal receptors (Lewis and Morris, 2006). However, it has been hypothesized that bird performance can be manipulated using particular wavelengths of the spectrum (Archer, 2015a). The performance of birds under different commercial colored lamps have been found to be different (Rozenboim et al., 2004). Use of monochromatic blue or green light has resulted in a superior growth of broilers increasing body weight and breast muscle weight (Rozenboim et al., 2004; Zhang et al., 2012).

2.2.3 Introduction of light to incubation of hatching eggs

2.2.3.1 Sources of lights used in the poultry industry

Providing light during incubation enhances physiological and anatomical development of birds (Rozenboim et al., 2004). Creating a favorable light environment for poultry production depends on the response of birds to different light sources (Archer, 2015b). For many years, the poultry industry has used incandescent light for illumination. However, this practice is now eliminated from the industry because of high power consumption (Burrow, 2008). Incandescent bulbs produce light by heating a filament of wire inside a bulb using an electric current. A nonreactive gas is filled into the glass bulb to prevent the wire from burning. However, compact

fluorescence lamps (CFL) and light emitting diodes (LEDs) are currently being used in the poultry industry. The CFLs discharge low pressure mercury-vapor gas using an electric current and fluorescence is used to produce visible light. CFL lights have a lower level of power consumption (Burrow, 2008) but they may cause toxicity to birds if the bulb gets broken accidentally (Archer, 2015b). Researchers have shown that CFLs and incandescent lights used in incubators may produce heat and change incubation environment. The excess heat produced by incandescent bulbs may depress the hatchability and increase embryo mortality (Rozenboim et al., 2003).

Currently with the advancement of technology, poultry producers focus more on using LEDs which are semiconductor devices that emit visible light at a particular wavelength (monochromatic) and consume less power than incandescent and CFLs (Huth & Archer, 2015b). Therefore, use of LED is better in incubators as they produce little to no heat. In addition, it has been shown that LEDs produce the best matching spectrum for the birds (Huth & Archer, 2015b). Consequently, based on the findings, (Huth and Archer, 2015a) it can be suggested that the use of LEDs during incubation and during poultry rearing is more reasonable than introducing incandescent and CFLs (Archer, 2015b).

2.2.3.2 Effect of photoperiodic incubation on embryo development

Effect of light on the embryo development differs according to the growth stage of retinal photoreceptors, the hypothalamic pacemaker that regulates rhythmicity of birds and the pineal gland. Embryo development is primarily affected both before and after formation and maturation of these organs (Dawson et al., 2001). Providing light during the first two days (prior to pineal gland formation) of incubation stimulates mitosis in the neural crest mesoderm that encourages the somite development or embryonic cell proliferation (Isakson et al., 1970; Ghatpande et al., 1995). In a recent study, it has been showed that exposure of embryos to green light (560 nm) supports

mesodermal differentiation into myoblast cells at embryonic day (ED) 05 (the age of the incubation from the day of eggs set in the incubators) which was assumed to be activated by the photic cues from retinal or pineal photoreceptors acting on the neuroendocrine system (Halevy et al. 2006). This accelerated growth of the embryo upon provision of light during the first 5 days of incubation (Shafey 2004).

The pineal gland is a light sensitive organ in brain that captures light and is formed on the third day of incubation (Erwin et al., 1971). During incubation, light passes through the eggshell and the neurohormone melatonin production is stimulated during the time that embryo does not receive light (Dawson and Van't Hof, 2002). Melatonin is synthesized mainly in the pineal gland (Underwood et al., 2001). During the time that the embryo receives light, production of melatonin is reduced (Akasaka et al. 1995). Photoperiodic information is transformed into neuroendocrine signals (circadian rhythm) through melatonin. Thus, provision of a photoperiod during incubation produces melatonin rhythms in the embryos. Also, researchers have shown that melatonin rhythm appears as early as 13 days of embryo development (Ozkan et al., 2012a).

Photoperiodic light affects the embryonic metabolic rate (Preda et al. 1962). During the incubation of pigeon (*Columba livia*) embryos, photoperiodic signals change in the metabolic rate. Higher metabolic rates of 17% have been detected with the presence of light compared to the dark. These higher metabolic rates enhance the embryo development (Prinzinger & Hinninger 1992). Accordingly, embryonic growth and development including cell proliferation and metabolic are affected by photo-stimulation during incubation. Therefore, evidence suggests that providing a photoperiod during incubation helps to enhance the development of poultry embryos.

2.2.3.4 Effect of photoperiodic incubation on hatch window

The time gap between the first hatched chick to the last chick is termed the hatch window (Romanini et al., 2013). This gap varies from 24-48 h (Løtvedt & Jensen, 2014). In the hatch window, the early hatched chicks have delayed access to water and feed (Careghi et al., 2005). This dehydration affects the growth and physiology of chicks after hatch (Løtvedt & Jensen, 2014). Therefore, narrowing of the hatch window is important to prevent adverse effects on growth due to delayed introduction to the feed.

When day-old birds (DOB) are in their thermo-neutral zone (TNZ) (rectal temperature 40-40.6 ºC at 60-70% of RH) they lose 1–2 g of moisture within a day (Hill, 2011). When they are overheated, (rectal temperature over 41.1 ºC) birds lose 5-10 g of moisture within a day (Hill, 2011). This is applicable for the DOBs that do not have access to water, from pull out of the incubator to placement in the barn. When chicks exceed the TNZ (hot), they become dehydrated. Therefore, it is essential to monitor and narrow hatch window and pull out chicks immediately after hatch (Hill, 2011).

Establishing a narrower hatch window will result in chicks hatching in a shorter time which facilitate removal of the birds earlier from the incubators, reduce the number of chicks that are dehydrated and improve early access of birds to feed and water. Fairchild and Christensen (2000) reported that turkey eggs incubated under white incandescent light with a 12L:12D photoperiod had a narrower hatch window. Therefore, the photo stimulation during incubation reduced the hatch window and hatch time in a positive manner, making the hatch window shorter. Ultimately, narrow hatch windows will enable the hatchery to achieve a more homogenous and well-performing batch of chicks (Løtvedt & Jensen, 2014).

2.2.3.5 Effect of photoperiodic incubation on chick quality at hatch

Incubation environment have proved to affect the chick quality at hatch. Chick quality is an important aspect in the production field, because it can affect the early post-hatch survival, production performance, effective feed conversion ratio, etc. The chick quality at hatch can be evaluated using chick body weights, chick body length, navel closure and leg defects (Archer, 2015a).

Chick weight and chick length signifies quality of incubation and is a predictor for development of birds (Mukhtar et al, 2013). Body weight of day-old chicks is associated with availability of yolk to be absorbed into the body. Therefore, measuring yolk free body weight is a good parameter that evaluates how much yolk is consumed by the chick. Higher the consumption is better as the chick gains weight (Mukhtar et al, 2013). Chick length is measured placing the chick alongside a ruler and the length is measured from the tip of the beak to the end of the middle toe (Willesmen et al., 2008). Chick length represents the rate of absorption of yolk after hatching in the body cavity of the chick. Higher lengths are an indicator for very well-developed chicks (Mukhtar et al, 2013).

Yolk sac uptake/ yolk sac retraction takes place through the navel of chicks. Open navels are vulnerable to bacterial and fungal infections resulting in poor post-hatch performance (Verschuere, 2016). Therefore, acquiring chicks with fully closed and dried navels is critical for good chick quality.

Embryos provided with light during incubation show higher weights at hatch over embryos incubated in complete darkness (Walter & Voitle, 1972; Rozenboim et al., 2003; Zhang et al., 2012). Specific colors of light had varying influences on hatchling quality. Providing red light to laying hen eggs (White leghorn -Hyline) during incubation produced 25% more chicks with healed navels and lowered the number of chicks hatched with leg defects in 2% (Archer, 2015a). However,

Cobb 500 broiler chicks hatched in white light showed greater chick lengths (190.21 mm, compared to the dark hatched chicks 189.97 mm) and 30% more chicks with healed navels. While the same commercial broiler line hatched in red light, had higher weights (47.64 g average body weight, compared to white LED light hatched chicks 46.29 g) with lower number of leg defects (4% less) (Archer, 2015a). Thus, incubation photoperiods, especially the color of light, had a considerable influence in obtaining good quality chicks.

2.2.4 Effect of lighting during incubation on the post-hatch performance

Chicks are more readily adapted to the rearing environment through epigenetic adaptation (Tzschentke and Plaggeman, 2006). It has been shown that incubation temperature manipulation increased the bird's ability to cope with temperature fluctuations in the rearing setting. Muscovy duck eggs incubated in low incubation temperatures (34.5 ºC) and higher incubation temperatures (38.5 ºC) had the ability to adapt to post-natal cold and heat adaptation consecutively (Tzschentke et al., 2004). Incubation with light can also improve the adaptation of chicks to a known rearing environment by reducing the fear in post-hatch environment. Lighting during incubation reduces the corticosterone release from the body in 31% in relation to a stress response compared to dark hatched birds (Ozkan et al., 2012a).

Light affects physiological functions closely associated with circadian rhythm through regulation of melatonin and transforming photoperiodic information into neuroendocrine signals. Melatonin hormone is synthesized in the pineal gland and regulates of body temperature, feeding and digestion, and immune function (Apeldoorn et al., 1999; Brennan et al., 2002). Development of melatonin rhythms can manipulate prenatal ontogeny that helps to cope with rhythmic physiological patterns (circadian patterns) in the post-hatch environment (Zeman et al., 1999).

In terms of welfare aspects, reduction of response to stress during post-hatch period facilitates growth and development. Lighting, during incubation, reduced post-hatch stress and fear, lowers blood corticosterone and improved the adaptation of chicks to a novel environment (Ozkan et al., 2012a). Thus, provision of a photoperiod during incubation and development of cyclic rhythms of melatonin, give chicks a better start in the early post-hatch environment.

2.3 Management practices used for pullet and layer rearing stages

2.3.1 Management practices used for pullet rearing phase

Designing a shelter for pullets is a challenge for providing a better post-hatch environment. Good quality chicks that are hatched and in optimum environment should need to rear in a housing facility that can improve the performance including body weight gain and egg production of birds. Therefore, housing design, management system and strain of chicken should be taken into consideration when designing a housing system for pullets. A house gives a protection for birds from predatory animals such as rodents, wild birds etc; also maintains a favorable climate for birds. Materials used for housing and equipment should not be harmful or toxic to the birds. In a rearing system flooring, feeders and waters and space allowance and lighting are the main features that are paid a higher attention (Lohmann LSL lite layer management guide, 2005).

Pullets can be reared on wire, slats, or litter. Flooring provides support for feet, and gives an opportunity for scratching, foraging and dust bathing. Floor should not cause the birds to have injuries and deformities to the legs. However, conventional cages are used commonly in poultry facilities to rear pullets. For birds reared in cages, the gap between floor wires must be a maximum of 2.5 cm (Lohmann LSL lite layer management guide, 2005). During the first couple of days' newspapers, paper plates and fiber egg trays can be used as floor covering. These floor coverings

promote the foraging and scratching behavior of birds (Lohmann LSL lite layer management guide, 2005).

In a poultry house, birds have access to feed and water at all times. Feeding space allowed for birds is mainly dependent on their age and body weight. Birds are provided with at least two waterers in case of one break down. Cups and nipple drinkers are most commonly used waterer types in poultry houses. Water temperature should be maintained at 20-25 °C (Lohmann LSL lite layer management guide, 2005). Space allocation for feeders and waters are as indicated in the Table 01.

Table 1. Minimum feeder and waterer space allowances for birds and maximum number of birds allowed per waterer at different stages of life.

Bird type and age	**Minimum feeder space/ bird (cm)**	**Maximum number of birds/ waterer**	**Minimum waterer space/ bird (cm)**
Chicks: 0 – 2 weeks	1	30	2.5
Pullets: 2 – 8 weeks	2	24	2.5
Pullets: 8 weeks to layer barn	4	12	2.5

The minimum area allocated for a bird depends on the age and weights of the bird. The space provided per bird is increased as the bird reaches mature weight. For each strain of bird, the breeder management guide provides the minimum requirements for space allowances for birds. Space allowance for Lohmann Lite pullets are as indicated below – Table 02 (Lohmann LSL lite layer management guide, 2005).

Table 2. Minimum required space allowances for chicks and pullets housed in cages, at different stages of life.

Bird type and age	Space allowance per bird (cm^2)
Chicks: 0 – 2 weeks	64.5
Pullets: 2 – 8 weeks	129.0
Pullets: 8 weeks to layer barn	283.9

Environmental conditions in the poultry facility affect the animal wellbeing and performance. Some of the important environmental components are temperature, humidity and ventilation. Before the chicks are brought into the barn, the temperature of the air should be maintained at 35-36 ºC (Lohmann LSL lite layer management guide, 2005). Once the required temperature is achieved, minimum ventilation can be supplied in order to avoid temperature differences in the house. Relative humidity should be at 60% (Lohmann LSL lite layer management guide, 2005). At the placement of chicks in barn environment, they can be safely unloaded to the cages and they must be watched carefully for the first couple of days. When the environmental conditions are optimum, the chicks are spread out and move freely. If the temperature is too cold, the chicks tend to huddle together. The chicks that are panting and lying on the floor with their wings spread out indicate higher temperatures than optimal. The desired temperature varies as the bird grows. The relative humidity should be maintained at 60-70% when the chicks grow (Lohmann LSL lite layer management guide, 2005). Table 03 shows the environmental temperature at each age.

Table 3. Desired environmental temperatures at bird level for cage rearing and floor rearing systems, at different stages of age.

Age	Cage rearing (°C)	Floor rearing (°C)
Day 1-2*	35	36
Day 3-4	33.4	34
Day 5-7	31	32
Week 2	28	29
Week 3	26	27
Week 4	22	24
From week 5	18-20	18-20

**Body temperature of 40-41 °C are the optimum for the chicks*

Beak trimming is a common practice that is used against cannibalism and feather pecking. Unbalanced environment with higher light intensity, unbalanced feed, poor ventilation and overstocking can increase cannibalism and feather pecking. Beak treatment can be performed when the chicks are around 7-10 days of age. Hot blade treatment can be used for beak treatment. Before beak trimming, the temperature of the blade is adjusted according to the beak size and strength. After treating the beaks, *ad-libitum* feed should be available for chicks. Adding vitamins to the drinking water will help recover from this stress (Lohmann LSL lite layer management guide, 2005).

Following a suitable vaccine program is important as bird immunity and healthiness is a key factor determining production performance. There are three different vaccination methods that can be performed for laying hen pullets. They are individual, drinking water and spray vaccination.

Injections and eye-drops are considered as individual vaccine. These are well tolerated by the birds and considered very labor intensive. Drinking water method is not very intensive and the water used must not contain any disinfectants to be effective (Lohmann LSL lite layer management guide, 2005). Amount of vaccine used should be calculated for complete consumption within 2-4 hours. For spray vaccine, distilled water is used to dilute the vaccine and coarse spray can be used until 3 weeks of age. Marek's vaccine is given individually to each bird at the hatchery (or as in ovo injection is also practiced) to help protect against Marek's disease which suppresses the immune function and reduces the growth performances (Lohmann LSL lite layer management guide, 2005; Egg inject: in ovo vaccination, 2019). Recommended vaccination program for the Lohmann Lite birds are as the table below (table 04).

Table 4. Different types of vaccines and method of applications used during grow out phase.

Disease	Application method	Remarks
Marek	Subcutaneous injection/intramuscular Injection	Injected at day 01/ ED 18 of incubation
Newcastle	Drinking water/ spray/ Subcutaneous Injection/ intramuscular Injection	Number of vaccinations performed according to disease pressure
Gumboro	Drinking water	Two live vaccinations recommended
Salmonella	Drinking water/ spray/ intramuscular Injection	Vaccine birds before transfer to the layer cages

2.3.2 Factors to consider when moving pullets to laying hen cages

Pullets can be transferred to the laying hen cages 15-17 weeks of age, before they reach the of sexual maturity. The laying hen facilities are designed to ensure that the hens get a comfortable environment for laying. Conventional cages for layers are designed to have 4-8 hens in a cage. Conventional cages protect the hens from injuries and provide a controllable environment. However, these cages restrict the bird's ability to engage in natural behaviors mostly due to the limited space provided. The birds should be weighed prior to transfer and after transfer (Hyline management guide, 2017).

The feed and water consumption and mortality should be monitored frequently. In a laying hen facility, the waterer and feeder systems are very similar to the pullet rearing setting. Birds must achieve their pre-transfer water consumption within 6 hours after transfer. The mortality should not exceed 0.1% per week (Hyline management guide, 2017). Conventional cages typically have a slope in the floor to facilitate the eggs moving in to the egg belt. However, the slope should not exceed 8 degrees (Code of practice for the care and handling of hatching eggs, breeders, chickens and turkeys, 2016). All the birds in a barn are transferred in the same day (Hyline management guide, 2017).

2.3.3 Nutrition of poultry

2.3.3.1 Early nutrition

In a commercial hatchery, the chicks that hatched early, must stay until the remaining eggs hatch. After hatching, chicks are exposed to a series of processing methods. They are sexing, sorting, vaccination, packaging and transporting. These processing techniques take time and delay the access of chicks to feed (Batal and Parsons, 2002). During this period until the chicks are introduced to feed, the yolk sac provides the energy and protein is for immediate post-hatch body

maintenance. Absorption of residual yolk sac is completed within 4 days of hatching. The residual yolk absorption is essential as it carries the maternal antibiotics and essential nutrients for early survival of birds (Noy and Sklan, 1999). When the chicks start to feed, the presence of feed in the gut, stimulates antiperistalsis movements of the yolk material to the duodenum (Panda and Reddy, 2007). Introduction of easily digestible proteins and carbohydrates to the chicks in early hours of age is early chick nutrition (Prabakar et al., 2016).

Performance is reduced when holding chicks for more than 24 hours without feed and water (Batal and Parsons, 2002). In a newly hatched chick, yolk comprises around 10-15% of the chick. Yolk provides energy during the early post-hatch period. Yolk contains around 16-35% fat and 20-25% protein at hatch (Moran and Reinhart 1980; Reidy et al., 1998). Yolk lipids contain triglycerides, phospholipids and small amounts of cholesterol and free fatty acids (Noble and Ogunyemi, 1989; Noy and Sklan 1998). These nutrients provide the energy required for the hatchling until they are housed and fed. Even though the protein content of the yolk is partly consisting of albumin, a large portion of protein consists of antibodies. Maternal antibodies are not digested during the incubation process and the immunoglobulins are left intact. Antibodies start to fully function at the time of hatch and are used for passive immunity (Prabakar et al., 2016). They are not utilized as amino acids. However, manipulation of incubation strongly affects the yolk consumption of chicks. For example, incubation higher temperatures of 38.8 °C reduces the yolk consumption rate in 5% (Ozaydin and Celik, 2014). Therefore, maintaining optimum incubation condition are essential for better yolk consumption. Even though the embryos consume optimum percentage of yolk, leaving only 10-12% of yolk as a ratio of body weight (Sklan, 2001), the nutrient supply from yolk is inefficient for the newly hatched chicks. And they are required to depend on external supply of nutrients as the digestive organs are not developed. Providing

nutrients in early life helps increasing the growth of digestive organs, increases mechanical activity of the intestine, develops immunity and overall growth performance (Sklan, 2001; Prabakar et al., 2016).

2.3.3.2 Effect of early nutrition on muscle development

Early post-hatch feeding positively affects the development of muscle (Uni and Ferket, 2004). In a newly hatched chick, the primary source of energy is carbohydrate in the form of glycogen and is reduced during the hatching process due to higher energy consumption. Therefore, providing energy is important (John et al., 1988). Early nutrition improves the development of the intestinal tract and organs that help increase the uptake of nutrients for muscle development. Development of muscle is extremely important for developing layer pullets as reaching an optimal developed pullet body weights before starting the laying cycle is one the main issues, currently in the layer industry (Prabakar et al., 2016).

Muscle cells are multi-nucleated and within each cell, the area of the cytoplasm is controlled by each nucleus. Therefore, muscle mass can be increased if the number of nuclei or the amount of cytoplasm is increased. Since there is limited time available to donate nuclei into an existing cell immediately after hatch, there is limited time available to impact muscle potential by increasing the number of nuclei within the cell. This process occurs by activated satellite cells that donates nuclei to the muscle. After this period of donation, muscle mass is increased through the cytoplasm which is the main method of increasing muscle mass (Prabakar et al., 2016). Satellite cell activity is closely correlated with early nutrition. Longer delays of access to the feed reduce the satellite cell activity, reducing the number of nuclei donated to the muscle. Due to this circumstance, the potential for developing muscle mass is reduced in the mature birds. Providing nutrients during this period enhances muscle mass potential by activating satellite cells. Studies

have demonstrated that feeding poultry during early post-hatch periods enhances the satellite cell proliferation and stimulates skeletal muscle growth (Halevy et al., 2000). Having a rigid system of skeletal muscle mass helps the developing layer pullets to reach optimal body weights before starting the laying cycle (Prabakar et al., 2016).

2.3.3.3 Effect of early nutrition on skeletal integrity

Bone development starts in developing embryo beginning from the formation of a cartilage matrix. This matrix begins to calcify, and the mineralization of tibia and femur bones are rapidly increased from ED 14-21 (Brown, 1982). The skeleton of the chick will be a well-formed miniature version of the frame that can be seen in an adult chicken (Evans, 2015).

There is only a limited amount of nutrients available in eggs at hatch. Yolk phosphorus, zinc, iron and copper are almost totally depleted at hatch. The nutrient recovery of the digestive system is also immature in the developing digestive system during the early post-hatch period (Evans, 2015). Chicks are in danger of being inadequately calcified because of the rapid growth of the body. This can lead to more porous bones and leg problems that will result in a less rigid skeleton at the age of sexual maturity and mortality up to 6% during the early production stages (Evans, 2015).

