Impact of light during laying hen egg incubation, on hatch traits, growth and behaviour

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Submitted by

Celma Eliete Ferreira dos Santos

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General Abstract

While the impact of various components of light have been studied in broilers and egg production hens, little is known about the impact that light wavelength and duration have on embryo development and welfare in the incubation phase. The overall objective of this research was to evaluate the effect of light wavelength and photoperiod during the incubation of fertile egg production hen eggs. In experiment 1, 640 Lohmann LSL (LSL) and 640 Lohmann Brown (LB) eggs were randomly distributed in 8 incubators. Two incubators per treatment were outfitted with red, blue, or white LED lights, and a 12L (Light):12D (Dark) photoperiod was used throughout incubation. The final two incubators remained dark. At hatch, 144 LSL and 144 LB pullets were placed in 3 brooding rooms under a Near Continuous (NC) or 3 rooms under an Intermittent (INT) lighting photoperiod. Post-hatch, pullet behaviour was video recorded on days 0, 2 and 4. The use of differing light wavelengths during incubation did not affect pullet behaviour post-hatch. A genotype effect was observed, as LSL pullets spent a greater percentage of time at the drinker on days 0 (P=0.012) and 2 (P=0.031), and at the feeder on days 2 (P<0.001) and 4 (P=0.005) compared to LB pullets. Brooding photoperiod also affected early behaviour, as pullets brooded under an INT photoperiod spent a greater percentage of time at the feeder on days 0 (P=0.036) and 2 (P=0.022), less percentage of time resting on days 0 (P=0.005), 2 (P<0.001) and 4 (P=0.004), and a higher percentage of time walking on days 2 (P=0.039) and 4 (P=0.041) than pullets under a NC photoperiod.

Experiment 2 was conducted with the objective of determining the effects of in-ovo photoperiod on hatch traits, growth and behaviour post-hatch. During incubation, 400 LSL eggs (n=3) were randomly distributed and exposed to one of 4 photoperiod treatments ((6L:18D (6L), 12L:12D (12L), 18L:6D (18L)) or 0L:24D (0L). At the hatch endpoint, males were evaluated for hatch traits (n=20/treatment), response to stress (heterophil to lymphocyte ratio (H/L), n=15 chicks/treatment) and composite asymmetry (sum of the difference between right and left; femur, tibiotarsus and metatarsus, n=20/treatment). Females (n=30/treatment) were evaluated for hatch traits at the hatch endpoint, and post-hatch growth to 21 days of age, early behavioural output, presence or absence of behaviour rhythms, H:L ratio, and composite asymmetry. Incubation time was reduced (P<0.05) with the use of light during incubation, with the greatest reduction occuring under 18L. However, the spread of hatch and hatchability were not affected. Stress tests (H/L ratio and composite asymmetry) of the male chicks were not affected by treatment. Body weight of female chicks at

day 0 (P<0.001), 7 (P=0.001), 14 (P=0.017) and 21 (P=0.027) was higher for chicks hatched from 0L incubation, but flock uniformity did not differ. Stress indicators (composite asymmetry and H:L ratio) at day 21 did not differ. On hatch day the percentage of time chicks from 18L spent walking (P=0.029) was higher than chicks from 6L. Standing (P=0.015) was also higher in chicks from 18L compared to chicks from 0L and 12L incubation. Running (P=0.003) on hatch day was higher in chicks from 18L than chicks from 0L, 6L, and 18L incubation. Time spent at the feeder, drinker, preening, performing aggressive and low incidence behaviours at day 0 were not affected by in-ovo photoperiod. Behaviour rhythm was present in chicks post-hatch for the following behaviours: resting (P=0.035) and walking (P<0.001) during the photophase on day 0; walking on day 1 (P=0.023), resting (P=0.001), and foraging (P=0.017) on day 2, regardless of exposure to light during incubation. In conclusion, regardless of light wavelength used during incubation, provision of darkness such as in the INT brood photoperiod used in this study at an early age, increased chick activity compared to those reared under near-constant light. Overall light wavelength does not affect chick behaviour post-hatch, and the greatest impact with the use of lighting on chick behaviour is due to photoperiod length. A photoperiod up to 18L under red light can be used during incubation without negative effects on stress measures and chick's behaviour, but it might reduce chick weight at hatch.

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List of Abbreviations

D	Dark
G	Genotype
H	Hour
H: L	Heterophil to Lymphocyte
INT	Intermittent
L	Light
LSL	Lohmann LSL Lite
LB	Lohmann Brown
LED	Light Emitting Diode
Min	Minute
Nm	Nanometer
NC	Near Continuous
Wk	Week

1.0 Chapter 1: General introduction

Artificial lighting is a management tool that can be used to improve the welfare and increase production efficiency of commercial poultry (Olanrewaju et al., 2006; Schwean-Lardner et al., 2013). This lighting can be provided by a number of sources, including fluorescent, light-emitting diode (LED) and incandescent. All these sources have been discussed in the literature with regards to bird development, growth, production efficiency and animal welfare (Rozenboim et al., 1999ab; Borille et al., 2013; Mendes et al., 2013; Olanrewaju et al., 2018).