Correct nutrition is important for the pullets from the day of hatch. Nutritional deficiencies of calcium, phosphorus, or vitamin D can lead to production of poor-quality eggshells and osteoporosis in laying hen phase due to bone loss attributable to osteomalacia (Fleming et al., 1998). Reduction in the amount of fully mineralized structural bone will result in increasing fragility and susceptibility to fracture leading to osteoporosis (Wilson and Duff, 1991). Early nutrition is important in maximizing bone content to prepare the bird for sexual maturity.

Supplementation of extra vitamin K during rearing is required for synthesis of osteocalcin, a protein involved in bone formation (Fleming et al., 1998).

2.3.3.4 Nutrition during the rearing phase

In order to get the highest performance of the birds, feeding a diet with full nutritive value is necessary. Layers in full production convert around one third of the nutrients that they consume into eggs. Providing ad-libitum feeding to the birds is not wasteful as the bird can adjust the intake of nutrients to the density of nutrients of the feed (North, 1984). Consumption of feed is mainly affected by body weight, performance of birds, feed texture, house temperature, level of energy and nutrient balance (Lohmann LSL lite layer management guide, 2005). Higher coarse textures of the feed increase the feed intake while texture reduces the feed intake. Low temperatures increase the maintenance requirement for energy. Therefore, the feed intake is increased in low environmental temperatures. Higher energy levels of feed reduce the feed intake. When there is a nutrient imbalance in the feed's hens try to compensate the nutrient deficiency by increasing feed consumption. This can be seen mainly in the latest stage of production (Hyline management guide, 2017).

A balanced nutrient diet is essential during the rearing stage as it helps the chick to grow and develop into a mature pullet. High proportions of fine components and coarse particles in the diet can lead to selective feeding. This will result in unbalanced nutrient supply to the bird. Lohmann LSL pullets have eight different stages of nutrient levels. They are namely, starter, grower, developer, pre-layer (17 weeks of age to 5% of production), pre-peak (approximately 18 weeks of age to 50% of production), layer phase 01 (approximately 50% production to 40 weeks), layer phase 02 (approximately 41-50 weeks), layer phase 03 (approximately 51-65 weeks) and layer phase 04 (approximately after week 65) (Lohmann LSL lite layer management guide, 2005).

From the placement of the chicks to the rearing environment until two weeks of age starter diet is fed. However, use of chick starter diet is recommended until the birds reach the standard body weight or if the recommended daily feed intake is low before introduction of a grower diet. When switching from grower diet to developer diet reduced nutrient density and increased crude fiber (5-6%) is recommended to increase the eating capacity (Lohmann LSL lite layer management guide, 2005). This will help the bird to reach the required body mass before the sexual maturity. Pre-layer diet consists of higher calcium levels, which is almost twice the amount of developer diet as well as higher protein and energy levels. Feeding such a diet for a period of 10 days before the start laying will benefit flock uniformity, provide essential and sufficient nutrients for late maturing birds and enable birds to obtain sufficient calcium for eggshell production of the first eggs (Lohmann LSL lite layer management guide, 2005).

Pre-layer diet is introduced for a short period before the pre-peak diet. Pre-layer diet enhances a smooth transition from low calcium and low nutrient dense developer diet to a diet with higher levels of calcium plus high amount of nutrients (Hyline management guide, 2017). Pre-layer diet contains around 2-2.5% of calcium, which is comparatively a high amount of calcium compared to a typical diet but not enough for a bird who is just about to start laying. Pre-lay diets should be given only for a short period because; it does not meet the complete nutrient requirement of a layer in full production (Lohmann LSL lite layer management guide, 2005).

After the onset of lay it is recommended to phase feed according to the feed intake and egg mass output per day. Hens with outstanding production will require higher amounts of calcium and lower amounts of phosphorous based on the age. All 5 phases of feed types depend on energy level of 2800 kcal/kg and a room temperature of 22 ºC. Under these conditions, a daily feed intake of 95-105 g per hen can be expected (Lohmann LSL lite layer management guide, 2005).

2.3.4 Lighting schedule during pullet rearing

Artificial lighting during pullet rearing affect many physiological processes of poultry. It affects regulation of feeding, growth, stress, sexual maturity, reproductive performance, eggshell quality, feed efficiency, and egg size (Etches, 1994).

Before the chicks arrive at the poultry house, they have gone through a series of processing steps. Once the chicks are placed in a barn, they are provided with 24 h of light. This will help find feed and water which help to help recover from the handling and transporting stress and adjust to the rearing environment. Presently, breeder companies are recommending intermittent/ recurrent lighting programs to keep the flock uniformity by encouraging feed and water intake. Such programs introduce day and night phases in the rearing facility to stimulate resting and activity in the growing chicks. Breeder companies suggest using intermittent lighting programs for the early stages of life and then the lighting program can be switched to the regular step-down program (Lohmann LSL lite layer management guide, 2005). Birds subjected to intermittent lighting during night tend to have a higher feed intake and body growth (Leeson et al., 2003). In addition, strong and active chicks stimulate the weak chicks for better performance. As a result, behavior of the flock is synchronized (Lohmann LSL lite layer management guide, 2005).

For the growth of birds, the color of light is also important. Introduction of 14L: 10D of red-light photoperiod (620 nm) to the rearing environment at age of week 12-22 of laying hens can reduce body weight compared to birds introduced to blue light (460nm) (Hassan et al., 2013). However, introduction of green/blue light can increase the growth and development of birds by promoting the proliferation of muscle satellite cells (Rozenboim et al., 2004). During the postnatal growth of broilers, around 95% of muscle fiber is derived from satellite cells. These cells differentiate and fuse with existing fibers during growth. Muscle fibers increase in size with

addition of nuclei from satellite cells. Introduction of green light to the early post-hatch period helps to promote satellite cell proliferation, increasing the muscle weight of birds (Halevy et al., 1998).

Green LED affect the growth hormone and Insulin like Growth factor-1 (IGF-1) levels during early post-hatch period (Zhang et al., 2014). IGF-1 regulates cell proliferation, differentiation and tissue growth. IGF-1 is involved in satellite cell proliferation and DNA synthesis (Florini et al., 1996). GH and IGF-1 are the primary factors that increase the early post-hatch performance and muscle mass of birds (Zhang et al., 2014). Therefore, many laying hen production systems incorporate green/blue light spectrum as a tool that supports growth increment during pullet stage (Once Innovations, 2016).

2.3.4.1 Effect of light on sexual development of pullets

The time to lay the first egg (sexual maturity) is greatly influenced by the length of day. Reduction of the day length (number of hours of light) given to the layers delays sexual maturity but increases the number of eggs produced during the first half of egg production. However, short days do not increase the total number of eggs produced during the production period. Increasing the day length reduces the age to lay first egg. Increasing of light for more than 11–12 h during the grow out (till 17 weeks of age), promotes sexual maturity and egg production (North, 1984).

Feeding program also affects the sexual maturity along with lighting program. Optimum feeding results in birds with the desired body weight and structure before the onset of sexual maturity (North, 1984). Introduction of intermittent lighting during the grow out period is another option to promote sexual maturity (Leeson et al., 2003). A well-developed body structure is an essential parameter to ensure the reproductive fitness of birds. The ratio of body weight to shank length, measures the body condition. It is also a measure of fleshing. Birds with a higher ratio are

overweight and the ones with a lower ratio are underweight. Overweight and underweight birds are considered to be birds with potentially poor reproductive fitness (Casey, 1970).

Sexual maturity is regulated by hypothalamic stimulatory hormone (gonadotropin releasing hormones, GnRH) and inhibitory hormone (gonadotropin inhibitory hormone; GnIH). Light stimulates the anterior pituitary gland to secrete GnRH that stimulates the release of gonadotropins (follicle stimulating hormone/FSH and luteinizing hormone/LH). At the time that the first egg is laid, the chicken ovary has 5-10 large follicles that have a hierarchy of size (larger than 1 cm). The largest follicle is the closest to ovulation. A sufficiently mature follicle on the verge of ovulation will synthesize progesterone. This takes place in response to the pre-ovulatory surge of LH. The small non-matured ovarian follicles are the source of estrogens and androgens. Flux of these steroids are LH responsive. The GnRH neurons are directly innervated by opsin containing photoreceptor cells (Shap, 1993). However, during the dark phase release of melatonin from retina and pineal gland stimulates GnIH release. GnIH acts on both the hypothalamus and anterior pituitary to prevent the release of GnRH and gonadotropins, respectively (Baxter et al., 2014). With the provision of higher amounts of light during this stage of age, GnRH and gonadotropins releasing is stimulated. Since the birds receive lower number of dark periods, production of GnIH in the bird's body is less stimulated (Tsutsui et al., 2010).

With release of gonadotropins, a series of physiological responses that take place in a bird's body. Growth of small follicles is the first response after light stimulation. These follicles make large amount of estrogen. Estrogen stimulates most of the reproductive transformations related to sexual maturity (Robinson et al., 1990). Also, estrogen increases the yolk precursor production in the liver, resulting an enlarged and pale liver. The amount of fat in liver increases to support the production of egg yolk lipids. Then, the oviduct enlarges to receive ovulated follicles by the time

of ovulation. Next, estrogen affects the composition of bones, by mobilizing calcium daily to facilitate eggshell production (Wilson, 1996). Finally, estrogens and androgens affect the plumage, comb size and color, and sexual receptivity to males. Thus, sexual maturation of chicken is regulated upon light stimulation by a balance of hormones (Robinson et al., 1996).

2.3.4.2 Effect of characteristics of light on egg production

The intensity of light and wavelength or color are key factors that affect the puberty and egg production of chicken. The intensity of light during the laying period can affect egg production of birds. The intensity of light in laying hen houses should be maintained at 5-10 lux (Hyline management guide, 2017). Light provided in this range of intensity for a duration of 14 h is recommended for layers during the production period. Light intensity beyond the recommended range can trigger aggressive, hyperactive, and cannibalistic behavior in birds, resulting in high mortality (Lohmann LSL lite layer management guide, 2005).

Min et al. (2012) showed that introduction of monochromatic red LED light can stimulate the birds to mature earlier compared to green and white light, respectively (Lewis and Morris, 2000). Also, chickens raised under red light produced more eggs compared to blue light (Min et al., 2012). For quail, reared under red light from week 14-60 with 20 lux, they produced a higher number of eggs compared to birds reared under blue/green light (Woodward et al., 1969). Potential reasons for increasing egg production, the long wavelengths of light (like red light) penetrate the skin and skull more efficiently than short wavelengths (Solangi et al., 2004). This improves reproductive performance of birds.

Not only egg production efficiency but also egg quality is affected by the color of light in a hen house. Eggshell strength was significantly higher in the Hy-line brown hens reared in red light from week 19-52 of age at 16L; 8D at an intensity of 15 lux, compared to the birds in blue

light (Er et al., 2007). However, the authors concluded that eggshell thickness was not affected by the color of light. Moreover, blue LED light reduced the egg length and width, making more rounded eggs. In contrast, red LED light can reduce egg width, making elongated eggs, especially as hens grow old (Er et al., 2007). However, Pyrzak et al. (1987) found that eggshell weight as a percentage of egg was higher under blue light compared to red in the first half of laying. Interestingly, in the second half of laying red light reported higher values. The inconsistency of results may be due to the dependence of egg quality on many factors such as light source, photoperiod, strain of birds, etc. (Min et al., 2012).

2.3.5 Effect of temperature and relative humidity on laying hens

Ambient temperature and relative humidity provided during production period also affect the performance and egg quality of layers. Laying hens are highly sensitive to temperature fluctuations. Optimum temperature and RH that layers are maximally productive is 18-24 °C and 50-60 % (Holik, 2015). Environments outside this optimum temperature and RH range will result depressed natural behavioral patterns of layers, including reduced body weights, feed intake, reduced egg production, egg weight, shell quality and overall egg quality (Mohmoud et al., 1996; Yahav et al., 2000; Mashaly et al., 2004).

The egg production cycle involves secretion of protein, calcium, and bicarbonate in large quantities. During temperatures higher than TNZ, panting and cutaneous cooling systems activated in birds for thermoregulation (Purswell et al., 2012). Plasma calcium and bicarbonate levels fall due to the respiratory alkalosis and will result increased blood pH level with reduced blood Ca ion (Ca^{2+}) (Mohmoud et al., 1996). Imbalance of calcium and bicarbonate reduce the quality of eggs (Mahmoud et al., 1996). Humidity controls thermoregulation through evaporative cooling. Lower RH levels than 50% in the production facility increases the water loss causing dehydration and

suppressed performance. Higher RH levels over 60 % reduces efficiency of panting and reduces hen performance including feed intake, body weight gain, and egg production (Yahav et al., 2000). However, Yahav et al. (2000) also reported that during heat stress regardless of RH fluctuations, the temperature merely affected the egg weight, eggshell thickness and break strength. Therefore, not only providing the optimum environmental conditions during the incubation is important but also, during production cycle to improve the production performances of laying hens.

CHAPTER 3: PRODUCTION PERFORMANCE OF PULLETS UPON RECIEVING LIGHTING DURING EMBRYO DEVELOPMENT

ABSTRACT

Environmental conditions during incubation can significantly affect the chick quality, post-hatch and egg production performances of laying hens. Therefore, it was hypothesized that providing a photoperiod (day/night cycle) during incubation will positively affect the hatching, post-hatching and egg production performance of laying hens. Two incubation experiments were conducted in this project to test this hypothesis. In the first experiment (preliminary trial), 2400 hatching eggs from 2 hen lines (Lohmann Lite and Lohmann Brown) were incubated in 6 incubators. Three lighting treatments were assigned to 2 incubators each. Control eggs (n=300 eggs/hen line) were incubated in the dark. For the first 18 days, 600 eggs from each hen line were incubated under red LED light. For the last 3 days of incubation, one-half of these eggs were transferred to the dark and the others remained incubated in predominantly red light (dim to red). The remaining 300 eggs from each hen line were incubated in full spectrum white LED light for 21 days. For all lighting treatments, a day-night cycle of 12 h light: 12 h dark (12L:12D) was used. Hatched female chicks (n=512) were housed in 64 cages at 8 birds/cage and provided one of two rearing (post-hatch) light regimes. In the second experiment 640 eggs from each of two laying hen lines were incubated in 8 incubators. Eighty eggs from each hen line were randomly assigned to each incubator. Six incubators were installed with 3 different types of LED light and 2 were left in the dark. Blue LED, red LED (dim to red) and full spectrum white LED lights were installed in 2 incubators each. In each lighting treatment the photoperiod used was 12L:12D. Hatched female chicks (n=384) were housed in 64 cages at 6 birds per cage and were provided with the same post-hatch light regimes as experiment 01. Long day post-hatch lighting treatment had a photoperiod of 23L:1D from day 1-3 and 20L:4D from day 4-14 (control). Short day lighting treatments had 18 h of light including two 30 min phases during the dark period in first 3 days followed by 17 h of continuous light with two 30 min phases for day 4-14. For both regimes, day length was reduced over time to the point of 9 h of light at 7-16 weeks of age. Day length was increased at 17 weeks by 1 h of light/ week to 14 h of light by 21 weeks of age. During the study hatching, post-hatch growth and feed consumption were monitored, and egg production and quality were measured. The red-light incubation treatment reduced the total time taken to hatch and improved the navel quality, yolk free body weight and post-hatch early growth rate. It also reduced the age to lay the first egg and increased the egg production without negatively affecting the egg quality parameters. In conclusion, introduction of red LED as a photoperiod to hatching eggs during incubation was beneficial to increase the production performance.

Key words: red LED, layers, incubation, photoperiod, egg production

3.1 INTRODUCTION

Increasing the production performance of layers is dependent on manipulating the environment around incubation and laying hen facilities. Making an efficient and productive layer begins from before the chick's hatch. Environmental conditions provided during the incubation stage are critical and will last until the post-hatch performance of birds. With the advancement of technology, introduction of a photoperiod to incubation is a known factor that can produce healthy chicks that can cope with the vigorous environment and can increase the production performance (Huth and Archer, 2015a). Daily rhythms of light-dark cycle during incubation encourage melatonin synthesis in the pineal gland (Reed and Clark, 2011). Melatonin secretion from the pineal gland is stimulated during the dark period and this hormone converts photoperiodic signals into neuroendocrine signals (Underwood et al., 2001). Rhythmic production of melatonin in chicken embryos can be seen, as early as the third day of incubation (Zeman & Herichova, 2011). Early melatonin rhythms influence development of embryo and post-hatch growth (Reed and Clark, 2011). Archer, (2014) showed that white LED light provided for 12 h per day (12L:12D) during incubation improved hatchability in 3 percent compared to dark provided to the whole incubation (0L: 24D) and improved overall day-old chick quality. In a recent study, Huth and Archer (2015a) noted providing white LED light (12L:12D) could improve chicks hatched with no defects by 19 percent compared to the dark (0L: 24D) and resulted in less vocalization in an isolation test (less fear to environmental stressors) of broiler birds. These effects are enhanced due to the prenatal circadian rhythms that improve epigenetic adaptation. This epigenetic adaptation improves the ability of chicks to adjust to the post-hatch environment, providing a good start in life (Özkan et al., 2012a). However, studies have also shown that provision of a photoperiod to the entire incubation period is not necessary to have to increase the post-hatch performance of birds. A study was done by Shafey et al. (2003) showed that introduction of cool white florescent (16L:

8D) from the ED 01-18 (followed by 3 days of dark) could increase the embryo weight gain of broilers from 120 mg/ day compared to the dark/ control (24D: 0L from ED 01-21). Introduction of a photoperiod from the initiation of incubation and throughout the incubation is not obligatory to have the influence of a circadian rhythm. If there is a diurnal rhythm of melatonin established during the first 18 days of incubation, evidence from Shafey et al. (2003) shows that, this diurnal rhythm continues to improve the growth of the embryo even during the last 3 days of incubation.

Since the photo-stimulation of hatching eggs during incubation can advance the post-hatch performance, researchers have focused on improving egg production performances through manipulating incubation conditions. Incubation temperature and X-ray radiation are known to be factors that can affect the egg production performance of egg layers (Glatz, 1997; Essenberg, 1935). For example, the age to reach sexual maturity of layers has changed depending on the amount of X-ray radiations received during incubation (Essenberg, 1935). During the peak production of layers, they reach maximum egg production of one egg/ hen/ day (Wolc et al., 2011). What differs among the strains could be when they start laying or reaching the sexual maturity and how long they lay at peak. However, reduction of age reaches the sexual maturity and higher continuous egg production will put the birds in risk, as the birds face a jeopardy of reducing the amount of mineralized structural bone and increasing the bone fragility and susceptibility to fracture. When they reach peak egg production, birds do not get enough calcium (Ca) for production of eggshells as the bones start depleting Ca reserves from young ages (Whitehead, 2004). Poor quality eggshells are a major economic concern of commercial egg producers as they reduce the number of table eggs available for sale (Sandilands et al., 2009).

Van der Pol, (2017) showed that providing a photoperiod during the incubation increased the strength and leg bone health of broilers. Having high bone strength is beneficial, minimizing

the calcium loss in the skeleton of layers during calcium resorption from bone and protects against negative effects on egg quality (Wistedt, 2013). Therefore, while reducing the age to reach the sexual maturity, providing a photoperiod during incubation would be a tool to provide a rigid skeleton to the layers and increase the egg quality.

Behavior and aggression of birds also affect the egg production performance. Aggressive behaviors such as feather pecking (both peaking at and pulling out feathers of group mates), and environmental stressors (changes in temperature, RH etc.) can cause poor feather condition of laying hens eventually increasing feed intake through loss of insulation capacity (Tauson and Svensson, 1980), reducing the egg production (Johnsen et al., 1998) and increased mortality (Blokhuis & Wiepkema, 1998). Feather pecking of birds indicate negative welfare aspects and it could lead to serious losses of production performance (Riedstra and Groothuis, 2003). Providing a photoperiod in incubation was reported to reduce the impact of stressors during the rearing period increase the welfare of birds (Huth and Archer, 2015a). To increase egg production performance, flock uniformity and welfare; introduction of a photoperiod to the incubation could be helpful as it has shown to decrease stress and fearfulness of birds. However, to our knowledge, no research has focused on introducing light as a physical factor to incubation environment to evaluate hatching, post-hatching and egg production performance of egg layers.

We investigated the effects of an incubation photoperiod on egg production performance under the following hypothesis. Introducing light to the incubation will positively affect hatching, post-hatching and egg production performance of commercial egg layers. This report will discuss incubation lighting as an environmental factor that can affect the production performance of layers.

3.2 Materials and Methods: Experiment 01

The animal care and use committee of Dalhousie University, Nova Scotia, Canada approved all procedures in the study.

3.2.1 Experimental design

Hatching eggs were obtained from two hen lines (Lohmann Brown and Lohmann Lite) and incubated under 4 hatchery lighting treatments using a completely randomized design. Out of the 4 treatments, 3 treatments had same type of treatments for the entire 21-day period. This included incubation in the dark (control), white LED light (W21) and red LED light (R21). The fourth treatment was red LED light provided from the start of incubation followed by a transfer to incubators with no light at day 18. The effects of incubation lighting treatments on hatch weight were analyzed using a two-factor completely randomized design.

During the post-hatch period of the experiment, chicks were raised in 8 environmental control rooms under 2 different grow-out photoperiods; long days and short days. Long day post-hatch lighting treatment had a photoperiod of 23L:1D from day 1-3 and 20L:4D from day 4-14 (control). Short day lighting treatments had 18 h of light including two 30 min phases during the dark period in first 3 days followed by 17 h of continuous light with two 30 min phases for day 4-14. For both regimes, day length was reduced over time to the point of 9 h of light at 7-16 weeks of age. Day length was increased at 17 weeks by 1 h of light/ week to 14 h of light by 21 weeks of age. A split-plot design (4 X 2 X 2) was used to evaluate the measurements made following transfer to these environmental control rooms. In the split-plot design, grow-out lighting treatment was used as the main plot and the combination of incubation lighting treatments and genetic background of the birds were used as sub plots.