The idea of using lighting during incubation of fertile eggs is not new. In-ovo lighting studies can be found in the literature as far back as the late 1960s (Tamimie, 1967; Tamimie and Fox, 1967; Siegel et al., 1969), but these studies contain many inconsistencies regarding the lighting benefits and efficiency of the hatch. These inconsistencies may be due to a combination of components used in the study, such as the source of light, intensity, wavelength, photoperiod, the timing of the onset of light during incubation, and genotype of the bird. Another possible reason for the discrepancies in results may be related to the varying combinations of these variables. The industry has not widely implemented this technology, and one of the reasons may be the lack of information about the effects of these parameters, either individually, or in combination. In addition, few studies have examined the effects of light during incubation on behaviour of chick's post-hatch. Therefore, two objectives were set out for the current work: 1) to identify how wavelength affects post-hatch chick behaviour, and 2) to determine how the duration of photoperiod in-ovo impact hatch traits, bird well-being behaviour and growth.

2.0 Chapter 2 Literature review: Impact of light during laying hen egg incubation on hatch traits, growth, and behaviour

2.1 Lighting in poultry production

2.1.1 Light

Light is electromagnetic waves distributed in a range from short to long wavelengths (Ryer, 1998). Light is used in commercial poultry production to control development, reproductive maturity, and improve production efficiency. Avian species have a tetrachromatic colour vision (Osorio et al., 1999), and are very sensitive to light, and capable of perceiving ultraviolet lights (Prescott and Wathes., 1999; Osorio et al., 1999; Lewis and Morris, 2006) which is outside of the visible light range. The main components that can be adjusted when using artificial lights are light intensity, photoperiod, and wavelength, and can be adjusted accordingly to production objectives.

2.1.2 Light intensity

Refers to the brightness of light. Some light sources such as LED lights can be dimmed (the brightness of light can be regulated). Light intensity can affect chicken behaviour, dim light can decrease, and bright light can increase birds' activities (Blatchford et al., 2009), and can accelerate sexual maturity (Lewis et al., 2004, 2008).

2.1.3 Light photoperiod

Photoperiod is the length of light period received in a day. Photoperiod affects various production variables such as growth and reproductive maturity in chicken. Photoperiod in poultry production can be categorized in continuous (24L:0D), near continuous (23L:1D), intermittent (alternate periods of light and dark within a 24-hour period), or ahemeral (the day length cycle can be short or long, and can also differ from a 24-hour period).

2.1.4 Light wavelength

2.1.4.1 White lighting

White light has a broader spectrum distribution than coloured lights (Warrant and Nilsson, 1998), and this light is commonly used in poultry units because of previously available sources such as incandescent light bulbs. Although white light is composed of different wavelengths, its absorption by photoreceptors is lower than specific wavelengths produced by monochromatic lights (Warrant and Nilsson, 1998), and the reason behind the lower absorption of white light is due to the shape of the white light photoreceptors (Warrant and Nilsson, 1998).

2.1.4.2 Coloured lighting

The colour of light is dependent on the wavelength, and it operates on a sliding scale. For example, as shown in figure 2.1, violet light is seen when the wavelength ranges from 400-450 nm, blue from 450-500 nm, yellow from 570-590 nm, orange from 570-590 nm, and red 610-760 nm.

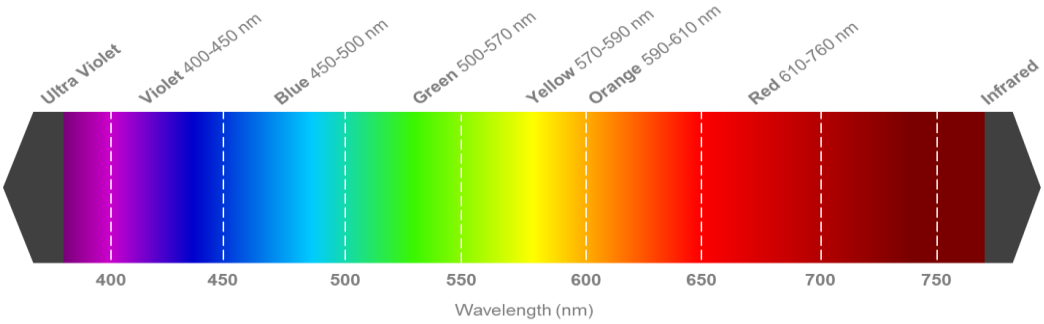

Figure 2.1. Light wavelength. Picture source (Pratti, 2016).

Prescott and Wathes (1999) used chicken behaviour to test bird sensitivity to light. The authors exposed the broilers to a panel with a specific light wavelength and another panel without light, and allowed the bird to make a choice to whether to peck the lit or the dark panel to receive a feed reward. The authors found that the perception of broiler birds to light was higher under wavelengths in the range 533 – 577 nm and lower under the wavelengths 415 nm and 600 nm.

2.1.4.3 Ultraviolet and infrared light

Ultraviolet and infrared lights are found on opposite sides of the visible light spectrum. Ultraviolet light is a short wavelength light distributed in three wavelength categories, UV-C from 100 - 280 nm, UV-B from 280 - 315 nm and UV-A from 315 - 400 nm (Ryer, 1998). Of these, UV-A is the least harmful to the body due to lower emission of energy (Ryer, 1998). The infrared spectrum has the lowest energy of all wavelengths, and it is not visible but can be sensed as heat (Ryer, 1998).

2.1.5 Light source

The most common artificial light sources used in the past in poultry barns were incandescent. However, these lighting systems are no longer available commercially for purchase (Blackwell, 2015), so it is necessary for producers to change. There are many choices for lighting systems including fluorescent and light emitting diode (LED) lighting systems.

The use of LED lights has increased in popularity due to the durability, low cost, and improved energy efficiency of the bulbs (Tabler and Wells, 2015). The LED bulbs are available in both white and monochromatic colours. The potential to use varying wavelengths is interesting from a production and welfare point of view. Many LED light bulbs are capable of being dimmed to allow the provision of a dawn-dusk transition period and control intensity of light.