3.2.2 Incubation lighting treatments

Hatching eggs from two commercial hen lines (Lohmann Brown and Lohmann Lite) were obtained for the experiment. From each hen line, 1200 eggs were randomly distributed to 6 Chick Master® G09 incubators. Two incubators were randomly assigned to an incubation lighting treatment.

Out of the 6 incubators, 2 were left in the dark (control) 2 were equipped with Red LED light strips marketed for lighting commercial laying hen facilities (620-670 nm, AgriShift® TLL for layers, Once Innovation, Plymouth, MN, USA) and 2 were equipped with white LED light strips (Canarm®, 4100K, Canarm LTD, Ontario, Canada). Lights were operated with a commercial dimmer and reduced to 40% of the maximum intensity.

Four light strips from each LED light type were vertically mounted on a metal rack that was attached to the left wall of each incubator. A timer was installed to control the photoperiod of 12 h of light and 12 h of dark (12L: 12D). Light intensity within the incubator averaged 170 lux at the egg level as measured using data loggers (Model OMYL-M62, Omega® Engineering, Quebec, Canada).

The control incubators and white LED incubators were loaded with 150 eggs from each hen line per incubator. Four trays were installed into each of these incubators with 75 eggs set into each tray. Holding a similar number of eggs, 8 trays were set in each red LED incubator. In total, 600 eggs from each hen line were placed in both red LED incubators. On day 18 of incubation, half the eggs from red LED incubators were transferred to the dark incubators for the next 3 days of incubation. Eggs that remained incubated in the red LED light until the end of incubation served as the third treatment (R21). Half of the eggs that received red LED light until embryonic day (ED) 18 followed by 3 days of dark period was the fourth treatment (R18).

3.2.3 Incubation procedure

All eggs were weighed prior to incubation. The incubators were maintained at 37.5°C and 85.0% relative humidity from ED 0 to ED 18 in accordance with the recommendations of the manufacturer. Any cracked, dirty, or malformed eggs were excluded from the study from the beginning. Data loggers were installed in the center of the first tray of each incubator to record the temperature, relative humidity, and light intensity of incubators in every 10 seconds during the course of entire incubation period. Every 15 minutes the egg trays were turned in a 90-degree arc from the start of incubation to 432 h of incubation (ED 18).

Eggs were candled and transferred into hatching baskets at ED 18. All eggs were returned to hatching trays in the same incubators if they came from other than eggs from the illuminated with red light. Half of the eggs from red LED incubators were randomly selected and placed into trays in the 2 dark incubators with the other half returned to the red LED incubators. During the last 3 days of incubation, the temperature was gradually reduced to 37.0°C. Relative humidity was progressively increased to 94.5% by 497 h. At 512 h, humidity was reduced to 55% to dry the chicks prior to removal.

Starting from 470 h into incubation the number of chicks that successfully hatched was monitored in intervals of 3 h. As the chicks hatched, they were moved to the back of the hatching tray separated from the un-hatched eggs by a barrier. Hatch window checks ended at 512 h of incubation.

At 512 h, all hatch trays were pulled out of the incubator to process the chicks. All hatched chicks were weighed and were sorted by gender. Lohmann Brown chicks were gender separated by the color of their down. Male chicks were yellow and female chicks were brown to red. Length of primary feathers on the edge of the outstretched wing was used to separate the genders of the Lohmann Lite birds (Lohmann-LSL Lite layer management guide, 2005).

3.2.4 Post-hatch environment

3.2.4.1 Lighting during grow-out period

Female chicks were transferred to the Atlantic Poultry Research Centre from the hatchery. Prior to placement, the chicks were vaccinated for Merek's disease by subcutaneous injection. The processed female birds were placed in the rooms of the Controlled Environment (CE) suite. Each room was equipped with conventional pullet rearing cages. Two portable cage units in each room held 8 cages (60X48cm) that were stocked with 8 birds per cage. Birds of the same hen line and incubation treatment were randomly assigned to a cage in each room. Dim to blue LED lights (440-470nm, AgriShift® TLP for pullets, Once Innovation, Plymouth, MN, USA) that are marketed for pullet rearing were used at an intensity of 10-20 lux for the first 27 days to illuminate these cages. Then intensity was dropped down from 28th day onwards to 4-6 lux progressively. A photoreceptor sensor of a digital light meter (Digital light Meter, model 61-686, Ideal Industries, Inc., Sycamore, IL) was used to measure the intensity of blue LED light at the feeder level.

Birds were weighed prior to placement in the cage and 6 h later to determine early post-hatch performance. Feed and water were given ad-libitum throughout the study period.

More readily adapted birds may not need extremely long photoperiods early in life to ensure water and feed and are accessed appropriately. To evaluate this, two lighting treatments (Figure 01) were applied during post-hatch period to the control environment rooms. Four rooms were randomly assigned to the control or long day treatment (Lohmann-LSL Lite layer management guide, 2005) and 4 rooms were assigned to the short-day treatment. The control rooms (long day photoperiod) were programed with the lighting program recommended for the white shelled layer by the primary breeding company. A photoperiod of 23L:1D was provided during the first three days followed by 20L: 4D from day 4–14. From day 15-21 photoperiod was 18L:6D, followed by at 22-28 days 16L:8D, 29-35 days 14L:10D, 36-42 days 12L:12D and by

day 43 it was 9L:15D per day. Treatment birds (short day photoperiod) were reared with a longer dark period as well as 2 intermittent periods of 30 minutes of light (0.5 L) given during dark period. The photoperiod allocation for day 1-3 was 18L: 6D (17L: 2D: 0.5L: 2D: 0.5L: 2D) and it was 17 L:7D (16L: 3D: 0.5L: 2D: 0.5L: 2D) from day 4–14, Day 15–21 it was 16.5L:7.5D (15L: 1.5D: 0.5L: 2D: 0.5L: 2D: 0.5L: 2D), day 22–28 was 14.5L:9.5D (13L: 2.5D: 0.5L: 2D: 0.5L: 2D: 0.5L: 3D), day 29-35 was 13L:11D (12L: 3.5D: 0.5L: 4.5D: 0.5L: 3D), day 36-42 was 11L:13D (10L: 4.5D: 0.5L: 4.5D: 0.5L: 4D), day 43 onwards 9L:15D. Intermittent periods of light were provided during the dark phase to encourage early feeding and drinking of the birds. For both photoperiods, day length was maintained at 9 h for light at 7-16 weeks of age. At 17 weeks of age (119 d) there was 10 h light allocated to pullets and was increased 1 h of light at the start of each week (weekly steps) to 14 h at 21 weeks of age.

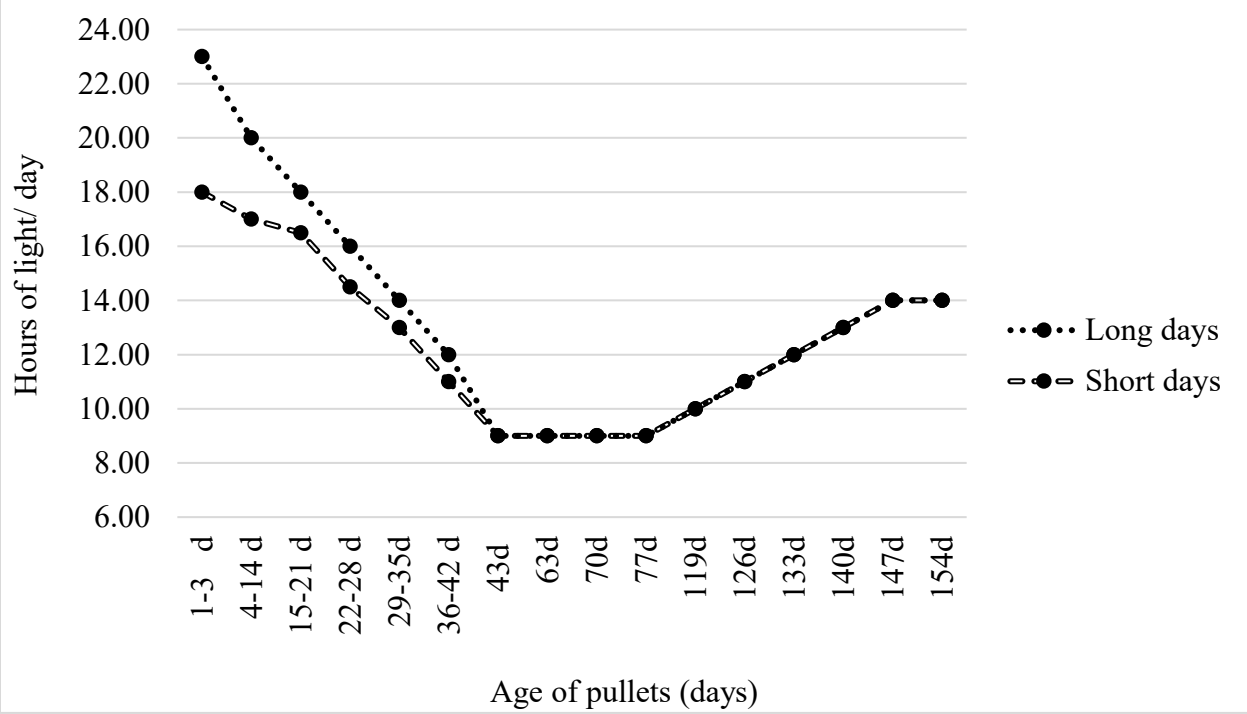

Figure 1. Lighting treatments used during the post-hatch pullet-rearing period. *Long day photoperiod was maintained according to the guidelines given by the primary breeder company*

for the white shelled layers (Lohmann Lite birds). Long day post-hatch lighting treatment had a photoperiod of 23L:1D from day 1-3 and 20L:4D from day 4-14 (control). Short day lighting treatments had 18 h of light including two 30 min phases during the dark period in first 3 days followed by 17 h of continuous light with two 30 min phases for day 4-14. For both regimes, day length was reduced over time to the point of 9 h of light at 7-16 weeks of age. Day length was increased at 17 weeks by 1 h of light/ week to 14 h of light by 21 weeks of age.

3.2.4.2 Pullet performance data

The body weights of pullets were measured at placement, followed by 6 h after placement. Mortality rate was also monitored of the pullets early in life, as a standard management practice, the beaks of the chicks were precision trimmed with a hot blade beak trimming device. The birds were weighed as a cage group on day 14, 21 and day 28. Starting from 28th day, birds were weighed every 4 weeks as a group, until end of the production period.

From week 1-4, birds were fed a starter diet and week 4-8 they were fed grower diet. Week 8-16 they were fed a developer diet and starting from week 16, a pre-layer diet was fed to the birds until they reach 5% of egg production. At that point the birds were fed a sequence of layer diets (Lohmann-LSL lite layer management guide, 2005).

At 16 weeks of age the shank length of each leg was measured and the relationship with body weights determined. Shank length is an indication if the bird's body frame development is in accordance with their weight. The shank length was measured from the top of the hock joint to the bottom of the footpad (Renema et al., 2007).

3.2.5 Laying hen facility

The birds were moved to a common production room equipped with layer cages at week 17, taking care to keep cage-mates together. Five birds were placed per cage in the production

room and the spare birds were transferred to the main production room. Day length was increased at 17 weeks of age to stimulate the birds to develop reproductively.

Production parameters include, the age that first egg was laid and daily egg production. Birds were monitored daily until each treatment combination reached 60% of hen day egg production (Equation 1. Hen day egg production) for 3 consecutive days. It took a total of 47 days to reach this stage. At the end of this period hen housed egg production was calculated (Equation 02).

Equation 1. Hen day egg production

$$Hen\ day\ egg\ production = \frac{Number\ of\ eggs\ produced\ on\ a\ day}{Total\ number\ of\ hens\ present\ on\ that\ day}$$

Equation 2. Hen housed egg production

$$Hen\ housed\ egg\ production = \frac{Number\ of\ eggs\ produced\ during\ the\ period}{Total\ number\ of\ hens\ present\ at\ the\ beginning\ of\ laying}$$

Additionally, feed consumption of the entire production cycle was monitored. Feed conversion ratio was calculated using the amount of feed a bird consumed during a period and the egg mass (average egg weight X number of eggs laid during the period) in a particular period of time.

Equation 3. Feed conversion ratio

$$\text{Feed conversion ratio} = \frac{\frac{Feed\ intake}{Number\ of\ birds\ per\ cage}\ \text{during the period}}{(Average\ egg\ weight\ per\ cage\ \text{X number of eggs laid during the period})}$$

For egg quality measurements, egg weight, specific gravity, shell breaking strength, yolk weight, albumen height, albumen weight, shell weight & thickness were measured at week 20 and 24. There were 3 eggs collected per cage. At the end of the production cycle feather cover was scored (Table 05) in head, neck, breast, abdomen, back, wing, tail areas of the bird. A 6-grade score system was used to rank the feather cover from full to bald with no feather cover (Renema et al., 2007).

Table 5. Six grade feather score system used to rank the feather condition of the birds.

Feather score	Description
0	Smooth, complete plumage
1	Ruffled, with no bare spots
2	Small bare spots (up to 5 cm wide at the widest part)
3	Large bare spots (greater than 5 cm wide)
4	Area completely bare
5	Area completely bare with injury to skin

3.3 Materials and Methods: Experiment 02

All procedures in this study were approved by the Animal Care and Use committee of Dalhousie University, Nova Scotia, Canada.

3.3.1 Experimental design

Using 8 incubators in Dal AC hatchery 4 lighting treatments were evaluated with 2 incubators per treatment. Six incubators were equipped with LED lights. Red, blue and white LEDs lights were installed for two incubators each. Eighty eggs from each hen line were randomly selected to be placed in each incubator. Lights were on for 12 h followed by 12 h of dark

(12L:12D). Completely randomized design was used to analyse the effects of lighting treatments on hatch performance.

The processed female chicks were placed in the Atlantic Poultry Research Center Controlled Environment (CE) suite. There were 8 CE rooms introduced with two different grow-out photoperiods. Randomly selected four rooms were introduced with one of two photoperiods. A split-plot design (4 X 2 X 2) was used to evaluate the bird post-hatch performance using grow-out lighting treatment as the main plot. Incubation lighting treatments and the two hen lines were two sub plots in each main plot.

3.3.2 Incubation lighting treatments

Hatching eggs from two commercial hen lines (Lohmann Brown and Lohmann Lite) were obtained for the experiment. From each hen line, a total of 640 eggs were randomly distributed in eight Chick Master® G09 incubators that are used in the Dalhousie Agriculture Campus' hatchery. Eighty eggs from each hen line were randomly selected to be placed in each incubator. Six incubators were installed with three different LED light types and two were left in the dark, which served as the control. Blue LED (440-470 nm, AgriShift® TLP for pullets, Once Innovation, Plymouth, MN, USA), red LED (620-670 nm, AgriShift® TLL for layers, Once Innovation, Plymouth, MN, USA) and full spectrum white LED lights (Canarm®, 4100K, Canarm LTD, Ontario, Canada) were installed in 2 incubators each.

Four light strips from each LED light type were vertically mounted on a metal rack that was attached to the left wall of each incubator. A timer was installed to facilitate the photoperiod of 12 h of light and 12 h of dark (12L: 12D) for the red blue and white LED lighting treatments. Light intensity within the incubator was maintained at 240 lux at the egg level as measured using

placing a data logger in the middle of the first tray of each incubator (Model OMYL-M62, Omega® Engineering, Quebec, Canada).

The incubators were loaded with 80 eggs from each hen line per incubator. From each of 2 hen lines of chicken, 20 eggs were placed on each tray. Four trays were set on each incubator. The same incubation procedure from experiment 01 was followed in this experiment.

3.3.3 Hatch day chick quality evaluation

On the day of hatch, the trays were pulled out following 512 h of incubation. The number of hatched chicks were counted as chicks from fertile eggs. The quality of chicks was evaluated and graded according to their physical appearance. The chicks with completely healed navels and dry chicks with no deformities denote that the chicks were in good quality (Verschuere, 2016).

Chick weight was the first measurement taken on the group of chicks in each tray per hen line. Day old chick weight in relation to their egg weight is an indicator of their quality Molenaar, 2010). Chicks were sorted by gender and each bird was examined for completeness of closure and condition of its navel. Fully healed navels were scored as one. Navels with up to a 2 mm gap and/or a spot of clotted blood and/or a remnant of the yolk sac material in the form of a black string visible outside the abdomen was scored as two. Unhealed navels with a gap of more than 2 mm (with or without black material in the center of the navel) were scored as a three (Molenaar, 2010).

All chicks were sorted by sex. From each tray 5 males and 5 female birds were randomly selected and were measured for chick length. Chick length reflects the degree of development of the bird (Mukhtar et al, 2013). When measuring chick length, the bird was placed along a ruler and the tip of the beak was placed on the end of the ruler. The bird's leg was grasped, and body stretched along the ruler. The length was determined as the distance from tip of the claw on the middle toe to the tip of the beak (Mukhtar et al, 2013).

One bird per gender was selected per tray and was euthanized by exposure to carbon monoxide in order to determine yolk free body weight. The body weight without residual yolk is the yolk free body mass. This is also an indicator of chick development (Mukhtar et al, 2013).

Before placement, chicks were vaccinated for Merek's disease with a subcutaneous injection. After all the birds are processed, the remaining unhatched eggs were cracked open, embryos were euthanized if alive and stage of development was determined.

3.3.4 Post-hatch environment

Hatched female chicks were taken to be placed in the poultry production room facility after 5 h from removal from the incubators. Prior to placement, the female chicks were vaccinated for Merek's disease by subcutaneous injection. The processed female birds were placed in the Atlantic Poultry Research Center Controlled Environment (CE) suite in pullet rearing cages at 6 birds per treatment combination per cage. Two portable cage units containing 8 cages (60 X 48cm) were installed in each room. The same lighting schedules (long days and short days) from experiment 01 were used for these birds.

3.3.4.1 Pullet performance data

The body weights of pullets were measured at placement and at 6 h after placement. Mortality rate was also monitored. As a standard management practice, the beaks of the chicks were precision trimmed with a hot blade beak trimming device at 5 days of post-hatch. The birds were individually weighed on day 7, 14, 21 and day 28. Starting from 28^{th} day birds were weighed every 4 weeks as a cage group until end of the production period.

From week 1-4, birds were fed with starter diet and week 4-8 they were fed with grower diet. Week 8-16 they were fed a developer diet and starting from week 16, a pre-layer diet was fed

to the birds until they reach 5% of egg production. At that point the birds were fed a sequence of layer diets (Lohmann-LSL lite layer management guide, 2005).

At 16 weeks of age the shank length of each leg was measured and the relationship with body weights determined. Ratio of body weight to shank length is a measurement of body condition or body flesh. Higher value for this ratio indicates that birds are overweight and lower values indicate the underweight birds (Casey, 1970). The shank length was measured from the top of the hock joint to the bottom of the footpad (Renema et al., 2007).

3.3.5 Laying hen facility

The birds were moved at week 17 to laying hen cages equipped with red LED (620-670 nm, AgriShift® TLL for layers, Once Innovation, Plymouth, MN, USA) lights that are marketed for layers. Five birds were placed per cage in the production room. Day length was increased at 17 weeks of age to stimulate the birds to develop reproductively.

Production parameters include, the age that first egg was laid. Egg production was monitored daily until 36 weeks of age. At the end hen housed egg production Equation 2. Hen housed egg production (Equation 04) was calculated over the course of the life of the bird.

Equation 4. Hen housed egg production

$$Hen\ housed\ egg\ prodcution = \frac{Number\ of\ eggs\ produced\ during\ the\ period}{Total\ number\ of\ hens\ present\ at\ the\ beginning\ of\ laying}$$

Additionally, feed consumption of the entire production cycle was monitored. To calculate the feed conversion ratio, the amount of feed a bird consumed during a particular period was divided by the egg mass (average egg weight X number of eggs laid during a particular period) laid over the course of 28-day period (Equation 05).

Equation 5. Feed conversion ratio

$$\text{Feed conversion ratio} = \frac{\frac{Feed\ intake \text{ during the period}}{Number\ of\ birds\ per\ cage}}{(Average\ egg\ weight\ per\ cage \text{ X number of eggs laid during the period})}$$

For egg quality measurements, egg weight, specific gravity, shell breaking strength, yolk weight, albumen height, albumen weight, shell weight & thickness were monitored in 4-week periods collecting 3 eggs per cage.

3.4 Statistical Analysis

Incubation and pullet performance data were subjected to mixed model analysis using the PROC MIXED procedure in SAS. Egg quality and feather score parameters were analyzed using PROC GLIMMIX procedure in SAS (version 9.4, 2012, SAS Institute Inc., Cary, NC, USA). PROC GLIMMIX method was used for the data that had a non-normal distribution.

3.4.1 Statistical model used to analyze incubation performance:

Completely randomized design

$Y_{ijk} = \mu + \text{incubation lighting treatment}_i + \text{henline}_j + \text{incubation lighting treatment X henline}_j + \varepsilon_{ijk}$

Where, Y_{ijk} = weight of the day-old chick, μ = overall mean, incubation lighting treatment (i = experiment 01: dark (control), White LED (W21), Red to dark (R18), Red LED 21 days (R21)/ experiment 02: dark (control), red LED, white LED, blue LED), ε_{ijk} = residual error.

3.4.2 Statistical model used to analyze hatch rate:

Non-linear regression model

A nonlinear regression model was used to analyze the effects of light wavelength on the spread of hatch (percentage of hatchability over the time). The nonlinear regression model used

here was 3-parameter logistic growth model. It was used to describe the relationship between the time of incubation and the accumulative hatchability. The nonlinear regression model of the spread of hatch was:

$$Y = \frac{\theta_1}{1 + \exp(-\frac{(X - \theta_2)}{\theta_3})} + \varepsilon$$

Where Y = cumulative percentage of hatched chicks; X = the hours of incubation; θ_1 = the asymptote; θ_2 = the time to half the asymptote and θ_3 = the interval from half to until 75% of the asymptote.

3.4.2 Statistical model used for pullet performance data:

Split plot design

A split plot design is a design with at least one blocking factor. The experimental units within each block are assigned to the treatment factor levels. The blocks are arranged at random to the levels of a further treatment factor. In a block design, the experimental units are nested within the blocks and within each block, treatments are randomly assigned. In a split plot design the experimental units are called split pot (sub plots) and are nested within whole plots (main plots) (Sehgal, 2009)

Sub plot – incubation photoperiod (IP), hen line (HL)

Main plot – grow out photoperiod (GOP)

Y_{ijkl} = μ + incubation lighting treatment$_i$ + hen line$_j$ + grow out photoperiod$_k$ + (lighting treatment x hen line$_{ij}$) + (lighting treatment x grow out photoperiod$_{ik}$) + (hen line x grow out photoperiod$_{jk}$) + (incubation lighting treatment$_i$ x hen line$_j$ x grow out photoperiod$_k$) + ε_{ijkl}

Where, Y_{ijkl} = performance of the pullets, μ = overall mean, incubation lighting treatment (i = experiment 01: dark (control), White LED (W21), Red to dark (R18), Red LED 21 days (R21)/ experiment 02: dark (control), red LED, white LED, blue LED), hen line (j = Lohmann Brown and Lohmann Lite), grow out photoperiod (k = long days and short days), ε_{ijkl} = residual error.

Incubator and cage were used as the experimental units for incubation lighting treatments and pullet performance data respectively. Incubation lighting treatment and hen lines were fixed effects of these experiments and grow out photoperiod was a random effect. Results with significant differences were further analyzed using Tukey-Kramer test. Effects were significant when $P \leq 0.05$ (if the P value was $P \leq 0.05$ the null hypothesis was rejected)

Repeated measures

As the pullets grow the weights and egg quality measures of the same group of birds were taken on the over multiple periods. This was effect was measured using with the repeated measures modeling with PROC MIXED.

3.5 Results for experiment 01

3.5.1 Results for incubation

3.5.1.1 General

Average fresh egg weight for Lohmann Brown and Lohmann Lite were 50.40 g and 47.41 g respectively. There were no initial egg weight differences (P>0.05) among the eggs assigned to the lighting treatments. No significant differences (P>0.05) were observed among the incubation lighting treatment for hatchability (Table 06).

Table 6. Hatchability percentages of different lighting treatments.

Incubation treatment	Mean ± SEM (%)
Control	86.6 ± 1.11
W21	90.6 ± 1.11
R21	89.2 ± 1.11
R18	89.6 ± 1.11

The results are Mean hatchability percentages ±SEM, and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean hatchability percentages are an average of two hen lines together per each lighting treatment. There were no significant differences seen for the hatchability percentages (P>0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).

3.5.1.2 Hatch weight

Initial hatch weight (following 512 h of incubation) did not differ among either the incubation lighting treatments or the hen lines (P=0.5103, Figure 02). There were no significant interactions observed between the main factors of interest. Therefore, the results are an average of two hen lines together.

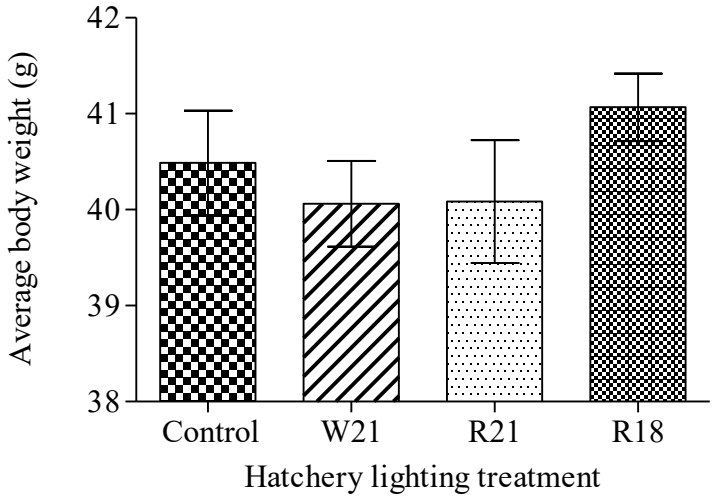

Figure 2. Average hatch weights (g) of the chicks for different incubation lighting treatments at 512 h of incubation. *Results show Mean weight (g) ±SEM. Initial hatch weight were not affected by the incubation treatment. There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period.*

3.5.2 Pullet post-hatch performance

Five hours after hatch, at the time of placement (Figure 03), chicks hatched without light had significantly higher body weight (P=0.001) compared with the lighting treatments. Chicks hatched from R18 treatment had the lowest body weight, whereas the effect was intermediate for chicks from the other two lighting treatments. There were no interactions (P>0.05) between hen line and incubation lighting treatments for body weights.

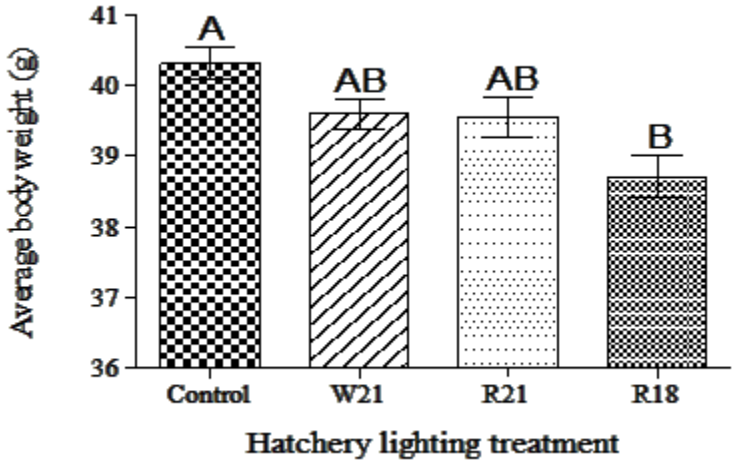

Figure 3. Average chick weight (g) for different incubation lighting treatments at placement (chicks were introduced to the post-hatch environment 5 h after pulled out from the incubators after 512 h of incubation). *Average placement body weights (Mean weight (g) ±SEM) were significantly higher in control chicks. R18 chicks had the lowest placement weight among the all incubation lighting treatments. Means with no common letters are significantly different (there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated*

in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).

Reports from other research groups have showed that broiler chicks hatched under LED had improvements in post-hatch performance. For instance, white LED light lowered susceptibility to stress (Huth and Archer 2015a), green LED light increased weight gain during the first 6 days' post-hatch (Zhang et al., 2016). Therefore, in the current study we anticipated to see chicks from LED light treatments had higher weight gains during the early post-hatch period.

The results from current study show that, body weight following 6 h (Figure 04) of placement was different (P=0.0396) among hatchery treatments. Chicks hatched with the R21 treatment had significantly higher body weights and chicks from R18 treatment had the lowest weight compared with R21 treatment. Birds from other two treatments had an intermediate weight at 6h after placement.

The weight gain percentages during the first 6 h after placement were significantly different (P=0.016, Figure 05) as well. Weight gain percentage was highest for R21 chicks. The birds hatched in the dark had the highest placement body weight but gained the least weight percentage 6 h post-placement. White LED and R18 treatments had an intermediate effect on body weight gain percentages. Chicks hatched under LED lighting treatments had lower weights at placement compared with the control treatment. However, in the current study, all the birds hatched under lighting treatments had accelerated the body weight gain and had higher body weight gain percentages during the early post-hatch period.

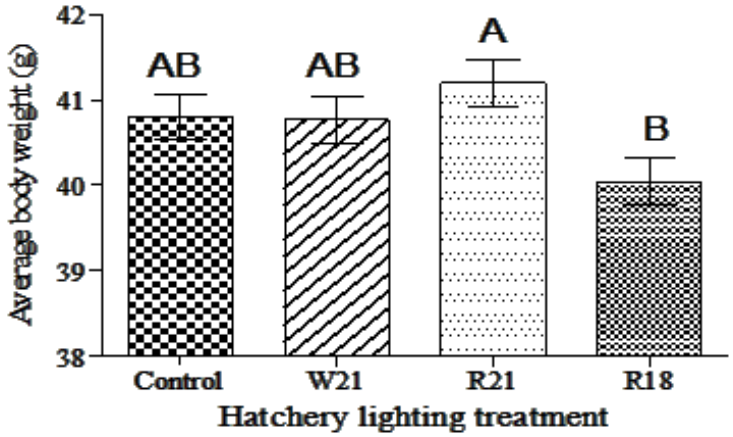

Figure 4. Six hours' post-placement average body weight (g) for different incubation lighting treatments. *Six hours after placement, average body weights (Mean weight (g) ±SEM) were significantly higher (Means with no common letters are significantly different) for R21 chicks and it was the lowest for R18 chicks. There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

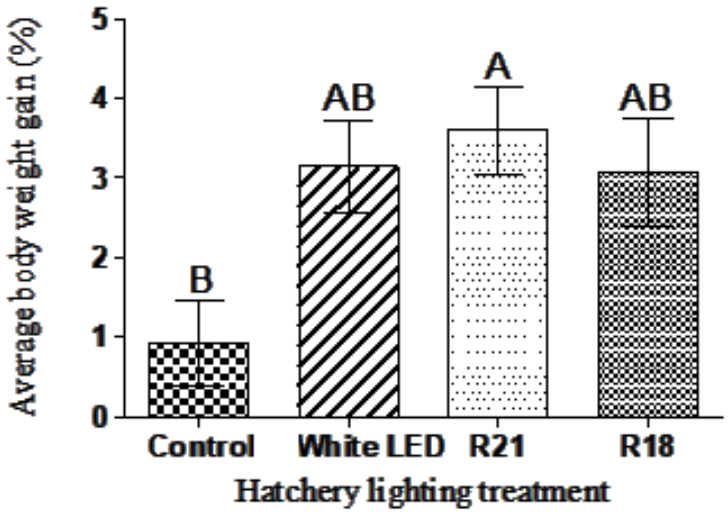

Figure 5. **Six hours' post-placement average body weight gain (%) for different incubation lighting treatments.** *Weight gain (Mean weight gain (%) ±SEM) after six hours of placement was highest (Means with no common letters are significantly different) for R21 chicks and chicks hatched in the control had the least weight gain. There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatments, control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

There were no treatment effects (P=0.060) on body weight by day 14 (Figure 06). However, if the eggs were exposed to either of the two red light treatments the chicks gained more weight (P=0.003, figure 07) during early post-hatch period. Birds from control group had the lowest body weight gain percentage at day 14 and W21 treatment was intermediate.

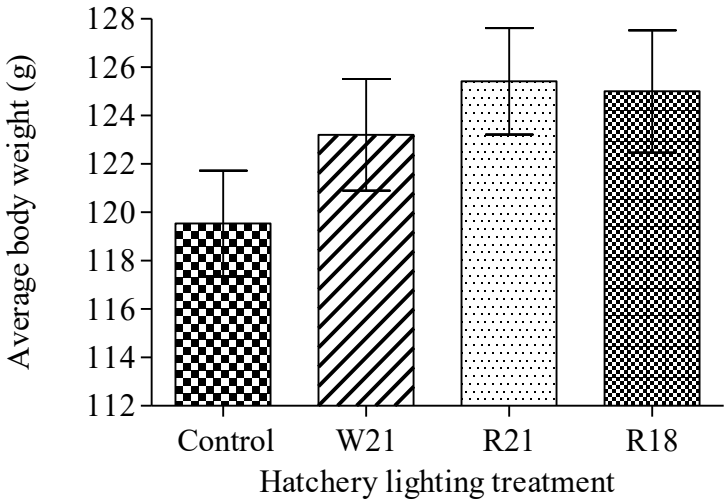

Figure 6. Average body weight (g) of chicks at the day 14 of age for different incubation lighting treatments. *Average 14-day body weight (Mean weight (g) ±SEM) did not significantly differ (Among the incubation lighting treatment, there was no significant (P>0.05) effect of interactions observed for the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

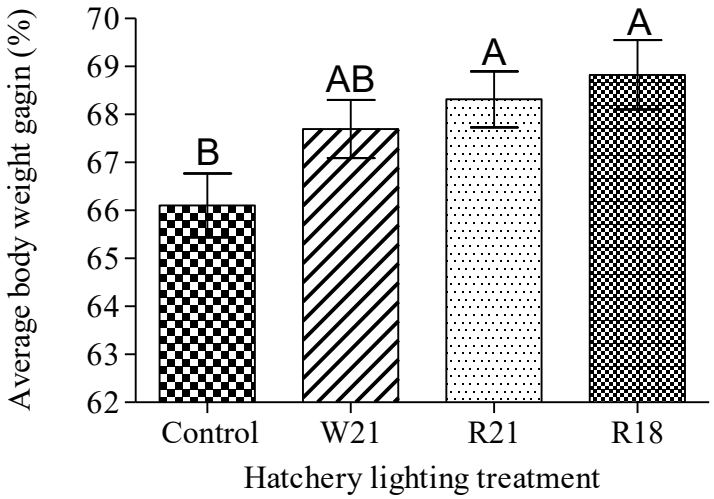

Figure 7. Average body weight gain (%) of chicks at the day 14 of age for different incubation lighting treatments. *At day 14 of rearing the average body weight gain (Mean weight (%) ±SEM) was higher (Means with no common letters are significantly different) if the chicks hatch from the red LED treatments. However, the control chicks had the least over the course of 2 weeks' period (There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights gain percentages are an average of two hen lines together per each lighting treatment. Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

Long day and short-day rearing lighting regimes (Table 07) did not influence (P=0.3823) the early body growth rate by day 14 of rearing.

Table 7. Effect of the interaction of incubation lighting treatments and grow-out photoperiod on the post-hatch weight (g) of pullets at day 14.

	Incubation lighting treatments			
Grow-out photoperiod	**Control**	**W21**	**R18**	**R21**
Long day	122.44 ±2.33	123.67 ±2.33	123.82 ±2.33	126.50 ±2.33
Short day	116.64 ±2.33	122.74 ±2.33	126.18 ±2.33	124.33 ±2.33

The results are Mean weight (g) ±SEM). There were no significant differences seen for the post-hatch weight (g) of pullets at day 14 (P>0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).

The weight gain difference persisted to day 21 (P=0.0222) where body weight gain was highest in the red to dark (R18) treatment (Table 08). The lowest weight gain was reported from the control treatment. Two other treatments were intermediate. By day 28 (Table 09), the difference for the body weight was marginally different (P=0.0567), indicating treatment effects were disappearing.

Table 8. Average body weight gain (%) at 21 days of age for different incubation lighting treatments.

	Incubation lighting treatments			
	Control	**W21**	**R21**	**R18**
21 days' average body weight gain (%)	79.7±0.30[b]	80.3±0.30[ab]	80.5±0.30[ab]	81.0±0.30[a]

The results are Mean weight gain (%) ±SEM), and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment. [ab] *Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

Table 9. Average body weight gain (%) at 28 days of age for the interaction of incubation lighting treatments, rearing photoperiod and the hen line.

		Incubation lighting treatments			
	Rearing photoperiod	**Control**	**W21**	**R21**	**R18**
LB	**Long photoperiod**	87.9±0.35	87.8±0.35	88.0±0.35	88.6±0.35
	Short photoperiod	87.4±0.35	88.0±0.35	88.0±0.35	88.3±0.35
LL	**Long photoperiod**	86.1±0.35	87.1±0.35	86.4±0.35	85.8±0.35
	Short photoperiod	86.0±0.35	86.4±0.35	86.5±0.35	87.1±0.35

The results (Mean weight gain (%) ±SEM) showed a marginal difference for the post-hatch weight gain of pullets at day 28 (P=0.0567). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period, LB=Lohmann Brown, LL= Lohmann Lite).

3.5.3 Body weights from week 05 to 24 of age

At the end of the grower stage (week 08, Table 10) there was an interaction (P=0.0072) observed for bird body weight that involved the light treatments in the hatchery, the post-hatch light treatments and type of hen line used. Lohmann Brown birds hatched from any hatchery treatment and raised in the long day photoperiod or incubated with white LED or red LED 21-day treatment and raised with short day photoperiods had highest body weights during this period compared to Lohmann Lite birds. Regardless of post-hatch photoperiod used, Lohmann Lite birds hatched under any hatchery lighting treatment had the lowest body weights during the grow out photoperiod. During the developer stage (Table 11) there was an interaction (P=0.0155) between grow out photoperiod and hatchery lighting treatments. At the end of this period, at week 18, birds incubated in the dark and raised in long day photoperiods had the highest body weights and the birds from the same hatchery treatment raised under short day photoperiod had the lowest body weights. During the pre-lay stage (Table 12) birds had slight body weight differences (P=0.0448) attributed to an interaction with incubation lighting, grow out photoperiod and hen line. However, this interaction has been disappeared by week 24 of age. By week 24 of the trial only hen line differences were seen (P<0.0001) (Table 13).

Table 10. Average body weights (g) of birds at the end of grower stage (Week 08).

		Incubation lighting treatments			
	Rearing photoperiod	**Control**	**W21**	**R21**	**R18**
LB	Long photoperiod	812±15.5[a]	779±17.2[a]	786±15.5[a]	801±15.5[a]
	Short photoperiod	758±17.2[a]	802±15.5[a]	796±15.5[a]	772±15.5[a]
LL	Long photoperiod	654±15.5[b]	663±15.5[b]	697±17.2[b]	643±15.5[b]
	Short photoperiod	655±15.5[b]	644±15.5[b]	645±15.5[b]	651±15.5[b]

The results are Mean weight (g) ±SEM, and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment. [ab] *Means with no common letters are significantly different (P<0.05). Results show that, Lohmann Brown birds hatched from any hatchery treatment and raised in the long day photoperiod or incubated with white LED or red LED 21-day treatment and raised with short day photoperiods had highest body weights during this period compared to Lohmann Lite birds. Regardless of post-hatch photoperiod used, Lohmann Lite birds hatched under any hatchery lighting treatment had the lowest body weights during the grow out photoperiod. (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in*

red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period, LB=Lohmann Brown, LL= Lohmann Lite).

Table 11. Body weights (g) of birds at the end of the developer stage (Week 18).

Rearing photoperiod	Incubation lighting treatments			
	Control	**W21**	**R21**	**R18**
Long photoperiod	1376±10.1^{a}	1363±10.1^{ab}	1344±10.1^{abc}	1342±10.1^{abc}
Short photoperiod	1314±10.1^{c}	1327±10.1^{bc}	1345±10.1^{abc}	1326±10.1^{bc}

The results are Mean weight (g) ±SEM, and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment. ^{ab} *Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

Table 12. Body weights (g) of birds at the end of the pre-lay stage (Week 20).

	Treatment	Control	W21	R21	R18
LB	Long photoperiod	1732±124	1734±124	1701±124	1721±124
	Short photoperiod	1705±124	1692±124	1639±124	1722±124
LL	Long photoperiod	1727±124	1751±124	1682±124	1723±124
	Short photoperiod	1631±124	1621±124	1670±124	1714±124

Body weights of birds at the pre-lay stage, did not show letter differences from the Tukey Kramer test (P<0.05, Mean weight (g) ±SEM). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).

Table 13. Body weights (g) of birds at 24 weeks of age for the commercial hen lines.

Hen line	LB	LL
Body weight	1992±14.2[a]	1632±14.2[b]

The results are Mean weight (g) ±SEM. [ab] Means with no common letters are significantly different (P<0.05, LB=Lohmann Brown, LL= Lohmann Lite).

3.5.4 Body weight to shank length ratio

Body weight to shank length ratio is a parameter that indicates aspects of the body condition of the birds. However, there were no differences recorded for the hatchery lighting

treatment or grow out photoperiod. There was only a difference observed between the hen lines (P <0.0001, Table 14). where Lohmann Lite birds had a lower average ratio than the Lohmann Brown birds. The provision of light for the incubation and rearing photoperiods did not negatively affect the body frame development of pullets in the current study.

Table 14. Body weight to shank length ratio of commercial hen lines (week 16 of age)

Hen line	Body weight to shank length ratio (g/mm)
LB	15.56±0.46[a]
LL	12.16±0.46[b]

The results are body weight to shank length ratio - Mean ratio (g/mm) ±SEM. [ab] Means with no common letters are significantly different (P<0.05). (LB=Lohmann Brown, LL= Lohmann Lite).

3.5.5 Age at first egg

There were no interactions seen among incubation treatments, type of bird and grow out photoperiod for the age at first egg. However, age at first egg was different between the commercial hen lines (P<0.0001, table 15). Lohmann Brown birds laid their first egg on average before the Lohmann Lite birds.

However, data indicated that there is a trend for being different for incubation lighting treatments (P=0.0678, table 16). Red LED 21 days took the shortest time to begin laying with the longest time for the birds from the white LED hatchery treatment. Two other treatments were intermediate. Rearing photoperiod did not affect the age at first egg (P>0.05).

Table 15. Age of the birds at the first egg, for two commercial hen lines

Hen line	Age at first egg (days post-hatch)
Lohmann Brown	128 ± 0.91 days[a]
Lohmann Lite	132 ± 0.91 days[b]

Age of the birds in days ± SEM. [ab] *Means with no common letters are significantly different (P<0.05, two commercial hen lines were used in the experiment which were Lohmann Brown and Lohmann Lite).*

Table 16. Age of the birds at first egg, for different incubation lighting treatments

Incubation lighting treatment	Age at first egg (days post-hatch)
Control	131 ± 1.2 days
W21	132 ± 1.2 days
R21	128 ± 1.2 days
R18	129 ± 1.2 days

Age of the birds in days ± SEM and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, results are an average of two hen lines together per each lighting treatment. (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).

3.5.6 Hen housed egg production

Egg production was different for hatchery lighting treatments (P=0.0232, Table 17). Birds hatched from R21 hatchery treatment laid the highest number of eggs during the first 47 days. Birds from white LED hatchery treatment gave the fewest eggs. However, the post-hatch photoperiod made no difference to the hen housed egg production. The hen lines were different (P<0.0001) for hen housed egg production. Lohmann Brown birds gave highest number of eggs during the laying period of 7 weeks.