2.2 Bird biological characteristics impacted by light

2.2.1 Embryo development

There is increasing interest regarding the use of lighting programs during incubation of fertile poultry eggs, as it is thought that light during incubation can influence the bird during embryogeneses (Özkan et al., 2012a; Dishon et al., 2017) and beyond (Rogers and Krebs, 1996; Archer et al., 2009; Özkan et al., 2012b; Huth and Archer, 2015). However, it is important to mention that before the light reaches the embryo it has to penetrate through the eggshell. Shell thickness affects the transmission (i.e. light passing through the shell) of light through the shell and the degree of effect is affected by light wavelength. The greatest transmission of light through the eggshell occurs in the equator portion of the egg from the wavelength range 200-800 nm, while the visible and near-infrared lights have a higher transmission of light through the small pole of the egg compared to the large pole. But there is no difference in near ultraviolet light transmission between the eggshell`s large and small poles (Shafey et al., 2004). The eggshell itself impacts how the embryo utilizes light, as the shell pigmentation can influence the amount of light directed onto the shell surface that is reflected and the amount of light that passes through the shell (Shafey et al., 2002; Maurer et al., 2015). The blue-green wavelengths have higher reflectance on the shell and lower light transmission (Maurer et al., 2015), and the transmission of light through the eggshell is higher as wavelength increases (Shafey et al., 2002; Maurer et al., 2015).

Light transmission through eggshells is affected by eggshell pigments and thickness, which are affected by bird`s genotype (Shafey et al., 2002, 2004, 2005; Maurer et al., 2015), and other factors. In some studies, dark pigmented (Shafey et al 2004, 2005; Maurer et al., 2015) and thick eggshells (Maurer et al., 2015) showed lower light transmission. The pigmentation of the eggshell also acts as a protective layer for the embryo against ultraviolet radiation (Maurer et al., 2015).

Another interesting factor is that the timing of the onset of exposure of the fertile eggs to light seems to play an important function on the impact of light on embryo development (Siegel et

al., 1969). These authors observed higher multiplication and differentiation of somite cells (primary cells in which originates tissues and organs) when White Rock heritage chicken embryos were exposed to light throughout the early stages of development compared to embryos exposed at a later phase. Other studies reported that exposing embryos to light during the last three days of incubation stimulated physical and functional lateralization of the brain (Rogers and Krebs, 1996). The differentiated results observed when embryos were exposed to light at an early developmental stage might relate to effects at a cellular level (Siegel et al., 1969; Coleman and McDaniel, 1976). Exposure of eggs to white light (18L:6D) throughout incubation increased metabolism in house sparrow embryos (Cooper et al., 2011), exposure to green light (15 minutes (min) L:15 min D) increased plasma growth hormone (GH), insuline growth factor (IGF-1) (Zhang et al., 2014), prolactine hormone levels (Dishon et al., 2017), and increased growth hormone-releasing hormone (GHRH), liver growth hormone receptor (GHR), and IGF-1 gene expressions in broiler embryos (Dishon et al., 2017).

2.2.2 Photoreceptors

Photoreceptors are the functional cells capable of receiving light stimuli and transmitting it through neural pathways to the brain. Photoreceptors are concentrated in specific areas of the body such as the retinae, hypothalamus, and pineal gland (Csernus and Mess, 2004a; Lewis and Morris, 2006). The eye is a pathway for light to enter the retinae, where photoreceptors are responsible for absorbing light. The development and maturation of photoreceptors starts during embryo incubation. The first signs of photoreceptor development in the broiler embryo retinae can be observed on the 8^{th} day of incubation, and by the 10^{th} day those photoreceptors can be differentiated from single and double cones or rods. By the 19^{th} day, the photoreceptors are mature and functional (Wai and Yew, 2002; Wai et al., 2006).

2.2.3 Melatonin synthesis, secretion and biological effects

Melatonin synthesis occurs in many body organs, including in the pineal gland, hypothalamus and the retinae (Gwinner and Hau, 2000). Melatonin is a hormone that influences many physiological and behavioural patterns such as thermoregulation (Zeman and Herichová, 2011), behaviour (Golombek et al., 1996), the immunity (Xie et al., 2008; Markowska et al., 2017), oxidative stress (Escribano et al., 2014), and the cardiopulmonary system (Farias et al., 2012). The enzymes involved in the syntheses of melatonin in the chicken retinae are NAT (N-

acetyltransferase) and HIOMT (hydroxyindole-O-methyltransferase) (Espinar et al., 1994). These enzymes develop and become functional during incubation. During incubation, retinal NAT activity in chicken embryos can already be observed by day 7 and 8. Embryonic retinae (NAT) activities on the 7^{th} and 8^{th} day of incubation are equally high in either light or dark incubated chicks suggesting that retinae melatonin enzyme activity is not controlled by light during this period (Espinar et al., 1994). The NAT and HIOMT enzymes are at their lowest activity on day 13, and the highest activity on day 21 of incubation (Espinar et al., 1994). The enzyme HIOMT, which is also responsible for synthesis of melatonin, exhibits higher activity from day 17, reaching its maximal level by day 21 of incubation (Espinar et al., 1994). These authors found melatonin hormone in chicken embryo retina on day 19 of incubation, but observed that the highest concentration of melatonin was present on day 21. The visual processes in chicken embryos becomes functional around the 18^{th} day (Tong et al., 2013), which coincides to the time the enzymes responsible to the production of melatonin increases its activities. On the last two days of incubation, NAT enzyme and melatonin syntheses in the embryo retinae can be inhibited by light (Espinar et al., 1994). In summary, the synthesis of melatonin in the embryos' retinae at the end of incubation is regulated by light and exposure of the embryos to light in the last two days of incubation decreased the retinae synthesis of melatonin (Espinar et al., 1994).

2.3 Impact of in-ovo lighting (wavelength) vs standard dark incubation

According to Shafey et al. (2002) who measured light wavelength (ranging from 200 to 1100 nm), absorption through non-pigmented (White Leghorn eggs) and pigmented eggshells (broilers), found that, in the pigmented shell, the lowest light absorption (99.93%) occurred under a $590<\lambda<630$ nm and the highest (99.96%) under a $380\leq\lambda<470$ nm. The highest (99.91%) light absorption in non-pigmented eggs occurred under a light wavelength equal or lower than 380 nm and the lowest (99.85%) light absorption under a $630<\lambda<780$ nm. The highest light absorption occurs in pigmented broiler eggs and at lower wavelengths. However, the light transmission is lower in brown shelled eggs at higher wavelengths, while for non-pigmented shells the light transmission is higher compared to brown shelled eggs but the light transmission in non-pigmented eggshells reduces at lower wavelengths (Shafey et al., 2002). These results show that less than 0.15 % of light gets through the eggshell, and the percentage of light transmitted is eggshell pigmentation and light wavelength dependent.

2.3.1 Hatch traits

2.3.1.1 Temperature

Light exposure can affect the temperature of incubating eggs and the degree of effects is dependent on the length of exposure to the light (Rozenboim et al., 2004) and the distance of the light bulbs to the eggs (Gold and Kalb, 1976). Rozenboim et al. (2004) reported that the exposure of broiler eggs to green (560 nm, LED) light for 15 min increased the egg yolk temperature by 0.01% within 1 min of exposure to the light. The effects of light on egg yolk temperature, however, can vary depending on the position and distance of the lamps in the incubator to the egg position and the source of light used (Gold and Kalb, 1976). The increment of heat on the yolk or air cell is related to the area of the eggshell surface that received the direct light. For example, when the lights were positioned parallel to the eggs, the increment of heat on the egg yolk was higher compared to when the light was directed towards the air cell (Gold and Kalb, 1976).

The incandescent (140-350 lux) light has a greater impact on the increase in egg yolk and air sac temperature during incubation than warm white fluorescent (700-1100 lux) lamps (Gold and Kalb, 1976). These authors reported that maintaining the fertile eggs at a minimum distance of 15 centimetres (cm) from the light bulbs minimizes the heat effect on the yolk and air sac. However, Coleman et al. (1977) observed that for light to influence White Leghorn embryo development, the distance of the light source to the egg should not be over 12 cm from the top of the eggs, and the light source should be from cool white fluorescent lamps.

2.3.1.2 Duration and pattern of incubation

The pattern of light does not appear to have significant impacts on incubation. For example, the use of intermittent (INT) lighting with a 3 min L (Light): 3 min D (Dark) photoperiod under green light did not affect fertile turkey eggs incubation length in relation to those eggs incubated in a dark environment (Rozenboim et al., 2003). Extending the INT photoperiod length to a 15 min L: 15 min D still did not affect the incubation length of turkey eggs' under green or white light incubation in comparison to dark incubation (Rozenboim et al., 2003). Exposure of broiler eggs to a 15 min L: 15 min D photoperiod under green light from day 5 to 21 of incubation also did not affect the incubation length compared to those eggs incubated in a dark environment (Rozenboim et al., 2004). But white (fluorescent, Biolux) light reduced the incubation length of broiler eggs when they were continuously photostimulated on the last week of incubation compared to exposure

of the fertile eggs to yellow, green, red, or blue colours, or dark during incubation (Hluchý et al., 2012).

Comparing monochromatic colours, broiler eggs under yellow light hatched earlier than the eggs incubated under green or red lighting, and the most lengthy incubation occurred for chicks incubated under blue light or without light (dark) (Hluchý et al., 2012). Additionally, the use of green (fluorescent) light for the first 18 days of broiler egg incubation reduced the incubation length compared to those eggs incubated under darkness (Shafey and Al-mohsen, 2002). Other poultry species were also affected by the lighting condition during incubation. Green lighting also reduced the incubation length of Japanese quail chicks in comparison to dark or blue lighting (Sabuncuoğlu et al., 2018). Quail eggs incubated in darkness had a shorter hatch window than eggs exposed to green and blue lighting (Sabuncuoğlu et al., 2018). However, Shutze et al. (1962) observed a reduction in incubation time for White Leghorn eggs when continuously photostimulated by white (incandescent) light throughout the incubation than eggs incubated under blue, yellow, red lighting, or without light.