Table 17. Average hen housed egg production (mean number of eggs per hen) for 47 days period.

	Mean value (Eggs)
Incubation treatments	**P=0.0232**
Control	26.0±0.67^{ab}
R21	27.6±0.67^{a}
W21	25.1±0.67^{b}
R18	26.0±0.67^{ab}
Hen lines	**P<0.0001**
LB	29.0±0.55^{a}
LL	24.0±0.59^{b}

Mean number of eggs per hen during 47 days' period ±SEM. ^{ab} Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period, LB=Lohmann Brown, LL= Lohmann Lite).

3.5.7 Egg quality

Egg quality parameters measured were egg weight, specific gravity, shell-breaking strength, yolk weight percentage, albumen height, albumen weight percentage, shell weight percentage and shell thickness (Table 18). Differences were observed only between the hen lines for egg weight, specific gravity, albumen weight percentage, shell weight percentage and shell thickness. There were no significant differences seen for any of the egg quality parameters in the hatchery treatments and grow out photoperiods.

Table 18. Egg quality parameters; egg weight, specific gravity, shell-breaking strength, yolk weight percentage, albumen height, albumen weight percentage, shell weight percentage and shell thickness for the main factors of interest.

Main factors of interest	**Egg weight (g)**	**Specific gravity (g/L)**	**Shell breaking strength (g)**	**Yolk weight as a percentage of egg weight (Mean±SEM)**	**Albumen height (mm)**	**Albumen weight as a percentage of egg weight (Mean±SEM)**	**Shell weight as a percentage of egg weight (Mean±SEM)**	**Shell thickness (mm)**
Incubation	(P=0.2794)	(P=0.9824)	(P=0.5956)	(P=0.4990)	(P=0.9032)	(P=0.9625)	(P=0.8313)	(P=0.7914)
Control	50.2±0.84	1.095±0.00	5851.9±141	10.3±0.22	9.74±0.18	68.4±0.32	10.5±0.12	0.39±0.00
R21	49.0±0.81	1.095±0.00	5667.6±137	10.2±0.21	9.74±0.18	68.3±0.31	10.4±0.12	0.38±0.00
W21	50.4±0.86	1.095±0.00	5607.6±137	10.6±0.23	9.91±0.19	68.5±0.34	10.4±0.12	0.39±0.00
R18	48.4±0.77	1.095±0.00	5723.1±137	10.1±0.21	9.77±0.18	68.4±0.31	10.5±0.12	0.39±0.00
Grow out	(P=0.4530)	(P=0.5783)	(P=0.4381)	(P=0.4564)	(P=0.7486)	(P=0.1408)	(P=0.2200)	(P=0.9912)
Long	49.8±0.58	1.095±0.00	5676.1±121	10.3±0.15	9.76±0.13	68.1±0.23	10.5±0.12	0.39±0.00
Short	49.1±0.58	1.095±0.00	5749.1±122	10.4±0.15	9.82±0.13	68.7±0.23	10.4±0.12	0.39±0.00

Main factors of interest	Egg weight (g)	Specific gravity (g/L)	Shell breaking strength (g)	Yolk weight as a percentage of egg weight (Mean±SEM)	Albumen height (mm)	Albumen weight as a percentage of egg weight (Mean±SEM)	Shell weight as a percentage of egg weight (Mean±SEM)	Shell thickness (mm)
Lohmann Brown	50.6±0.55[a]	1.097±0.00[b]	5774.6±104	10.3±0.16	9.84±0.13	69.3±0.22[a]	10.1±0.10[b]	0.383±0.00[b]
Lohmann Lite	48.4±0.61[b]	1.093±0.00[a]	5650.5±111	10.4±0.15	9.74±0.13	67.6±0.24[b]	10.7±0.10[a]	0.398±0.00[a]

Egg quality parameters are in average egg weight (g)± SEM, average specific gravity(g/L)± SEM, average shell breaking strength (g)± SEM, average yolk weight % ± SEM, average albumen height (mm) ± SEM, average albumen weight % ± SEM, average shell weight % ± SEM, average shell thickness (mm) ± SEM. [ab] Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period, LB=Lohmann Brown, LL= Lohmann Lite, two grow out photoperiods used were long day photoperiod and short day photoperiod).

3.5.8 Feed conversion ratio

Feed conversion ratio was significantly different (Table 19, P=0.0138) among the hatchery lighting treatments during period of week 20-24. Birds hatched under the R21 days lighting treatment had the highest feed conversion ratio. The lowest feed conversion ratio was recorded for dark treatment. Two other treatments had an intermediate feed conversion.

Table 19. Feed conversion ratio of the incubation lighting treatments applied

Incubation lighting treatment	Feed conversion ratio
Control	2.1 ± 0.05^{b}
R21	2.31 ± 0.06^{a}
W21	2.2 ± 0.05^{ab}
R18	2.21 ± 0.06^{ab}

Results are in Feed conversion ratio ± SEM and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean weights are an average of two hen lines together per each lighting treatment. ^{ab} *Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

3.5.9 Feather score

There were no interactions (P>0.05) observed for feather score measurements among the three main factors of interest (Table 20). There were hen line differences seen for feather cover on breast and head areas (P<0.05). However, for tail area, there was a hatchery treatment difference observed (P<0.05). Feathers in tail area were superior for birds from white LED hatchery treatment (1.13±0.09, a lower feather score signifies that the feather condition is better) and birds from control hatchery treatment had the poorest feather cover in the tail area (1.47±0.09). An interaction was observed (P<0.05) for grow out photoperiod and hatchery treatment for feather cover in neck area (P=0.0002, table 21). Birds from white LED treatment and raised in short-day photoperiod had a superior feather cover (1.94±0.1). However, birds raised in long day photoperiods and hatched under R18 (2.44±0.10) and W21 hatchery treatments (2.64±0.11) gave the poorest score feather cover in neck area. Also, birds form control hatchery treatment and raised in short days had a poor neck feather cover (2.5±0.10).

Table 20. Feather score of the birds for the main factors of interest. Feathers were scored different areas of the body including breast, head, abdomen, back, wing, tail and neck.

Main factors of interest	Breast (Mean±SEM)	Head (Mean±SEM)	Abdomen (Mean±SEM)	Back (Mean±SEM)	Wing (Mean±SEM)	Tail (Mean±SEM)	Neck (Mean±SEM)
Incubation	(P=0.1666)	(P=0.4438)	(P=0.5357)	(P=0.6496)	(P=0.9560)	(P=0.0434)	Refer table 21 for the interaction between incubation lighting treatment and grow out photoperiod.
Control	2.46±0.16	1.63±0.10	2.00±0.13	1.94±0.13	1.41±0.10	1.47±0.09[a]	
R21	2.38±0.16	1.66±0.10	1.83±0.13	1.96±0.13	1.34±0.10	1.38±0.09[ab]	
W21	2.03±0.16	1.46±0.10	1.81±0.13	1.91±0.13	1.34±0.10	1.13±0.09[b]	
R18	2.44±0.16	1.47±0.10	1.78±0.13	1.75±0.13	1.35±0.10	1.23±0.09[ab]	
Grow out	(P=0.3019)	(P=0.6850)	(P=0.6421)	(P=0.6322)	(P=0.6473)	(P=1.0000)	
Long	2.22±0.12	1.53±0.07	1.89±0.11	1.85±0.09	1.39±0.09	1.29±0.06	
Short	2.21±0.12	1.58±0.07	1.89±0.11	1.92±0.09	1.33±0.09	1.29±0.06	

Main factors of interest	Breast (Mean±SEM)	Head (Mean±SEM)	Abdomen (Mean±SEM)	Back (Mean±SEM)	Wing (Mean±SEM)	Tail (Mean±SEM)	Neck (Mean±SEM)
Lohmann Brown	2.57±0.23[a]	1.68±0.07[b]	1.83±0.23	1.91±0.09[b]	1.45±0.08	1.23±0.07	2.48±0.05[a]
Lohmann Lite	2.08±0.12[b]	1.42±0.07[a]	1.88±0.12	1.86±0.09[a]	1.27±0.08	1.38±0.07	2.19±0.05[b]

Mean feather score parameter± SEM. [ab] Different letters indicate significant differences (P<0.05). A six grade feather score system was used to rank the feather condition of the birds where 0 indicated a highest quality of feathers and 5 indicated poorest quality of the feathers (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period, LB=Lohmann Brown, LL= Lohmann Lite, two grow out photoperiods used were long day photoperiod and short day photoperiod).

Table 21. Feather score for the interaction of incubation lighting treatments and grow-out photoperiod for the neck area of birds

	Grow out photoperiod	
Incubation lighting treatment	**Long**	**Short**
Control	2.17±0.10^{ab}	2.50±0.10^{a}
R21	2.39±0.10^{ab}	2.38±0.10^{ab}
W21	2.64±0.11^{a}	1.94±0.10^{b}
R18	2.44±0.10^{a}	2.25±0.10^{ab}

Results show, mean feather score for neck area ± SEM and there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore, mean scores are an average of two hen lines together per each lighting treatment. ^{ab} *Different letters indicate significant differences (P<0.05). A six grade feather score system was used to rank the feather condition of the birds where 0 indicated a highest quality of feathers and 5 indicated poorest quality of the feathers (Control: eggs were incubated in the dark during the whole incubation period, W21: eggs were incubated in white LED light with a photoperiod of 12L:12D, R21: eggs were incubated in red LED light with a photoperiod of 12L:12D, R18: eggs were incubated in the first 18 days in red LED light with a photoperiod of 12L:12D followed by a 3 days of dark period).*

3.6 Results for experiment 02

3.6.1 Results for incubation

3.6.1.1 General results for incubation

Initial egg weights differed between the hen lines (P=0.0245). Lohmann Brown had an initial egg weight of 59.63 g and for Lohmann Lite it was 58.75 g. There were no initial egg weight differences (P>0.05) observed among the lighting treatments. No differences (P>0.05) were observed among the incubation lighting treatment for the hatchability (Table 22).

Table 22. Hatchability percentages for incubation lighting treatments.

Incubation treatment	Mean Hatchability ± SEM (%)
Control	88.9 ± 2.7
White LED	95.2 ± 2.7
Red LED	94.7 ± 2.7
Blue LED	92.3 ± 2.7

The results (Mean hatchability (%) ±SEM) are an average of two hen lines together per each lighting treatment. There were no significant differences (P>0.05) seen for the hatchability percentages. (Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).

3.6.1.2 Hatch window

The percentage of chicks hatched over the time (Figure 8) was marginally differed among the treatments applied during incubation (P=0.0675). Birds hatched from red LED light completed their hatch window 4 h earlier than control and blue LED birds. They also hatched 3 h before the white LED birds. Results show that red LED birds had a trend of having a shorter hatch window compared to other treatments.

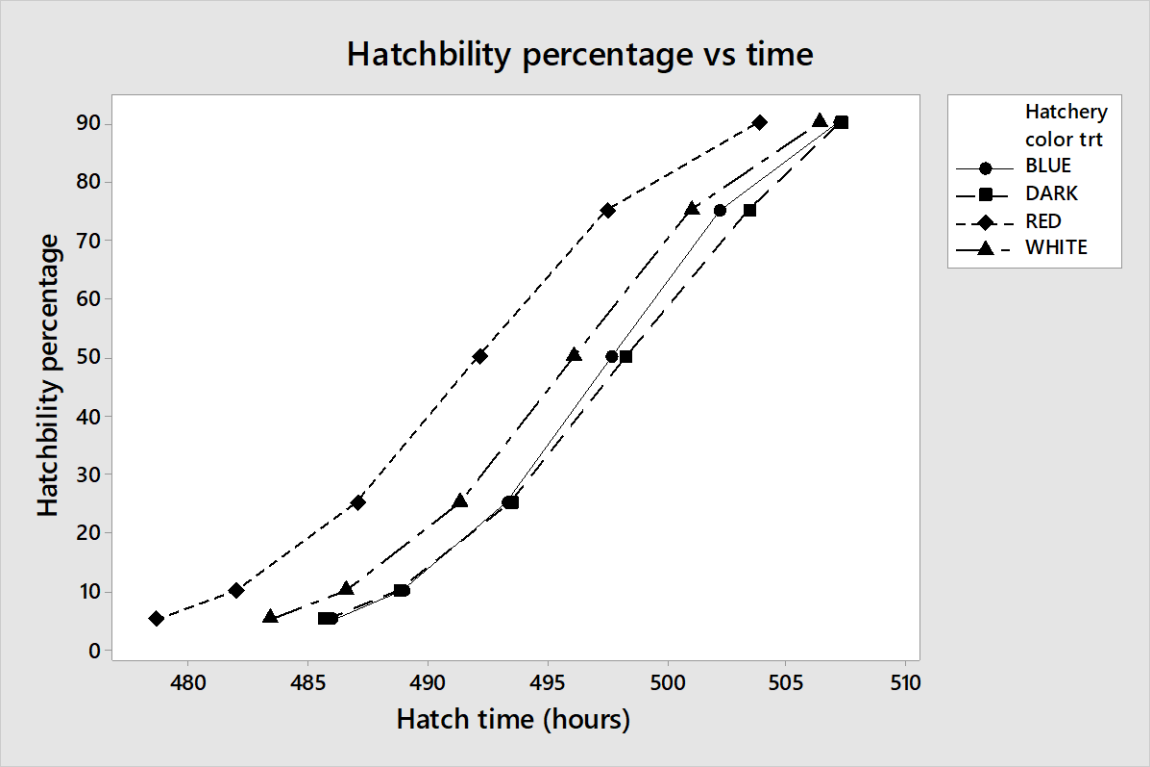

Figure 8. Percentage of chicks hatched during the hatch window from 472 – 512 hours of incubation. *Chicks hatched from red LED light finished the hatch window earlier than other three treatments. Chicks hatched under control, Blue and white LED light treatments spent longer time to reach the maximum percentage of hatchability compared to the red LED light treatment. (Results are an average of two hen lines together per each lighting treatment. Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED were provided to each treatment with a photoperiod of 12L:12D).*

3.6.1.3 Chick quality

Even though there were differences in the hatch window among the hatchery treatments, the initial chick weights at the 512 h of incubation were not different (P=0.144; Figure 9). The highest quality navels that had a score closer to one (P=0.0304) or best navels was recorded from the chicks hatched under red LED light (if the navel score is lower the higher the quality of navels). The chicks hatched under blue LED had the poorest navels compared with other hatchery treatments (Figure 10).

Chick lengths (Figure 11) were not significantly different (P=0.2685) among the treatments applied. However, yolk free body weights were marginally different (P=0.068). Looking at the results, Figure 12 shows there was a trend that shows birds form red LED had the highest yolk free body weight. These results show that birds form the red LED hatched earlier with more quality, healed navels and had utilized their yolk sac very efficiently than the birds from other treatments.

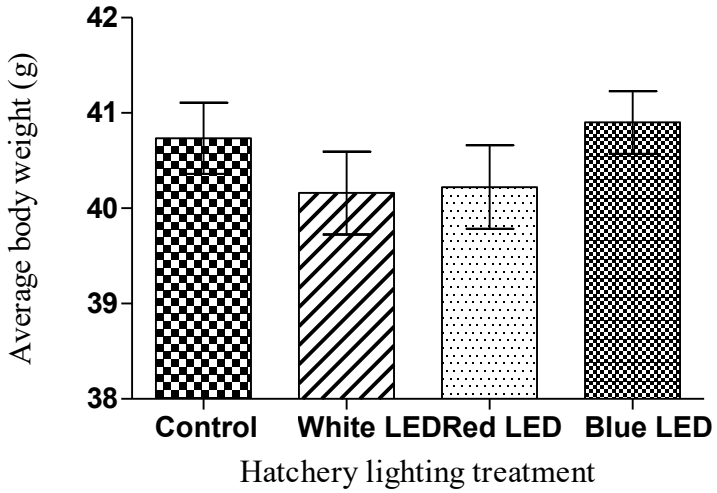

Figure 9. Average hatch weights (g) of the chicks for different incubation lighting treatments. *Results show Mean weight ±SEM. Initial hatch weight were not affected* (P>0.05) *by the incubation treatment (Results are an average of two hen lines together, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

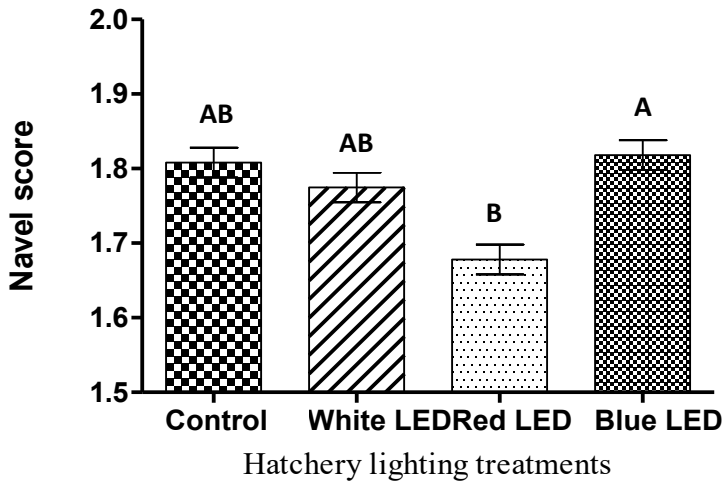

Figure 10. Navel scores of the hatchlings for different incubation lighting treatments. *Navel scores (Mean navel score ±SEM) showed significant differences* (P<0.05) *among lighting treatments. In the scoring system, fully healed navels were scored as one. Navels with up to a 2 mm gap and/or a spot of clotted blood and/or a remnant of the yolk sac material in the form of a black string visible outside the abdomen was scored as two. Unhealed navels with a gap of more than 2 mm (with or without black material in the center of the navel) were scored as a three. Best navels were recorded from the chicks hatched under red LED light. The chicks hatched under blue LED had the less healed navels compared with other hatchery treatments (There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment. AB Means with no common letters are significantly different (P<0.05), control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

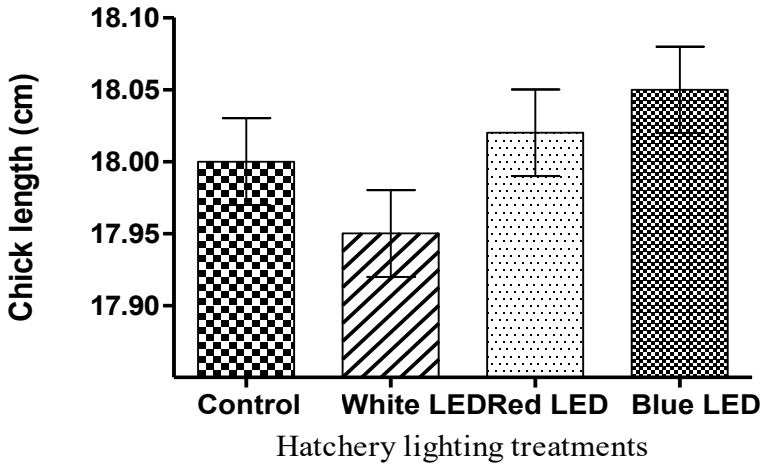

Figure 11. Lengths (cm) of the chicks for different incubation lighting treatments. *Chick lengths (Mean chick length (cm) ±SEM) of day-old birds were not significantly different among the treatments. (There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

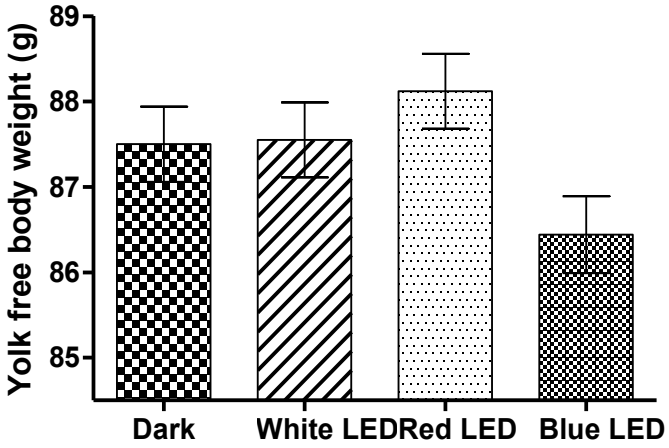

Figure 12. Yolk free body weights (g) of day-old birds for different incubation lighting treatments. *The yolk free body weight had a marginal difference (P=0.068) and the trend of the yolk free body weight shows that the birds form red LED had the highest yolk free body weigh compared to the other lighting treatments (There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

3.6.2 Post-hatch performance

At the time of placement, chick weights (Figure 13) were significantly different (P=0.0399). Chicks from the dark hatchery treatment had the highest body weight and the birds from red LED treatment had the lowest body weight. Two other treatments had intermediate body weights. However, the body weights after the 6 h of placement were not significantly different (P=0.2279) (Figure 14). Over those 6 h, birds incubated in red LED had the highest (P=0.0001,

Figure 15) weight gain percentage. The chicks from the dark treatment did not have accelerated weight gain compared to other lighting treatments. The body weights and weight gain percentages were not different at week 01, 02 03 and 04 for incubation lighting treatments and grow out photoperiods (Table 23). There were only hen line differences observed during these periods.