2.3.1.3 Spread of hatch

The incubation length and hatching time are controlled by environmental (temperature, humidity), physiological, and behavioural mechanisms (Tong et al., 2013). The time needed from start to end of hatch is approximately 24 to 48 hours in a standard dark incubation (Løtvedt and Jensen, 2014). The mechanisms involved in determining the hatching time are related to vocalization synchrony, and the levels of thyroid and corticosterone hormones (Romanini et al., 2013). The thyroid and adrenal gland are already functional in the chicken by the 7^{th} day of incubation (De Groef et al., 2008). The exposure of broiler eggs to a monochromatic wavelength (continuous green light) during incubation did not affect the concentration of plasma thyroid hormones triiodothyronine (T3) and thyroxine (T4) on embryos during incubation (embryonic days 15, 17 and 19) nor post-hatch (days 1,3,5,7,21,35, or 42) (Zhang et al., 2014). Sabuncuoğlu et al. (2018) reported a shorter hatch window for quail chicks hatched from a dark incubation than the chicks hatched from a green or blue lighting incubation. There are minimal information in the literature regarding in-ovo lighting and hormonal responses, and, to date, the author has no knowledge of in-ovo lighting wavelengths studies regarding the impact of monochromatic or white light colours on the production of thyroid hormones, or the effects of hormonal changes on the spread of hatch of fertile eggs photostimulated by various wavelengths of light.

2.3.1.4 Embryo and chick mortality

The exposure to in-ovo lighting appears to have varying impacts on embryonic mortality. As an example, the continuous use of white (incandescent) light to incubate White Leghorn egg incubation did not affect embryonic mortality on the first 4.5 days of incubation compared to those embryos incubated under dark (Lauber, 1975). In contrast, broiler embryos incubated under dim to blue or dim to red ((dim to blue/red technology have capabilities to shift the spectrums of light when dimmed (Once®, n/a) wavelengths (12L:12D) had a lower percentage of mortality at the early stage of incubation compared to embryos incubated in dark conditions (Archer, 2018). However, broiler embryonic mortality in the middle, at the end of incubation, and total mortality at hatch were not affected by exposure to 12L:12D dim to red or dim to blue (LED) lighting for the first 18 days of incubation (Archer, 2018). This author also had previously reported that using a red (LED) light under the same conditions mentioned above to incubate broiler and White Leghorn eggs resulted in no differences in mid and late embryo mortality when compared to embryos from dark incubation (Archer, 2015b). The use of a white (LED) light emitting high levels of red wavelength reduced White Leghorn, broiler and Pekin ducks' embryos' mortality at the early phase of incubation and decreased total embryo mortality of Leghorn and Pekin duck embryos at hatch (Archer et al., 2017).

Higher mortality appears with the use of blue, or green (LED) lights. Sabuncuoğlu et al. (2018) observed that using blue (480 nm), or green (560 nm) (LED) lights, or no light continuously throughout incubation of Japanese quail fertile eggs resulted in higher mortality at the early stage for those incubated under blue light followed by embryos incubated in the dark. The lowest mortality among all treatments occurred under green light incubation (Sabuncuoğlu et al., 2018). But a decrease in mortality occurred at the end of incubation, when those quail eggs were exposed to green light or incubated under no light (dark incubation), while the embryos under blue lighting incubation had the lowest percentage of dead embryos (Sabuncuoğlu et al., 2018). However, the overall mortality at the end of hatch did not differ among the lighting treatments (Sabuncuoğlu et al., 2018).

2.3.1.5 Hatchability

Hatchability off eggs is affected by environmental and physiological factors, including bird genotype, breeder health, egg contamination, embryo developmental stage during oviposition, and

egg storage (Reijrink et al., 2008). The exposure of the eggs to light during incubation also affect hatchability and the level of impact is affected by light source, intensity, and wavelength. More recently, it was published that when combining light wavelengths, a white (LED) light with a high red wavelength output used on the first 18 days of incubation (12L:12D) resulted in an increased percentage of fertile White leghorn, broiler, and Pekin duck eggs hatched compared to the hatchability of their counterparts incubated in darkness (Archer et al., 2017). The use of only white (fluorescent) light during incubation of broiler eggs also increased the percentage of eggs hatched compared to eggs incubated in darkness (Hluchý et al., 2012; Archer, 2015b; Huth and Archer, 2015) or red lighting environment (Archer, 2015b).

In some cases, bird type has impacted results when using only one wavelength during incubation. Hatchability of layer eggs given red lighting (12L:12D), from day 0 to 18 of incubation increased compared to eggs from white light or dark incubation (Archer, 2015b). On the other hand, white light (12L:12D), from day 0 to 18 increases fertile broiler eggs hatchability in comparison to red light or dark incubation (Archer, 2015b).

Exposure of broilers eggs during the first 18 days of incubation to 'dim to red' or 'dim to blue' light (12L:12D) also increased the percentage of eggs hatched compared to those eggs from dark incubation, however, blue and red lighting treatments did not differ in the percentage of hatched chicks (Archer, 2018). Those findings disagree with Copper (1972) where the author reported that the onset of exposure of eggs (turkey) to light affected hatchability. For example, when the eggs were incubated under dark and transferred to an hatcher under cool white (fluorescent) light (24L:0D) the hatchability of those eggs increased compared to eggs exposed to light during the incubation period and left without light during the hatching phase. However, Hluchý et al. (2012) observed that exposure of fertile leghorn eggs to cool white (fluorescent) light during the last week of incubation increased the percentage of hatched chicks compared to eggs exposed to yellow, green, red, or blue lighting or dark incubation.

The use of green light from day 0 to 18 of broiler egg incubation increased the percentage of hatched chicks compared to incubation under dark (Shafey and Al-mohsen, 2002). However, green or blue lights did not affect hatchability of fertile quail eggs compared to hatchability of eggs from dark incubation (Sabuncuoğlu et al., 2018). Additionally, green (LED) light under an INT (Intermittent) lighting schedule (3 min L: 3 min D) used during incubation of fertile turkey eggs did not impact the percentage of hatched poults when compared to turkey eggs incubated in

darkness (Rozenboim et al., 2003). Even when the length of time that light is on under an INT lighting schedule is increased (15 min L:15 min D), fertile turkey eggs hatchability was not affected compared to eggs incubated in dark or white lighting (Rozenboim et al., 2003). Exposure of fertile broiler eggs to the green (LED) light (15 min L:15 min D) from day 5 to 21 of incubation also did not affect hatchability when compared to broiler eggs incubated under dark conditions (Rozenboim et al., 2004). This finding suggests that the combination of factors such as composition of light and onset of exposure of the eggs to light might play an important role on the level of in-ovo lighting effect on the hatchability. In addition, the intensity of light can also affect hatchability, as high (1430-2080 lux) light intensity decreases hatchability compared to lower (900-1380 lux) light intensities (Shafey et al., 2005).

2.3.2 Chick quality

2.3.2.1 Navel quality

The condition of the chick navel at hatch is critical. Broiler chicks that hatch with unhealed navels can impact productivity by reduced final body weight and increased mortality over the production cycle (Fasenko et al., 2003). Additionally, increasing automation used in hatcheries can result in a higher difficulty in identifying chicks with unhealed navels (Fasenko et al., 2003). Therefore, it is very important that all chicks hatch with a well-healed navel to decrease susceptibility to diseases and improve efficiency in all stages of production. In reviewing the literature, some studies suggest an improvement in navel healing with the use of specific light wavelengths during poultry eggs incubation. The exposure of fertile broiler eggs to white light (12L:12D) or exposure of leghorn eggs to white or red (LED) light (12L:12D) during the first 18 days of incubation, increased the percentage of hatched chicks with healed navels over chicks from 0L:24D incubation (Archer, 2015b). White Leghorns tested in the same study, reacted similarly, but photostimulation under white light resulted in the greatest percentage of chicks with healed navels as compared to red light photostimulation, or dark incubation (Archer, 2015b). However, exposure of broiler embryos to white or red wavelength under a 12L:12D photoperiod increased the percentage of chicks with healed navels at hatch compared to chicks from dark incubation (Archer, 2015b). No differences between light wavelengths for broilers were observed, indicating a different response by bird type. The exposure of the eggs to dim to red and dim to blue (LED) lights from day 0 to 18 during incubation under a 12L:12D photoperiod also appeared to improve

navel healing over chicks hatched from a 0L:24D incubation (Archer, 2018). The better healing in navels may result from the increased bird development due to the exposure to in-ovo lighting, as the early hatched chicks reached maturation earlier.

2.3.2.2 Body weight

Reports on the effect of light wavelength during incubation on embryo/chick weight are contradictory. Some results showed that exposure to specific wavelength of light can affect chick/embryo weight. The photostimulation of White Leghorn embryos during incubation by white light (incandescent) increased embryo weight by day 4.5 of incubation compared to embryos incubated under dark (Lauber, 1975). The use of green light during incubation also resulted in an increased percentage of breast muscle to body weight from day 9 to 21 in broiler embryos compared to embryos incubated under dark (Rozenboim et al., 2004). The use of red (LED) (12L:12D) light from day 0 to 18 of incubation reduced White Leghorn chicks' weight at hatch compared to chicks incubated under white lighting or dark incubation (Archer, 2015b). The exposure of White Leghorn embryos continuously to green (566 nm) or blue-violet (400 nm) lighting during incubation resulted in higher embryo weight on the 4^{th} day of incubation compared to embryos incubated under the wavelengths of 433 nm or 500 nm (Lauber, 1975). Continuous photostimulation of broiler embryos with green (fluorescent) light from set (day 0) to day 18 of incubation stimulated weight gain in embryo at 11, 13 and 15 days of age in contrast to embryos from dark incubation, however, at hatch broiler chicks from green lighting incubation presented a lower absolute body weight and relative body weight to egg weight (Shafey and Al-mohsen, 2002). Green (560nm, LED) light (15 min L: 15 min D) exposure from day 5 to 21 of incubation increased broiler embryo weight at days 14, 15, 17, and 20 compared to those embryos incubated under a dark environment (Rozenboim et al., 2004). However, at hatch there were no differences in male chick weight from neither green or dark incubation (Rozenboim et al., 2004). Huth and Archer (2015b) found no difference in chick weight at hatch for broiler eggs incubated for 21 days under white (LED) light or incubated under daknesss. Additionally, an in-ovo photostimulation from 0 to 18 days through red or white (LED) lighting (12L:12D) had no affect on broiler chick weight at hatch compared to dark incubated eggs (Archer, 2015b). Similarly, exposure of White Leghorn or broiler eggs to white light with high red wavelength output for 12 hours per day from 0 to 18 day of incubation had no affect on chick weight at hatch (Archer et al., 2017). However, Pekin duck eggs' incubated under the same variables, the ducklings hatched with lower body weight than ducklings hatched from a

dark environment (Archer et al., 2017). The use of continuous blue or green lighting, as compared to no lighting during incubation of Japanese quail eggs did not affect chick weight at hatch (Sabuncuoğlu et al., 2018). Suggesting that bird species may be important. The continuous photostimulation of various sized broiler fertile eggs from flocks of different ages with cool white (fluorescent) light for the first 18 days of incubation did not affect chick weight at hatch compared to chicks from eggs incubated in complete darkness (Zakaria, 1989). A photostimulation using dim to red or dim to blue (LED, 12L:12D) lighting from day 0 to 18 of incubation also did not affect broiler chick weight at hatch compared to dark incubation (Archer, 2018). Most of the literature reported no effects of light on chick weight at hatch with the exception of a few contrasting results showing increased chick weight under white or green lighting and reduced chick weight at hatch when exposed to red light. These results appear to be species-specific. The mechanisms behind the impact of light on chick weight is not clear. However, the increase in body weight might result from the increase in multiplication and growth of myofiber and myoblast cells due to light stimulation (Halevy et al., 2005).