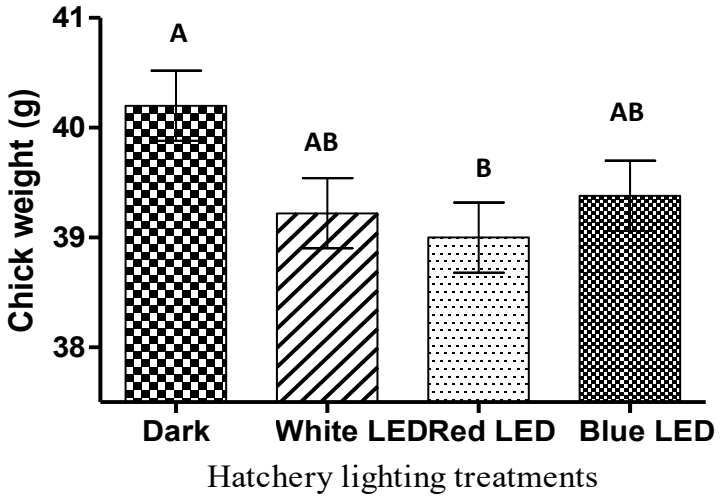

Figure 13. Average chick weights (g) at the placement for different incubation lighting treatments. *Chicks from the dark treatment had the highest weight at the placement and the red LED chicks had the lowest weight (P=0.0399, there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment. AB Means with no common letters are significantly different, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

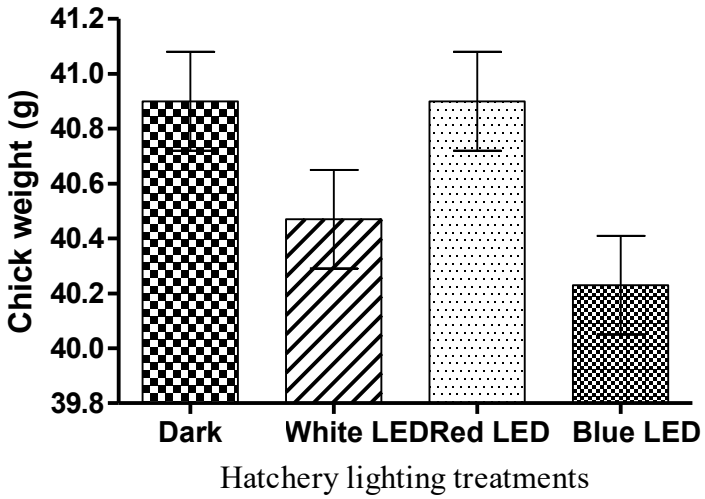

Figure 14. Average chick weights (g) after 6 h of placement for different incubation lighting treatments. *Chick weights after 6h of placement did not differ among the treatments. There was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment. AB Means with no common letters are significantly different, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

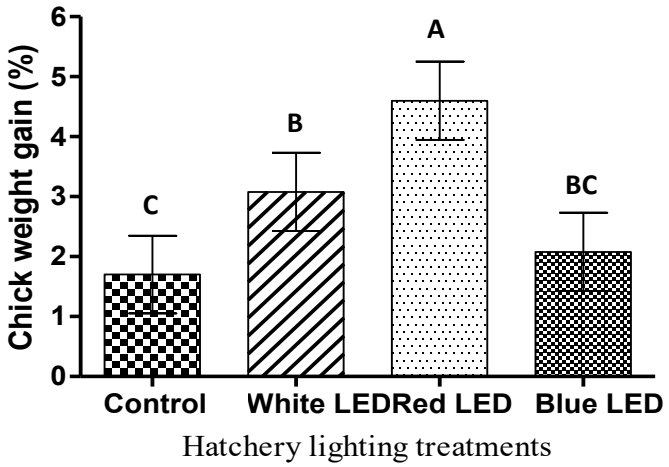

Figure 15. Chick weight gain percentages (%) of body weight after 6h of placement for different incubation lighting treatments. *Chick weight gain percentages were highest for the red LED treatment. Control/ dark chicks had the lowest weight gain percentage (*P<0.05*, there was no significant (P>0.05) effect of interactions observed between the main factors of interest. Therefore,* results *are an average of two hen lines together per each lighting treatment. ABC - Means with no common letters are significantly different, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D).*

Table 23. Average body weights (g) for incubation lighting treatments, grow out photoperiods and hen line at week 1, 2, 3 and 4.

Growing stage	Main factors of interest	Average body weights (g) ±SEM	Main factors of interest	Average body weight gain (%) ±SEM
Week 1	**Incubation**	P=0.7928		P=0.0877
	Control	64.77±0.86	Control	37.71±0.65
	White LED	64.64±0.86	White LED	39.18±0.65
	Red LED	65.04±0.86	Red LED	39.94±0.65
	Blue LED	64.01±0.86	Blue LED	38.23±0.65
	Grow out	P=0.1280		P=0.0512
	Long day	65.93±0.68	Long day	39.79±0.46
	Short day	63.84±0.68	Short day	37.74±0.46
	Hen lines	P<0.0001		P<0.0001
	LB	66.86±0.68 [a]	LB	40.23±0.65 [a]
	LL	62.37±0.68 [b]	LL	37.29±0.65 [b]
Week 2	**Incubation**	P=0.4474		P=0.4750
	Control	120.95±2.17	Control	10.98±0.10
	White LED	118.69±2.17	White LED	10.88±0.10
	Red LED	116.16±2.17	Red LED	10.77±0.10
	Blue LED	117.28±2.17	Blue LED	10.82±0.10

Growing stage	Main factors of interest	Average body weights (g) ±SEM	Main factors of interest	Average body weight gain (%) ±SEM
	Grow out	P=0.3611		P=0.3669
	Long day	119.27±1.54	Long day	10.91±0.07
	Short day	117.26±1.54	Short day	10.82±0.07
	Hen lines	P<0.0001		P<0.0001
	LB	124.32±1.54 [a]	LB	11.15±0.07 [a]
	LL	112.22±1.54 [b]	LL	10.59±0.07 [b]
Week 3	**Incubation**	P=0.4396		P=0.3702
	Control	192.59±4.32	Control	37.77±0.47
	White LED	194.70±4.32	White LED	37.92±0.48
	Red LED	185.10±4.32	Red LED	37.04±0.48
	Blue LED	189.77±4.32	Blue LED	38.09±0.47
Week 3	**Grow out**	P=0.6846		P=0.0756
	Long day	191.51±3.05	Long day	37.10±0.35
	Short day	189.57±3.05	Short day	38.31±0.35
	Hen lines	P<0.0001		P<0.0001
	LB	201.87±3.05[a]	LB	38.67±0.35[a]

Growing stage	Main factors of interest	Average body weights (g) ±SEM	Main factors of interest	Average body weight gain (%) ±SEM
	LL	179.21±3.05[b]	LL	36.74±0.35[b]
Week 4	**Incubation**	P=0.2859		P=0.4005
	Control	99.94±2.67	Control	292.53±5.11
	White LED	92.65±2.67	White LED	287.34±5.11
	Red LED	95.40±2.67	Red LED	280.51±5.11
	Blue LED	94.92±2.67	Blue LED	284.69±5.11
	Grow out	P=0.1293		P=0.5478
	Long day	92.94±1.89	Long day	284.46±3.79
	Short day	98.51±1.89	Short day	288.08±3.79
	Hen lines	P<0.0001		P<0.0001
	LB	105.7±1.89[a]	LB	307.57±3.67[a]
	LL	85.76±1.89[b]	LL	264.97±3.67[b]

The body weights were not significantly different (P>0.05). Only the hen line differences were seen, [abc] Different letters indicate significant differences. (P<0.05, Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, two commercial hen lines used were Lohmann Brown and Lohmann Lite).

3.6.3 Body weights at week developer, grower, pre-lay, pre-peak and phase 01

The grower stage (week 04-08) body weights (Table 24) were not different (P>0.05) among any of the treatments applied. During the developer, stage (Table 25) there was an interaction observed (P=0.0012) among the hatchery treatments, the grow out photoperiod and the hen lines. During this stage, Lohmann Brown birds from white and blue LED hatchery treatments raised in long days had the highest body weights. All the Lohmann Lite birds had lower body weights during this stage compared to Lohmann Brown birds. However, this difference did not continue into the pre-lay, pre-peak or phase 01 stage (Table 24) where no body weight differences were seen (P>0.05) for the hatchery lighting treatments and grow out photoperiod. There were only hen line differences seen at these time points.

Table 24. Average body weights (g) of birds for incubation lighting treatments, grow out photoperiods and hen lines at grower, pre-lay, pre-peak and phase 01 stages.

Growing stage	Main factors of interest	Average body weights (g) ±SEM	Growing stage	Main factors of interest	Average body weights (g) ±SEM
Grower	**Incubation**	P=0.1513	**Pre-peak**	**Incubation**	P=0.1119
	Control	745.95±9.43		Control	1956.80±32.80
	White LED	747.99±9.43		White LED	1870.12±32.80
	Red LED	721.29±9.43		Red LED	1934.88±32.80
	Blue LED	746.97±9.43		Blue LED	1890.77±32.80
	Grow out	P=0.6045		**Grow out**	P=0.9984
	Long day	743.27±6.67		Long day	1925.40±25.97
	Short day	737.83±6.67		Short day	1925.33±25.97
	Hen lines	P<0.0001		**Hen lines**	P<0.0001
	LB	817.39±6.67[a]		LB	2121.86±25.97
	LL	663.71±6.67[b]		LL	1728.87±25.97
Pre-lay	**Incubation**	P=0.1037	**Phase 01**	**Incubation**	P=0.2521
	Control	1701.74±23.47		Control	2049.34±32.05
	White LED	1714.05±23.89		White LED	2011.43±32.05
	Red LED	1676.54±23.89		Red LED	2076.77±32.05
	Blue LED	1730.17±23.47		Blue LED	2090.67±32.05
	Grow out	P=0.5128		**Grow out**	P=0.9665

Growing stage	Main factors of interest	Average body weights (g) ±SEM	Growing stage	Main factors of interest	Average body weights (g) ±SEM
	Long day	1712.76±22.04		Long day	2057.72±24.41
	Short day	1698.49±21.78		Short day	2056.38±24.41
	Hen lines	P<0.0001		**Hen lines**	P<0.0001
	LB	1966.10±21.09[a]		LB	2256.10±24.41[a]
	LL	1445.15±21.09[b]		LL	1858.00±24.41[b]

The body weights were not significantly different (P>0.05) at grower, pre-lay, pre-peak and phase 01 stages for incubation lighting treatments and grow out photoperiods. Only the hen line differences were seen, [ab] Means with no common letters are significantly different. (P<0.05, Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, two commercial hen lines used were Lohmann Brown and Lohmann Lite).

Table 25. Average body weights (g) of birds for developer stage.

	Lohmann Brown hen line		Lohmann Lite hen line	
Grow out photoperiod	**Long days**	**Short days**	**Long days**	**Short days**
Incubation lighting treatments				
Control	1525.8±17^{ab}	1473.4± 17^{b}	1116.2± 17^{c}	1131.1± 17^{c}
White LED	1564.5±17^{a}	1521.6± 17^{ab}	1123.4± 17^{c}	1136.9± 17^{c}
Red LED	1463.5± 17^{b}	1472.2± 17^{b}	1159.0± 17^{c}	1099.2± 17^{c}
Blue LED	1558.5±17^{a}	1501.6± 17^{ab}	1148.9± 17^{c}	1119.7± 17^{c}

There was an interaction among commercial hen line, grow out photoperiod and the incubation lighting treatments. Mean weight (g) ±SEM. ^{abc} Means with no common letters are significantly different. (P<0.05, Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, two commercial hen lines used were Lohmann Brown and Lohmann Lite).

3.6.4 Body weight to shank length ratio

Body weight to shank length ratio showed no differences (P>0.05) for the hatchery lighting treatments and grow out photoperiods. There was only a difference between the hen lines seen (P<0.0001) where Lohmann Lite birds had a lower body weight to shank length ratio (13.29±0.5) than the Lohmann Brown (17.87±0.5) birds (Table 26). Lighting during incubation and rearing photoperiod did not negatively affect the body frame development of pullets in this experiment similar to the preliminary experiment.

Table 26. Body weight to shank length ratio of the commercial hen lines.

Hen line	Body weight to shank length ratio (g/mm)
Lohmann Brown	17.87±0.5[a]
Lohmann Lite	13.29±0.5[b]

Body weight to shank length ratio - Mean ratio (g/mm) ±SEM, [ab] Means with no common letters are significantly different (P<0.05, two commercial hen lines were used in the experiment which were Lohmann Brown and Lohmann Lite).

3.6.5 Age at first egg

There was an interaction of hatchery treatments and grow out photoperiod on time to lay the first egg (P=0.0425, table 27). Combined effect of red-light hatchery treatment and long day photoperiod favors reducing the age to lay first egg. Birds of the blue light treatment and reared under short days took the longest time to lay the first egg. All other treatments were intermediate.

Table 27. Age of the birds at the first egg for incubation and grow-out treatments

Incubation lighting treatments	Grow out photoperiod	
	Long	Short
Red LED	132.7±1.4 days [d]	138.3±1.4 days [abc]
White LED	138.5±1.4 days [ab]	136.5±1.4 days [bcd]
Blue LED	137.1±1.4 days [abc]	140.2±1.4 days [a]
Control	134.5±1.4 days [cd]	137.2±1.4 days [abc]

Age at first egg in days of age ±SEM. [ab] Means with no common letters are significantly different (P<0.05. Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

3.6.6 Hen housed egg production

Since the birds from red LED hatchery treatment and long day photoperiod had a decrease in the age to lay first egg, they have also had the highest number of eggs produced (110.7±1.23 eggs) during this time. Inversely, birds of blue light and reared under short days had the lowest (105.1±1.23 eggs) hen housed egg production (Table 28).

Table 28. Average hen housed egg production (mean number of eggs produced per bird) for the period of egg production (from week 18-36).

	Grow out photoperiod	
Incubation lighting treatments	**Long**	**Short**
Red LED	110.7±1.23 eggs [a]	105.9±1.33 eggs [ab]
White LED	105.8±1.23 eggs [ab]	108.7±1.23 eggs [ab]
Blue LED	107.1±1.23 eggs [ab]	105.1±1.23 eggs [b]
Control	110.5±1.23 eggs [ab]	105.8±1.33 eggs [ab]

Mean number of eggs produced per hen for 18 weeks period ±SEM. [ab] Means with no common letters are significantly different (P<0.05). Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

3.6.7 Egg quality

The egg quality measurements did not show significant differences (P>0.05) among the hatchery and grow out treatments applied for any of the parameters of interest (Table 29). There were only hen line differences were observed for egg weight, specific gravity, yolk weight, albumen weight and shell weight and percentages.

Table 29. Egg quality parameters; egg weight, specific gravity, shell-breaking strength, yolk weight percentage, albumen height, albumen weight percentage, shell weight percentage and shell thickness for the main factors of interest.

Main factors of interest	Egg weight (g)	Specific gravity (g/L)	Shell breaking strength (g)	Yolk weight as a percentage of egg weight	Albumen height (mm)	Albumen weight as a percentage of egg weight	Shell weight as a percentage of egg weight	Shell thickness (mm)
Incubation	(P=0.4063)	(P=0.2738)	(P=0.9927)	(P=0.4449)	(P=0.4719)	(P=0.1265)	(P=0.2833)	(P=0.5957)
Control	59.2±0.43	1.094±0.00	6470.5±273	24.9±0.19	8.70±0.19	64.9±0.19	10.13±0.04	0.4761±0.00
White LED	59.5±0.43	1.093±0.00	6445.5±272	24.9±0.19	8.89±0.19	65.0±0.19	10.03±0.04	0.4788±0.00
Red LED	60.1±0.43	1.094±0.00	6534.7±268	24.6±0.19	8.81±0.19	65.3±0.19	10.09±0.04	0.4691±0.00
Blue LED	60.1±0.43	1.094±0.00	6543.3±278	24.6±0.19	9.06±0.19	65.5±0.19	10.01±0.04	0.4743±0.00
Grow out	(P=0.9583)	(P=0.2603)	(P=0.9511)	(P=0.1026)	(P=0.7849)	(P=0.0828)	(P=0.5199)	(P=0.4589)
Long	59.7±0.34	1.095±0.00	6489.4±194	25±0.14	8.89±0.15	64.9±0.15	10.08±0.03	0.4768±0.00
Short	59.7±0.34	1.095±0.00	6507.6±191	25±0.14	8.84±0.15	65.4±0.15	10.04±0.03	0.4724±0.00
Hen lines	(P<0.0001)	(P=0.0149)	(P=0.0.5709)	(P<0.0001)	(P=0.5189)	(P<0.0001)	(P<0.001)	(P=0.1724)
LB	60.79±0.31[a]	1.097±0.00[b]	5774.6±104	25.18±0.14[b]	8.81±0.15	65.78±0.15[a]	10.24±0.03[a]	0.3833±0.01
LL	58.65±0.31[b]	1.093±0.00[a]	5650.5±111	24.36±0.14[a]	8.91±0.15	64.56±0.15[b]	10.7±0.10[a]	0.3989±0.01

Egg quality parameters are in average egg weight (g)± SEM, average specific gravity(g/L)± SEM, average shell breaking strength (g)± SEM, average yolk weight % ± SEM, average albumen height (mm) ± SEM, average albumen weight % ± SEM, average shell weight %

± SEM, average shell thickness (mm) ± SEM. [ab] Means with no common letters are significantly different (P<0.05). (Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, LB=Lohmann Brown, LL= Lohmann Lite, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

3.6.8 Feed conversion ratio

There were no significant differences in feed conversion ratio for any of the treatments applied during the pre-peak period of laying (week 20-24). From 25-28 only strain differences were observed for the feed conversion ratio (Table 30, P=0.0002). Interaction between the photoperiod applied during the rearing and the hatchery treatments showed a marginal difference during the laying hen period between 29-32 weeks (Table 31, P=0.0456). However, by the week of 36, only the strains were differed significantly (Table 32, P=0.0003). Other treatments were not significantly different.

Table 30. Feed conversion ratio of the birds for two commercial hen lines from week 25-28.

Hen line	Feed conversion ratio
Lohmann Lite	2.07±0.01[a]
Lohmann Brown	1.98±0.01[b]

Mean feed conversion ratio ±SEM, [ab] Means with no common letters are significantly different (P<0.05, two commercial hen lines were used in the experiment which were Lohmann Brown and Lohmann Lite).

Table 31. Feed conversion ratio of the birds from week 29-32 for the interaction of incubation lighting treatments and grow-out photoperiod.

Incubation lighting treatments	Grow out photoperiod	
	Long	Short
Red LED	2.01±0.04	2.03±0.04
White LED	1.97±0.04	2.08±0.04
Blue LED	2.09±0.04	2.00±0.04
Control	2.03±0.04	2.05±0.04

Mean feed conversion ratio ±SEM. The results for the feed conversion ratio were not different for the interaction of incubation lighting treatment and grow out photoperiod. (Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each LED light treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

Table 32. Feed conversion of the birds for two commercial hen lines from week 33-36.

Hen line	**Feed conversion ratio**
Lohmann Lite	2.03±0.01[a]
Lohmann Brown	1.93±0.01[b]

Mean feed conversion ratio ±SEM. [ab] Different letters indicate significant differences (P<0.05, two commercial hen lines were used in the experiment which were Lohmann Brown and Lohmann Lite).

3.7 Discussion

Provision of a photoperiod to incubation is a factor that impacts embryonic development. Studies have shown that use of a photoperiod during the incubation have increased hatching and post-hatch growth performance of commercial broilers (Archer, 2015a). Nonetheless, this effect is enhanced depending on the spectral characteristic of light (Rozenboim et al., 2004), length of photoperiod (Ozkan et al., 2012b), hen lines and type of light used (Archer and Mench, 2014). For this reason, reports on chick growth and physiology are not consistent among studies. A recent study showed that green LED light illumination from day 5 of incubation until hatch (0.1 W/m2 intensity) did not accelerate the time of hatch (Rozenboim et al., 2004). However, during the current study we found that chicks hatched from red LED light (wavelength of 620-670 nm) had an accelerated hatch time and completed the hatch window earlier compared to the chicks hatched from other lighting treatments (blue light treatment of 440-470 nm wavelength, white and dark). Rozenboim et al (2013) showed that stimulation of the opsins in the retina and extra retinal photoreceptors are correlated with the color of light that the embryos are exposed and the green light exposure down regulates the expression of green and red opsins, whereas red light enhances the expression of green and red opsins. With that, the hatchability is enhanced and accelerated with the red-light exposure to the embryos and green light exposure will diminishes the speed of hatchability. Normal hatch window ranges between 24-48h (Løtvedt & Jensen, 2014). Wider spread of hatch window delays the early hatched chicks to access water and feed (Romanini et al., 2013). Establishing a synchronized hatch window reduces the spread of hatch. This will facilitate removal of the birds earlier from the incubators and will reduce the number of chicks that are dehydrated. In addition, it will synchronize the activities that lead to early access of birds to feed and water (Fairchild and Christensen, 2000). With these results, shorter spread of hatch window with red LED light is beneficial in commercial practice.

Chick quality reflects the performance of incubation and is a predictor for development of birds (Mukhtar et al, 2013). High quality chicks are also important handling the environmental stressors that they face after hatch (Ozkan et al., 2012a). Chick quality at hatch can be evaluated using a variety of qualitative and quantitative parameters including hatch weights, navel condition chick length and yolk sac utilization (Archer, 2015b).

Incubation photoperiods affect quality parameters of chicks in many ways. Archer (2015b) reported White leghorn (Hy line) eggs incubated under red light had higher hatchability, higher number of healed navels, chick lengths and higher hatch weights. Also, this study showed that chicks hatched under white/red light had the lowest percentage of chicks with defects. In agreement with the results of this study, birds hatched from red LED had the best healed navels in the current experiment. Yolk sac uptake/ yolk sac retraction takes place at the navel of chicks. Therefore, healed navels are important against the vulnerability to bacterial and fungal infections that can cause poor post-hatch performance (Verschuere, 2016).