2.3.2.3 Chick length

Chick length at hatch has been suggested as a predictor for meat yield potential at slaughter age (Molenaar et al., 2008; Petek et al., 2010). Willemsen et al. (2008) reported that chick length and weight at hatch were correlated regardless of breeder flock. However, at slaughter age Willemsen et al. (2008) did not note any correlation between chick length at hatch and final body weight in broilers at 42 days of age.

The use of different lighting during incubation does not seem to affect the length of the chick at hatch as observed from studies of different wavelengths of light and the different onset of exposure of the embryo to the light wavelengths (Rozenboim et al., 2004; Archer, 2015b). For example, the exposure of fertile broiler eggs to green (LED) light (15 min L:15 min D) from day 5 to 21 did not affect chick length at hatch compared to chicks derived from dark incubation (Rozenboim et al., 2004). Not even exposure of fertile broiler eggs to white or red (LED) lighting from day 0 to 18 of incubation affected the length of chicks at hatch when compared to chicks from dark incubation (Archer, 2015b). Results from in-ovo wavelength research show that light wavelength does not influence the developmental length of the embryo.

2.3.2.4 Organ and GIT absolute and relative weights

Organs smaller than normal in proportion to the size of the body decreases the welfare of the chicken and compromises production efficiency. For example, a proportionally smaller heart relative to body size may not supply enough blood and oxygen to the body, increasing the susceptibility of the chicken to diseases, and increasing bird mortality (Molenaar et al., 2011). Intestine size can affect metabolism, retention and excretion of nutrients affecting feed efficiency (Metzler-Zebeli et al., 2018). The liver has a critical function in regulation of the metabolism of proteins (Zheng et al., 2016), lipids (Nguyen et al., 2008), and carbohydrates (Zheng et al., 2016). Therefore, a liver size proportional to the body size is essential to maintain normal metabolic demands of the organism. In-ovo lighting does not appear to affect these parameters, as white or green (LED) lighting photostimulation did not affect broilers relative heart and liver weights to body weight at days 15, 17 or 19 of incubation or past incubation at days 1, 3, and 6 compared to chicks from dark incubation (Zhang et al., 2016). Additionally, photostimulation by green (560 nm) or blue (480 nm, LED) lighting during the entire incubation of Japanese quail eggs did not affect the absolute and relative weights to slaughter weights of liver, gizzard, heart, wings, legs, and breasts at 56 days of age post-hatch compared to quails hatched from dark systems (Sabuncuoğlu et al., 2018). The impact of lighting wavelength on the embryo during development should be further studied as the current literature is scarce. Therefore, solid conclusions are difficult to be made regarding the potential benefits or harms of light wavelength on organ development and its impact on the birds' post-hatch.

2.3.2.5 Growth post hatch

Incubation conditions can affect hatchling growth beyond hatch (Molenaar et al., 2008) therefore, it is crucial to provide the best environment during incubation to ensure healthy day-old chicks to maximize production efficiency post-hatch. The use of different lighting wavelengths during incubation has shown positive effects regarding the development of in-ovo lighting on birds' post-hatch. Rozenboim et al. (2003) exposed fertile turkey eggs to a green (560 nm, LED) light under an INT (3 min L: 3 min D) photoperiod during the entire incubation, they observed an increase in body weight gain from the age of 28 to 59 days post-hatch compared to white turkeys hatched from dark incubation. Furthermore, when exposure of the fertile turkey eggs to light increased to a 15 min L:15 min D (INT) photoperiod, the hatched poults from green (LED) light

incubation increased weight gain from day 28 until day 79 in comparison to those turkey poults from dark or white lighting incubation independent of gender (Rozenboim et al., 2003).

The incubation condition also seems to affect the quality of the carcass of turkeys, and in this case gender may be important. Male turkeys hatched from a dark incubation showed a decrease in absolute breast muscle weight in relation to light incubated birds. When considering the proportion of breast muscle weight relative to body weight in males, the white turkeys from green, white, or dark incubations resulted in no differences in relative breast muscle weight to body weight (Rozenboim et al., 2003). The female turkeys from green (560 nm) lighting incubation showed an increase in absolute breast muscle weight and relative breast muscle weight to body weight in comparison to those breast traits from the female turkey's hatched from a white lighting or dark incubation (Rozenboim et al., 2003).

Bird type may also result in differences in reaction to in-ovo lighting. The photostimulation of broiler eggs by green (LED, 560 nm) light under a 15 min L: 15 min D (INT) photoperiod from day 5 to 21 during incubation showed higher body weight at one-week post-hatch than those broilers hatched from dark incubation (Rozenboim et al., 2004). On the other hand, a continuous photostimulation using blue or green light during incubation of Japanese quails, did not affect the quails body weight from the age of 7 to 42 days post-hatch in comparison to quails from dark incubation (Sabuncuoğlu et al., 2018).