Hatch weights and chick lengths were not different in the current study among the treatments applied. The results for yolk free body weight show a trend for the birds from red LED to utilize the yolk earlier than the birds from other hatchery lighting treatments. Residual yolk in newly hatched chicks can account for as much as 10% (Vieira and Moran, 1998) of a chick's body weight. Residual yolk contents for the red lighting treatment were the lowest of all the lights investigated. The evidence from this experiment suggests that chicks incubated in red light had already converted yolk reserves into body tissues. Therefore, they had less residual yolk (Wolenski et al., 2004). Blue light incubated chicks have failed to consume the yolk as much as other treatments. Red light chicks had the lowest weight at the placement due to higher yolk utilization rate compared to other treatments. This is in line with the results of Ozkan et al, (2012a). They suggest

that lighting during incubation favors the embryo to increase energy utilization and activity rate of chicks, there by resulting lower body weight. Even though, the red LED birds had the lowest weight at the placement both the experiments showed that they had the ability to accelerate and reach the highest weight gain after 6 h of post placement. The chicks incubated under lighting environment are better able to adapt to the rearing environment lighting program, as they are already exposed to a lighting environment during the incubation. Introduction to an artificially lit production environment may cause stress to birds hatched in the dark (Ozkan et al., 2012a). In the current experiment higher post-hatch body weight gain of birds hatched under light, could indicate light during incubation improved adaptation of chicks to a novel environment (Ozkan et al., 2012a). This adaptation can decrease the stress and fear response of the birds during post-hatch that also help improving the post-hatch body weight gain of birds. Early acquisition of feed may be related to the improved feed conversion ratio identified (Zhang et al., 2012; Rozenboim et al., 2013). Results are positive for photoperiodic lighting during incubation as it appears to improve adaptation of chicks to the production environment (Ozkan et al., 2012a).

Short days were provided to the grow out period assuming that lighting during incubation had established a diurnal rhythm, that synchronizes newly hatched chicks and improves adaption. Therefore, extremely long days in early life may not be necessary for adapting to commercial feed and water systems (Lohmann-LSL Lite layer management guide, 2005). However, regardless of the grow out photoperiod, chicks from the R21 treatment had the highest body weight gain during first two weeks post-hatch. Thus, out of the incubation lighting treatments used, the red LED treatment facilitated a better start in life. However, in both experiments, the positive effect on body weights from incubation lighting disappeared with birds age.

Different research studies have shown that wavelengths have various effects on poultry behavior, growth and reproduction. These physiological aspects are manipulated through the signals of light received through both the retinal photoreceptors and extra retinal photoreceptors located in the eyes and bird's brain. Studies have shown that growth and behavior of poultry birds are manipulated by the shorter wavelength signals received through the retinal photoreceptors. Reproduction behavior is linked and manipulated through the long wavelength light (red light) by the extra retinal photoreceptors on the hypothalamus (Lewis and Morris 2000). Looking at the reproduction behaviour, Min et al. (2012) showed that monochromatic red LED light during the rearing phase had a positive effect on egg production of laying hens. Results from both the experiments in current study suggest that providing red light for the whole incubation period could increase egg production. Long wavelengths of light (towards red spectrum) penetrate the skin and skull more efficiently than the short wavelengths (towards blue-green spectrum) leading to an increased reproductive performance of layers (Solangi et al., 2004). Therefore, generally the red light efficiently increases the egg production of laying hens, whereas green or blue light has a low influence.

Compared to short wavelengths long wavelength can easily infiltrate deep tissues that increases the discharge of reproductive hormones from the hypothalamus. Hypothalamic photoreceptors capture the light and is transuded into nervous impulses. This process will stimulate the synthesis and release of gonadotropin-releasing hormone that triggers the actions of the hypothalamo-pituitary-gonadal axis which encourage the development of gonads stimulating the sexual maturity (Reddy et al., 2012). Min et al (2012) showed that white and red light post-hatch caused pullets to mature earlier compared to blue light. This resulted in more eggs over the production cycle for the red and white lit birds. These results are similar with the results of current

study regarding egg numbers. The birds hatched from the red LED light had taken the lowest time to lay the first egg eventually gave the highest number of eggs over the production cycle without negatively affecting the egg quality.

Reduction of age to reach the sexual maturity and higher continuous egg production will increase the risk of reducing amount of mineralized structural bone available in the layer hen body to produce eggshells and in some instances result in low bone fragility with a high potential to fracture. Starting to produce eggs from an early age will deplete the Ca reservoirs in the body. If bones are depleted of Ca reserves from very early ages poor quality eggshells can result. This is a major economic concern of commercial egg producers as it reduces the number of table eggs available for sale (Sandilands et al., 2009). However, providing a photoperiod during the incubation has shown to increase the leg bone health of broilers. Providing a light-dark cycle during incubation may establish a circadian pattern of melatonin release. Rhythmic melatonin secretion increases embryonic ossification, which ultimately improve bone development (Van der Pol, 2017). Van de pol et al. (2014) also reported that 12L:12D incubation increased post hatch tibia cortical area, cortical thickness, tibia length and femur weight which is an indicator of increasing whole bone strength (Augat and Schorlemmer 2006). Having high bone strength is beneficial making the bone highly resistive to break. This can minimize the harm on skeleton of layers during calcium resorption from bone and protects against negative effects on egg quality (Wistedt, 2013). Therefore, providing a photoperiod during the incubation could provide a strong skeleton to the layers making available of high dense Ca reservoirs for eggshell production. Results from these experiments show that providing light to the incubation did not negatively impact the quality of eggshells.

Feed conversion ratio is dependent on feed intake, metabolic efficiency, and the production environment. Feed conversion detects the amount of feed consumed and the amount of egg mass produced (Hurnik, 1978). A layer in full production can convert around one third of the nutrient's intake into eggs. However, in the current study when birds getting older, feed conversion ratios did not show significant differences among the treatments. These results suggest that bird's nutrient ingestion and conversion into eggs has carried out in a similar manner for all the treatments.

Feather condition depends on many factors including nutrition, health and environmental factors (light, temperature etc.) (Carrascal et al., 1998). In the current study feather condition was improved by introducing a photoperiod during incubation. Even though white LED light had both negative and positive effects on the feather condition red LED light generally, had a positive effect on the feather cover. In contrast to our results, Riedstra and Groothuis (2003) suggest providing embryos with light during the last week of incubation will cause serious damage to feather cover from increased feather pecking. However, improved feather cover from the results of the current study suggests that hatching birds under red LED light did not increase feather pecking behavior but may reduce it, thereby increasing the welfare of birds.

3.8 Conclusion

Introduction of a photoperiod to the incubation can greatly affect the production performances of layer birds. In this study, it was hypothesized that providing a photoperiod during incubation would positively affect the hatching, post-hatching and egg production performance of laying hens. To test the hypothesis the laying hen hatching eggs were photo-stimulated with three different color lights (blue light treatment of 440-470 nm wavelength, red light treatment of 620-670 nm wavelength and white light).

In conclusion, out of the wavelengths introduced to the incubation, red light stimulated hatching process and increased the chick quality. Introduction of red LED light to the incubation also enhanced the post-hatch early growth rate, reduced the time to lay the first egg and increased the egg production without negatively affecting the egg quality parameters and feather cover. Therefore, introduction of red LED to the laying hen chick incubation is beneficial to increase production performance.

CHAPTER 4 : RESPONSE OF LAYING HENS TO HEAT STRESS, PROVIDED A PHOTOPERIOD DURING INCUBATION

ABSTRACT

High environmental temperatures have deleterious effects on the productive performance and welfare of laying hens. Providing a photoperiod during incubation is expected to improve the tolerance of laying hens for stressful environments. Objective of the current study was to evaluate the influence of a heat stress on live bird performance and egg quality of the laying hens provided with a photoperiod during incubation. A total of 640 eggs from each of two laying hen lines, Lohmann Lite (LL) and Lohmann Brown (LB) were incubated in 8 incubators. Eighty eggs from each strain were randomly selected to be placed in each incubator. Six incubators were installed with 3 different LED light types and two were left in the dark, which served as the control. Blue LED, red LED (ONCE Innovation) and full spectrum white LED lights (4100K) were installed in 2 incubators each. In each lighting treatment the photoperiod used was 12 h of light followed by 12 h of dark (12L:12D). The lights were dimmed to a similar intensity for the incubators with lights. Hatched female chicks (n=384) were housed in 64 cages at 6 birds per cage density and were provided with two different light regimes. Predominantly blue LED was used to provide light during grow out. Control cages (n=32) were given a standard photoperiod of 23L:1D during the first 3 days and 20L:4D from day 4-14. Short day treatment cages (n=32) were given 18 h of light including two 30 min phases during the dark period in first 3 days. From day 4-14 light was reduced to 17 h of continuous light with two 30 min phases. For both regimes, the total day length was reduced over time. Both treatments had 9 h of light at 7-16 weeks of age. Day length was increased at 17 weeks by 1 h of light/week to 14 h of light by 21 weeks of age. Birds were moved to laying hen cages at week 18, placing 5 birds/cage. During the laying period, layers were reared under red LED light. At week 37 of age a heat stress was introduced to half of the flock. The temperature of this group was elevated up to 30 °C during day time and reduced to 23 °C overnight for 4 weeks. In the control group, the temperature was maintained at 23 °C. Body weights, feed consumption, egg production, egg quality, and feather condition were monitored during this period. Body weights were not significantly affected by the heat stress. Feed consumption was lowest in the LB birds hatched from blue LED light and raised in short days. A significant egg production drop was observed in heat stressed birds hatched from white LED and raised in long days. Egg weights were significantly lowered in the heat stressed birds hatched from control hatchery treatment. Feather condition of the back area of the birds were poorest in the birds that hatched under the white LED light. Taking the overall live bird performance in to consideration, it can be concluded that introduction of red and blue LED photoperiods in to the incubation does not negatively affect the body weight gain, hen housed egg production, egg quality and feather condition during a heat stressed period. However, hen housed egg production and feather condition were poor in the heat stressed birds if hatched with white LED light.

Keywords: heat stress, incubation, lighting, red LED lights, layers

4.1 Introduction

High environmental temperature during the laying hen phase is a critical factor that reduces egg numbers and quality. Optimum temperature that layers are maximally productive is 18-24 °C (Holik, 2015). Environments outside this optimum temperature range will result depressed natural behavioral patterns of layers, including reduced body weights, feed intake (Mashaly et al., 2004), reduced egg production, egg weight, shell quality and overall egg quality (Mohmoud et al., 1996; Mashaly et al., 2004). During high heat exposure, layers will require energy for thermoregulation, limiting available energy for other metabolic activities.

Interrupted calcium metabolism is also a factor affected by high heat. Interference in calcium metabolism eventually affect the egg production performance of birds (Mohmoud et al., 1996). Heat stress involves panting, that results in reduction of blood CO_2 level of birds. This will increase the blood pH level of laying hens and ultimately reduce the blood Ca ion (Ca^{2+}) level (Mohmoud et al., 1996). Reduced level of Ca^{2+} in blood will increase the parathyroid hormone secretion. This hormone helps to mobilize the Ca^{2+} from the medullary and cortical bones (Soares, 1984) which is a major source of Ca^{2+} for egg shell formation (Bar et al., 1978). Lower blood calcium levels stimulate secretion of parathyroid hormone which signals proximal tubule of the kidneys to reduce Ca^{2+} excretion and to produce 1,25-dihydroxyvitamin D, the active form of vitamin D that increase the Ca^{2+} absorption from the intestine. Other than parathyroid hormone, estrogen secretion before the onset of lay also helps to mobilize skeletal Ca^{2+} and stimulates 1,25-dihydroxyvitamin D production (Turner, 2009). However, long term exposure to high heat significantly reduces the Ca^{2+} resorption and Ca^{2+} reservoirs in the body, creating critical effects on egg production, egg shell formation (Mashalay et al., 2004) and skeletal health of laying hens (Mahmoud et al., 1996).

Photoperiodic conditioning during incubation can be an approach to reduce the negative effects of heat stress on laying hens. During the natural incubation chicken eggs are exposed to short bouts of light when the mother hen leaves the nest to feed and drink. Providing a light-dark cycle during incubation may establish a circadian pattern of melatonin release. Rhythmic melatonin secretion increases embryonic ossification, which ultimately improves bone development (Van der pol et al., 2014). Machida et al. (1995) showed that scoliosis developed in chickens that were pinealectomised at 3 days of age (eliminating circulating melatonin). However, this effect was reduced to 20% with injection of melatonin every other day. Thus, melatonin plays a major role in bone development. Van de pol et al. (2014) reported that white LED light provided with a 12L:12D photoperiod during incubation increased post-hatch tibia cortical area, cortical thickness, tibia length and femur weight which is an indicator of increasing whole bone strength (Augat and Schorlemmer 2006). Having high bone strength is beneficial making the bone resist to breakage. This can minimize the harm to the skeleton of layers in calcium resorption from bone and protects against negative effects on egg quality (Wistedt, 2013).

Therefore, we speculated that photoperiodic incubation increases the ability to have strong bones (Van de pol., 2014) that would benefit resorption of Ca^{2+} during laying hen period and secure egg production, reducing the negative effects of a heat stress.

4.1.1 Hypothesis

Introduction of a photoperiod during incubation will reduce the negative impact to laying hen production performance upon exposure to heat stress.

4.1.2 Research objective

To evaluate the production performance of laying hens exposed to a heat stress and were provided a photoperiod during incubation.

4.2 Materials and Methods

All procedures in this study were approved by the animal care and use committee of Dalhousie University, Nova Scotia, Canada.

4.2.1 Experimental design

(This study is a continuation of experiment 02 – refer to the chapter 03)

Using the 8 incubators in Dal AC hatchery, 4 lighting treatments were evaluated with 2 incubators per treatment. Six incubators were equipped with red, blue, and white LED lights that are marketed for the commercial poultry industry. Red, blue and white LEDs lights were mounted in each of 2 incubators for replication of each treatment. A photoperiod of 12 h of light followed by 12 h of dark (12L:12D) was used for LED lighting treatments. Two incubators were left without light which was served as the dark control.

The hatched female chicks were placed in the Atlantic Poultry Research Center Controlled Environment (CE) suite. There were eight CE rooms introduced with two different grow-out photoperiods. Four rooms were randomly assigned to one of the two photoperiods. A split-plot design (4 X 2 X 2 X 2) was used to evaluate the bird post-hatch performance using incubation photoperiod and grow-out lighting treatments as main plots. Incubation lighting treatments and the two hen lines were two sub plots in each main plot. There were 4 main plots used in this study and two main plots with a heat stress and the other two were left at the original breeder recommended temperature (controls).

4.2.2 Laying hen facility

Birds from the second experiment (refer to the chapter 03) were used for this trial. Layers of age of week of 37 were introduced to heat stress. Two rooms were randomly selected for each grow out photoperiod for a total of 4 rooms. These were considered the control groups, which were given a constant temperature of 23 °C throughout the whole period. To introduce heat stress,

the remaining 4 rooms were exposed to cyclic daily temperatures (Figure 16). In this group temperature was elevated to 30 °C from 10 a.m. to 5 p.m. and reduced overnight from 5 p.m. to 12 a.m. From 12 a.m. to 10 a.m. the temperature was maintained at 23.0 °C for both the groups. Temperature was increased at rate of 1 °C/ h up to 30 °C and dropped at the same rate to reach 23.0 °C. This temperature fluctuation cycle was continued for 4 weeks with laying hens to induce heat stress.

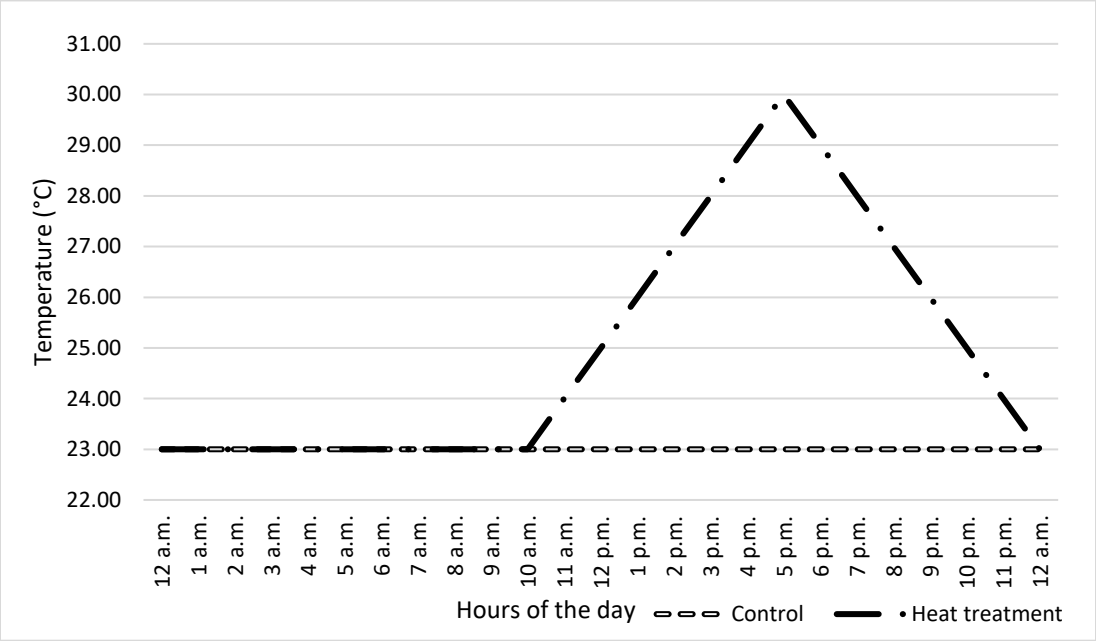

Figure 16. Temperature fluctuation cycle applied for control and treatment groups during the heat stress period. *Control birds had a constant temperature of 23 °C and the temperature for the heat stressed group (treatment) was raised at 1 °C/ h rate to 30 °C during the day time. Temperature of this group was reduced to 23 °C over the night.*

Body weights of the birds were measured at the end of 28-days period. Daily feed consumption and egg production were monitored during the entire cycle of heat stress. Feed conversion ratio was calculated using (Equation 6) the amount of feed a bird consumed during a period and the egg mass (average egg weight X number of eggs laid) in a particular period. For

egg quality measurements, egg weight and specific gravity were monitored weekly. A full egg quality test was run at the end of heat stress period. The measurements including egg weight, specific gravity, break strength, yolk weight, albumen height, albumen weight, shell weight & thickness were monitored at the end of heat stress period. Three eggs were collected from each cage to do the egg quality measurements. At the end of production cycle feather cover was scored (Table 33) in head, neck, breast, abdomen, back, wing, tail areas of the bird. A 6-grade score system was used to rank the feather cover from full to bald with no feather cover (Dennis et al., 2009).

Equation 6. Feed conversion ratio

$$\text{Feed conversion ratio} = \frac{\frac{Feed\ intake\ \text{during the period}}{Number\ of\ birds\ per\ cage}}{(Average\ egg\ weight\ \text{X number of eggs laid per period})}$$

Table 33. Six grade feather score system used to rank the feather condition of the birds.

Feather score	Description
0	Smooth, complete plumage
1	Ruffled, with no bare spots
2	Small bare spots (up to 5 cm wide at the widest part)
3	Large bare spots (greater than 5 cm wide)
4	Area completely bare
5	Area completely bare with injury to skin

4.3 Statistical Analysis

Pullet performance data were analyzed by mixed model using PROC MIXED procedure in SAS (version 9.4, 2012, SAS Institute Inc., Cary, NC, USA) for body weights, feed conversion and hen housed egg production. Egg quality and feather condition data were analyzed by mixed model using PROC GLIMMIX procedure in SAS as the data were not normally distributed.

4.3.1 Statistical model used for performance data:

Split plot design

Sub plot – incubation photoperiod (IP), hen line (HL), heat stress (HS)

Main plot – grow out photoperiod (GOP)

$Y_{ijkl} = \mu + GOP_i + IP_j + HL_k + HS_l + (GOP \times IP_{ij}) + (GOP \times HL_{ik}) + (GOP \times HS_{il}) + (IP \times HL_{jk}) + (IP \times HS_{jl}) + (HL_k \times HS_l) + (GOP \times IP \times HL_{ijk}) + (GOP \times IP \times HS_{ijl}) + (GOP \times HL \times HS_{ikl}) + (IP \times HL \times HS_{jkl}) + (GOP \times IP \times HL \times HS_{ijkl}) + \varepsilon_{ijklm}$

Where, Y_{ijkl} = pullet performance, μ = overall mean, grow out photoperiod (i = long days and short days), incubation photoperiod (j = control, red LED, white LED, blue LED), hen line (k = Lohmann Brown and Lohmann Lite), ($_l$ = control temperature, heat stress temperature) ε_{ijkl} = residual error.

Cage was used as the experimental unit for performance data. Results with significant differences were further analyzed using Tukey-Kramer test. Effects were significant when $P \leq 0.05$.

4.4 Results

4.4.1 Body weights

Body weights were not affected by any of the treatments applied. Only the hen lines showed a significant difference in average body weights (Table 34). LB birds had a higher average body weight 2229.6±26 g at the end of the period and the LL birds weighed 1864.4±26 g.

Table 34. Body weights (g) of the birds at the end of the heat stress period for main factors of interest.

Main factors of interest	Body weights at the end of 28-day period
Incubation (P=0.3417)	
White LED	2014.5±33 g
Control	2027.4±33 g
Red LED	2064.4±33 g
Blue LED	2081.7±33 g
Heat treatment (P=0.7927)	
Control	2052.7±30 g
Heat	2041.3±33 g
Grow out (P=0.6344)	
Long	2055.1±26 g
Short	2038.9±26 g
Hen lines (P=0.0020)	
LB	2229.6±26 g[a]
LL	1864.4±26 g[b]

Mean average body weight (g) ±SEM, [ab] Different letters indicate significant differences. (P<0.05, Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, control of heat treatment had continuous temperature of 23 °C throughout the whole period, heat treatment was provided with cyclic temperatures, LB – Lohmann Brown, LL – Lohman Lite).

4.4.2 Feed conversion ratio

An interaction was seen among hatchery treatment, hen line, grow out photoperiods and the heat stress condition for feed conversion ratio. Feed conversion ratio was significantly low (1.53±0.09) for the LB birds that were exposed to blue LED light treatment and were provided short day grow out photoperiod and control temperature (Table 35, P<0.0005). Lohmann Lite birds hatched in blue LED light, raised in short days and provided with control temperature during the heat stress period had the highest feed conversion ratio (1.92±0.09). All other treatments had an intermediate feed conversion ratio.

Table 35. Feed conversion ratio of birds for the interaction of hatchery treatment, grow out photoperiod, temperature treatment and hen line.

Incubation lighting treatment	Temperature treatment	Lohmann Lite hen line		Lohmann Brown hen line	
		Grow out photoperiod		Grow out photoperiod	
		Long	Short	Long	Short
Red LED	Control	1.60±0.09^{ab}	1.85±0.09^{ab}	1.58±0.09^{ab}	1.61±0.09^{ab}
Blue LED	Control	1.69±0.09^{ab}	1.92±0.09^{a}	1.67±0.09^{ab}	1.53±0.09^{b}
White LED	Control	1.81±0.09^{ab}	1.61±0.09^{ab}	1.57±0.09^{ab}	1.77±0.09^{ab}
Control	Control	1.63±0.09^{ab}	1.64±0.09^{ab}	1.58±0.09^{ab}	1.66±0.09^{ab}
Red LED	Heat	1.56±0.09 ^{ab}	1.62±0.09 ^{ab}	1.57±0.09 ^{ab}	1.64±0.09 ^{ab}
Blue LED	Heat	1.57±0.09 ^{ab}	1.61±0.09 ^{ab}	1.54±0.09 ^{ab}	1.77±0.09 ^{ab}
White LED	Heat	1.63±0.09 ^{ab}	1.57±0.09 ^{ab}	1.70±0.09 ^{ab}	1.59±0.09 ^{ab}
Control	Heat	1.64±0.09 ^{ab}	1.62±0.09 ^{ab}	1.61±0.09 ^{ab}	1.57±0.09 ^{ab}

Average feed conversion ratio ±SEM, ^{ab} Different letters indicate significant differences. (P<0.05,Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, control of heat treatment had continuous temperature of 23 °C throughout the whole period, heat treatment were provided with cyclic temperatures, LB – Lohmann Brown, LL – Lohman Lite).

4.4.3 Hen housed egg production

For hen housed egg production an interaction was observed among hatchery treatments, grow out photoperiod and temperature (Table 36, P<0.0001). The birds from the white LED hatchery treatment raised on long days and given a heat stress had significantly lower hen housed

egg production (24±0.3 eggs). A lower hen housed egg production of 25 eggs/bird (for 28 days) was recorded for birds from white LED light and control hatchery treatments that were reared on short days and given control temperatures (white LED treatment=25±0.7 eggs, control hatchery treatment=25±0.7 eggs). All other treatments resulted in hen housed egg production of 27 eggs/bird during the heat stressed period (28 days).

Table 36. The hen housed egg production (number of eggs produced per bird for 28 days period).

Incubation lighting treatment	Control temperature		Heat treatment	
	Grow out photoperiod		Grow out photoperiod	
	Long	Short	Long	Short
Red LED	27±0.3 [a]	27±0.3 [a]	27±0.3 [a]	27±0.3 [a]
Blue LED	27±0.3 [a]	27±0.3 [a]	27±0.3 [a]	27±0.3 [a]
White LED	27±0.3 [a]	25±0.7 [b]	24±0.7 [b]	27±0.3 [a]
Control	27±0.3 [a]	25±0.7 [b]	27±0.3 [a]	27±0.3 [a]

Mean hen housed egg production/bird/28 days ±SEM, results are an average of two hen lines together), [ab] Different letters indicate significant differences. (P<0.05, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

4.4.4 Egg quality measurements

4.4.4.1 Egg weights

Egg quality parameters were affected by the high heat exposure. Lohmann Lite birds incubated in the dark and exposed to high heat environments had significantly (P<0.0001) reduced egg weights (61±0.8 g, Table 37). Lohmann Brown birds hatched from red LED or white LED hatchery treatment and reared in control temperature had the highest egg weights of 65±0.8 g.

Table 37. Egg quality parameter; egg weights (g) of the birds during the heat stress period.

	Control temperature		Heat treatment	
Incubation lighting treatment	**LB hen line**	**LL hen line**	**LB line**	**LL hen line**
Red LED	65.0±0.8 [a]	64.1±0.8 [abc]	64.6±0.8 [ab]	63.0±0.8 [abc]
Blue LED	63.0±0.8 [abc]	63.4±0.8 [abc]	64.3±0.8 [ab]	63.0±0.8 [abc]
White LED	65.0±0.8 [a]	62.4±0.8 [bc]	63.0±0.8 [abc]	64.4±0.8 [ab]
Control	63.3±0.8 [abc]	63±0.8 [abc]	63.0±0.8 [abc]	61.0±0.8 [c]

Mean egg weight (g) ±SEM, [ab] Different letters indicate significant differences. (P<0.05, Control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, control temperature had continuous temperature of 23 °C throughout the whole period, heat treatment was provided with cyclic temperatures, LB – Lohmann Brown, LL – Lohman Lite).

4.4.4.2 Egg specific gravity, yolk weight, albumen weight, shell weight as a percentage of egg weight and albumen height

Weekly specific gravity (Table 38) measurements showed significant differences for the interaction of hatchery treatments and heat treatments provided (P=0.0209). Results showed that specific gravity (1.089) of the heat stressed birds hatched from red LED hatchery treatment was slightly lower than other treatments.

The treatments applied did not affect the yolk weight, albumen weight and shell weight percentages (Table 39) and these parameters were differed only between the two hen lines. A significant statistical difference was observed from the albumen height (Table 40) between hatchery treatments (P=0.0164) and grow out photoperiods. However, for albumen height Tukey letter difference was not provided.

Table 38. Egg quality parameter; weekly specific gravity of the birds during the heat stress period.

	Control temperature		Heat treatment	
Hatchery treatment	Long	Short	Long	Short
Red LED	1.093 [a]	1.092 [ab]	1.089 [b]	1.091 [ab]
Blue LED	1.092 [ab]	1.093 [a]	1.091 [ab]	1.091 [ab]
White LED	1.091 [ab]	1.092 [a]	1.091 [ab]	1.091 [ab]
Control	1.093 [a]	1.093 [a]	1.090 [ab]	1.090 [ab]

Mean weekly specific gravity ±SEM, results are an average of two hen lines together, [ab] Different letters indicate significant differences (P<0.05, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, control temperature had continuous temperature of 23 °C throughout the whole period, heat treatment was provided with cyclic temperatures).

Table 39. Egg quality parameters; yolk weight, albumen weight and shell weight as a percentage of egg weight during the heat stress period.

Main factors of interest	**Yolk weight as a percentage of egg weight (Mean**±SEM**)**	**Albumen weight as a percentage of egg weight (Mean**±SEM**)**	**Shell weight as a percentage of egg weight (Mean**±SEM**)**
Incubation	(P=0.3598)	(P=0.0816)	(P=0.5620)
Red LED	26.49±0.30	63.49±0.32	9.86±0.07
Blue LED	27.03±0.30	63.45±0.31	9.92±0.07
White LED	27.23±0.30	62.72±0.31	9.85±0.07
Control	27.03±0.30	62.69±0.32	9.98±0.07
Heat treatment	(P=0.3084)	(P=0.9444)	(P=0.0576)
Control	26.67±0.21	63.08±0.29	9.99±0.06
Heat	27.09±0.21	63.11±0.29	9.81±0.06
Grow out	(P=0.5587)	(P=0.8891)	(P=0.7585)
Long	27.04±0.21	63.11±0.25	9.92±0.06
Short	26.83±0.21	63.07±0.25	9.88±0.06
Hen lines	(P<0.0001)	(P<0.0001)	(P=0.0388)
Lohmann Brown	25.83±0.21[b]	64.40±0.24[a]	9.97±0.06[b]
Lohmann Lite	28.05±0.21[a]	61.78±0.25[b]	9.82±0.06[a]

Egg quality parameters are in average yolk weight % ± SEM, average albumen weight % ± SEM, average shell weight % ± SEM. [ab] *Different letters indicate significant differences (P<0.05, control: eggs were incubated in the dark during the whole incubation period, White LED, Red*

LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, control of heat treatment had continuous temperature of 23 °C throughout the whole period, heat treatment were provided with cyclic temperatures, two hen lines used in the experiment were Lohmann Brown and Lohman Lite).

Table 40. Egg quality parameter; albumen height (mm) of the birds during the heat stress period.

Incubation lighting treatment	Grow out photoperiod	
	Long	Short
Red LED	7.77±0.23[a]	7.10±0.23[a]
Blue LED	7.46±0.23[a]	7.68±0.23[a]
White LED	7.25±0.23[a]	7.81±0.23[a]
Control	7.23±0.23[a]	7.58±0.23[a]

Mean albumen height (mm) ±SEM,[ab] Different letters indicate significant differences (P<0.05, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod).

4.4.5 Feather score

Feather score results (Table 41) were different (P<0.05) in hen lines for neck area and back area. Lohmann Lite birds had better neck feather cover (0.66±0.07, a lower feather score signifies that the feather condition is better) than Lohmann Brown birds (0.88±0.07). Lohmann Brown birds had a superior feather cover (0.25±0.07) for the back area of feather cover compared to Lohmann Lite birds (0.81±0.07).

A significant difference was observed for incubation lighting treatments (P<0.05) for the feather cover in back area. Birds from white LED treatment showed the highest score (0.72±0.09)

for the back area indicating poor feather cover compared to other treatments. In addition, blue LED and control incubation treatments had a positive effect on the feather cover signifying a better feather cover for back area compared to the white LED light treatment.

Table 41. Feather score of the birds at the end of the trial. Feather cover was scored for different areas of the body including breast, head, abdomen, back, wing, tail and neck.

Main factors of interest	Head area	Neck area	Breast area	Abdominal area	Back area	Wing area	Tail area
Incubation	**(P=0.9524)**	**(P=0.2281)**	**(P=0.3671)**	**(P=0.3419)**	**(P=0.0372)**	**(P=0.4055)**	**(P=0.4055)**
Red LED	0.31±0.09	1.00±0.1	1.09±0.09	1.18±0.09	0.56±0.09^{ab}	1.00±0.02	1.00±0.02
Blue LED	0.31±0.09	0.71±0.1	0.91±0.09	1.06±0.09	0.43±0.09^{b}	0.97±0.02	0.97±0.02
White LED	0.31±0.09	0.78±0.1	1.03±0.09	1.28±0.09	0.72±0.09^{a}	1.00±0.02	1.00±0.02
Control	0.25±0.09	0.78±0.1	1.00±0.09	1.23±0.09	0.40±0.09^{b}	1.00±0.02	1.00±0.02
Heat treatment	**(P=0.4429)**	**(P=0.4406)**	**(P=0.5354)**	**(P=0.1539)**	**(P=0.2480)**	**(P=0.3248)**	**(P=0.3248)**
Control	0.34±0.07	0.86±0.07	1.06±0.10	1.25±0.06	0.58±0.06	0.98±0.01	0.98±0.01
Heat	0.25±0.07	0.78±0.07	0.95±0.10	1.12±0.06	0.48±0.06	1.00±0.01	1.00±0.01
Grow out	**(P=0.3235)**	**(P=0.2825)**	**(P=0.3041)**	**(P=0.1539)**	**(P=0.4385)**	**(P=0.3248)**	**(P=0.3248)**
Long	0.25±0.07	0.77±0.07	1.05±0.08	1.12±0.06	0.50±0.05	0.98±0.01	0.98±0.01
Short	0.34±0.07	0.87±0.07	0.97±0.08	1.25±0.06	0.56±0.05	1.00±0.01	1.00±0.01
Hen lines	**(P=0.1048)**	**(P=0.0025)**	**(P=0.5352)**	**(P=0.7174)**	**(P=<0.0001)**	**(P=0.3248)**	**(P<0.0001)**

Main factors of interest	Head area	Neck area	Breast area	Abdominal area	Back area	Wing area	Tail area
Lohmann Brown	0.38±0.07	0.88±0.07[a]	0.98±0.08	1.17±0.06	0.25±0.07[b]	0.98±0.01	0.98±0.01
Lohmann Lite	0.22±0.07	0.66±0.07[b]	1.03±0.08	1.20±0.06	0.81±0.07[a]	1.00±0.01	1.00±0.01

Mean feather score parameter± SEM. [ab] Different letters indicate significant differences (P<0.05, control: eggs were incubated in the dark during the whole incubation period, White LED, Red LED and Blue LED lights were provided to each treatment with a photoperiod of 12L:12D, two grow out photoperiods used were long day photoperiod and short-day photoperiod, control of heat treatment had continuous temperature of 23 °C throughout the whole period, heat treatment were provided with cyclic temperatures, two hen lines used in the experiment were Lohmann Brown and Lohman Lite).

4.5 Discussion

In this experiment we wanted to evaluate the effect of providing a photoperiod during incubation on the laying hens that were exposed to a high heat environment during egg production. Therefore, we hypothesized that introduction of a photoperiod during incubation will increase the tolerance to heat stress of laying hens. The laying hens were exposed to four different lighting treatments during incubation and were provided a control temperature or a heat stress during the egg production period. Overall egg quality and the body weights of the birds were not negatively affected by the photoperiodic incubation.

Providing temperatures outside the optimum temperatures is a known factor that reduces body weights (Mashaly et al., 2004). In the current experiment, application of a photoperiod during incubation using white/ red/ blue LED light did not influence body weights of laying hens during high environmental temperatures.

Rearing photoperiod of the birds is influential for production performance. Researchers are keen to know the effect of light duration on the production performance of birds. Abbas et al. (2007) showed that introduction of intermittent lighting (short days) of 1L:3D can increase heat tolerance in chronically heat stressed broilers by reducing the body temperatures and increasing the level of melatonin. They suggest that increased levels of melatonin during the post-hatch period had ameliorated immunosuppression associated for broiler birds exposed to a heat stress. However, in the current study, introduction of short days in early post hatch period did not benefit specifically the Lohmann Lite birds incubated in the blue LED lights, since they had higher feed conversion ratios.

Egg production performance is inversely related to high heat temperatures (Mashaly et al., 2004). Heat stress involves panting which results in reduction of blood

CO_2 level of birds. This will increase the blood pH level of laying hens and ultimately reduce the blood Ca ion level (Mohmoud et al., 1996). To minimize the negative impact of heat stress on shell quality, the hydrogen ions are released into the blood stream from bone resorption and bicarbonate ions are secreted into the shell gland lumen to produce the egg shells (Muller 1969). However, Mohmoud (1996) showed that heat stressed birds had persistent blood alkalosis with reduced blood Ca^{2+}, inducing delayed oviposition by impaired egg calcification. Due to this reason, the birds incubated in white light and the controls, raised in short days with control temperature and the white LED birds raised with long days and stressed with high temperatures had delayed oviposition reducing the overall egg production indicating a low tolerance for heat stress. The birds incubated under other treatments were capable of tolerating heat stress regardless the grow out photoperiod. In this experiment application of red and blue LED lights during incubation increased the tolerance to heat. They maintained optimum egg production upon a heat stress compared to the white LED light incubation treatment.

High heat exposure resulted in reducing several factors of egg quality. The egg weights of the heat stressed, Lohmann Lite birds hatched in the dark were significantly decreased. These results agree with the findings of Mashaly (2004). High environmental temperatures cause reduction in egg weights by limiting nutrient availability for egg formation by discouraging feed intake. High temperature during laying period decreases plasma protein concentration (Zhou et al., 1998) and calcium concentration (Mahmoud et al., 1996) that are required for egg formation. Decline in egg weight is likely affected by the reduced nutrient availability. The birds hatched with a photoperiod provided during incubation did not have reduced egg weights. This suggest that red, blue, and white LED

lighting had increased the ability of birds to tolerate the heat stress while helping to keep the egg weights at an optimum level.

Specific gravity measurements showed a slight reduction of specific gravity **(**1.089) for the heat stressed birds hatched from red LED hatchery treatment and raised in long day photoperiods. However, this level of specific gravity is reasonable for a laying hen flock at the age of 42 weeks (Tumova and Gouse, 2012). Yolk weight, albumen weight and shell weight percentages were not affected by any of the treatments applied. They were only differed between the two hen lines.

Heat stress can reduce the shell quality including specific gravity and shell weight (Mashalay 2004). Plasma calcium level is highly correlated with the egg shell quality in which a reduction is expected upon a heat stress (Mahmoud et al., 1996). High environmental temperature also reduces the calcium uptake from the duodenal epithelial cells (Mahmoud et al., 1996) and the bone. Hydroxyproline is a collagen metabolite, which is an indicator of bone resorption. Heat stressed hens excretes higher amounts of hydroxyproline with a significant reduction of blood Ca^{2+}. Due to lower amounts of hydroxyproline, the parathyroid hormone secretion is increased to restore the blood calcium ion level. However, prolonged heat exposure reduces bone resorption and eventually lowers the shell quality and bone strength during high heat (Magruder and Nelson, 1967). In a recent study, Van de pol (2014) suggested the potential of photoperiodic incubation in providing the birds with a strong cortical bone giving a rigid skeleton. Also, the birds from current study showed no significant difference for body weight to shank length ratio. This ratio is an indicator of well-developed skeleton system (Renema *et al*., 2007). Both of these factors explain that having a well-developed skeleton

have supported Ca resorption during high heat. Since, there has been an adequate amount of calcium reservoirs there have been no adverse effects made on eggshell weight and specific gravity by heat application on the birds that hatched under photoperiodic incubation in the current study. Also, these calcium reservoirs are helpful when the layers get older and start depleting the cortical bone, as judged by cortical bone thickness. This lost happens through the long bones of the wings, legs, keel, etc. Therefore, having a strong cortical bone/ rigid skeleton will also be helpful for birds preventing osteoporosis, fractures and weakening of the skeleton that occur when the birds get older.

Feather condition depends on many factors including nutrition, health and environmental factors (light, temperature, RH) (Carrascal et al., 1998). In the current study, birds from white LED treatment showed a poor feather cover for the back area, compared to other treatments. However, high environmental temperatures did not cause significant difference for the feather condition. In addition, introduction of red and blue LED light during the incubation had a positive effect on the feather cover. In contrast to our results Riedstra and Groothuis (2003) suggest providing embryos with light during the last week of incubation will cause serious damage to feather cover by feather pecking. However, from the results of the current study it can be suggested that birds hatched under red and blue LED light could tolerate the high heat stress without damaging the feather cover. Previous studies have also shown that providing a photoperiod during incubation will increase the tolerance of birds to environmental stressors finally increasing the welfare and production performance of birds (Huth and Archer, 2015a).

4.6 Conclusions

Heat stress during laying hen period can reduce the production performance of layer birds. In the current study, overall production performance varied among the hatchery treatments. Taking the overall live bird performance in to consideration, it can be concluded that introduction of red and blued LED photoperiods in to the incubation does not negatively affect the body weight gain, hen housed egg production, egg quality and feather condition during a heat stressed period. However, hen housed egg production and feather condition were poor in the heat stressed birds if they were incubated with white LED light.

CHAPTER 5: PROJECT CONCLUSIONS AND RECOMMENDATIONS

Use of a photoperiod in the incubator is a novel concept to the hatchery industry to increase the hatching performance, chick growth and egg production performance. This area of research is beneficial to improve the performance and well-being of layer birds produced by the hatching egg industry.

Hatch time synchronization is a key parameter to monitor the incubation performance. Chicks that hatch too early become dehydrated and those that hatch too late may not be included in the overall harvest (Hill, 2011). Traditionally, eggs are incubated in the dark and no development of a diurnal rhythm is observed, that could potentially synchronize the hatch process and provide the chicks with ability to adjust to the day and night rhythms of the rearing environment (Archer, 2015). In this study, introduction of light during incubation and establishment of a day and night cycle helped to reduce the hatch window. Specially, red LED photoperiod was more beneficial in reducing the hatching time compared to the birds hatched under dark/ no light. The chicks from red LED light were able to accelerate the hatch and complete the hatching process earlier. Accelerated hatch time will save the money and time spent on incubation for industry and reduces the number of chicks and their performance lost due to dehydration delay of access to feed.

It is difficult to encourage early feed consumption in newly hatched chicks, which maximizes early survival and improves uniformity of the flock. The provision of light during incubation could prepare the newly hatched chick to adjust more readily to the rearing environment through improved robustness and the establishment of a circadian rhythm early in life (Ozkan et al., 2012a). Improving the ability of pullets to adapt to their environment could benefit them with a good start during early brooding and improve their ability to cope with adverse conditions later in production (Archer and Mench, 2016). As

per the current study, improved post-hatch performance was observed when a red LED photoperiod was introduced during incubation.

Ability to adapt to the environment with encouraged feed intake during the early stages is also important for developing layer pullet's desired optimal pullet body weights before sexual maturity (Prabakar et al., 2016). The results of this study suggest that there was no negative impact on incubation lighting on development of body before sexual maturity. Providing a combination of breeder recommended lighting schedule to laying hen birds that were hatched from red LED light could also reduce the time to lay the first egg of birds increasing the hen house egg production without making negative effects to the egg quality, feed conversion ratio and feather cover.

The bird's ability to cope with stressful situations, especially to elevated temperatures were also monitored during this study. Introduction of red and blued LED photoperiods in to the incubation does not negatively affect the body weight gain, hen housed egg production, egg quality and feather condition during a heat stressed period. However, hen housed egg production and feather condition were poor in the heat stressed birds if they hatched from white LED light.

In future, if this study were to extend, we are anticipating selecting the red LED light treatment and include different photoperiods (18L:6D, 12L:12D, 6L:18D) to evaluate the performance of birds. To evaluate the post-hatch performances the feed, water consumption and crop fill should be monitored to get an idea of the consumption of the pullets.

The preliminary study shows that birds from the R21 treatment produces 1.6 eggs more than the control hatchery treatment during 47 days of period. From the other

experiment it can be shown that birds incubated in red LED and raised in long day photoperiods produce 0.2 eggs more than that of the control incubation treatment combined with long days. Accordingly, if a flock size of 1000 layers was taken into consideration, it can be predicted that the amount of eggs obtained from the birds hatched from red LED light is higher than that of control. For the industry of table egg production, this project is truly beneficial as introduction of red LED photoperiod to incubation not only increased hen housed egg production but also overall hatchling quality and post-hatch performance, including a reduction in the age to lay the first egg.

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