The stimulus in the growth of chicks incubated under specific lighting wavelengths might be related to hormonal changes stimulated by light (Zhang et al., 2014; Dishon et al., 2017). These authors observed stimulation of growth hormone (GH), insulin-like growth factor 1 (IGF-1) (Zhang et al., 2014), and IGF-1 gene expression (Dishon et al., 2017) when embryos were exposed to green lighting during incubation. Additionally, an additive effect might exist between in-ovo lighting and brood lighting wavelength for broiler chicks. For example, increased body weight throughout the growth period was observed for male broilers photostimulated by green (560 nm, LED) lighting (INT schedule of 15 min L:15 min) from day 5 to 21 of incubation, and brooded post-hatch under a white lighting schedule in comparison to those broilers hatched from dark incubation and brooded under a white lighting environment post-hatch, or hatched from green in-ovo lighting and brooded under a green lighting environment (Rozenboim et al., 2004). However, for females the increase in body weight was only observed on the last three weeks of the grow out period when they hatched from green in-ovo lighting and were brooded under white lighting (Rozenboim et al., 2004).

2.3.3 Health and welfare

Composite asymmetry and immunity

The impact of light wavelength during incubation on composite asymmetry and immunity is still limited. However, results reported by Archer (2017) showed that white or red (630 nm) light wavelength used during incubation can decrease composite asymmetry and increase humoral immunity response in broiler chicks at hatch compared to broiler chicks incubated under green (520 nm) light or dark incubation.

2.3.4 Behaviour

The use of lighting during incubation can impact functional lateralization of the brain causing changes in chicks' behaviour post-hatch (Rogers and Krebs, 1996; Riedstra and Groothuis, 2004). As a response to the exposure of the right eye to the light, which might affect behaviour post-hatch, chicks hatched from an in-ovo lighting incubation increased the use of the right eye over the left eye to investigate the surrounding and increased cognitive behaviour performance (Rogers and Krebs, 1996). However, this behavioural lateralization might come with a cost, as White Leghorn eggs exposed to light during incubation increased the early pecking behaviour post-hatch in relation to chicks that were not exposed to light during incubation (Riedstra and Groothuis, 2004). This increase in early pecking behaviour in chicks exposed to light during incubation occurred due to the incapacity of the chicks in recognizing their counterparts, choosing to peck at any bird in the cage while chicks that were not exposed to light during incubation pecked more at foreigner birds (Riedstra and Groothuis, 2004). The stimulus in functional lateralization of the brain is light wavelength dependent, and chicks exposed to white light showed higher lateralization than chicks exposed to green or red light (Rogers and Krebs, 1996). In addition to behaviour expression, birds can communicate via expression of a wide range of sounds (territoriality, fear etc.). In-ovo lighting wavelength affected quail response to threats, and these changes included communication. Japanese quails that hatched from green (560 nm, LED) lighting incubation reacted differently to a novel field challenge compared to those quails hatched from a blue (480 nm, LED) lighting or dark incubation (Sabuncuoğlu et al., 2018). Quails hatched from green (560 nm, LED) lighting showed higher vocalization in an open field test at the ages of 21 and 42 days of age than quails hatched from dark incubation, and quails hatched from blue lighting were intermediate (Sabuncuoğlu et al., 2018). The green or blue lighting incubation, however, did not affect the time

the birds spent in tonic immobility or a frozen state (related to fear) (Sabuncuoğlu et al., 2018). The time to produce the first noise, the time for the first movement, or the time to enter a novel space in an open field test was also not affected compared to those quails from dark incubation (Sabuncuoğlu et al., 2018). These confounding results make it difficult to conclude if those Japanese quails incubated under different in-ovo lighting wavelengths were more anxious or fearful than dark incubated quails. Additionally, Japanese quail chicks hatched from blue (480 nm) lighting incubation showed higher fearful behaviours (jumping activity and excretes elimination) when in a novel environment than those quails from green light incubation (Sabuncuoğlu et al., 2018).

2.4 Impact of in-ovo lighting incubation (photoperiod) vs standard dark incubation

The concept of using light during incubation has not been widely researched but has shown some promising results. One of the very early studies reported advanced development of the embryo as early as the first 10 hours of incubation in relation to embryos that were not exposed to light (Siegel et al., 1969). Other benefits that have been reported include stimulation of the multiplication of breast muscle cells, increase embryo weight, and reduction in incubation time (Shutze et al.,1962; Isakson et al., 1970; Cooper et al., 2011). The impact of a range of photoperiods during incubation of poultry, however, has not been widely studied, and the available literature has given limited conclusions.

2.4.1 Hatch traits

2.4.1.1 Temperature

A large increase or decrease of the standard temperature used in commercial incubation (37.8 °C) could result in increased embryo mortality and a decline in the quality of the hatched chicks (Lourens et al., 2005). The research discussing the increment of heat caused by the addition of light during incubation of fertile eggs is contradictory, and there are very few studies reporting the temperature among the eggs or the egg yolk temperature during incubation as impacted by light photoperiod. Previous research indicated that exposure of broiler eggs to green (560 nm, LED) light for over 15 min increased the egg yolk temperature (Rozenboim et al., 2003). Fairchild and Christensen (2000), however, did not observe any effect of incandescent light under a 12L:12D

photoperiod on the incubator temperature during incubation of fertile turkey eggs compared to an incubation under 0L:24D. Both studies cited above showed contradictions, although in each experiment a different light source was used. An incandescent and fluorescent light (Gold and Kalb, 1976), and even LED light (Rozenboim et al., 2003) have been reported as potentially able to emit some heat, although LED does not produce the same level of heat as other light systems. In their methodology, the authors (Fairchild and Christensen, 2000; Rozenboim et al., 2003) did not mention the distance between the light lamps and the eggs. As a previous study observed, a minimum distance of 15 cm from the egg to the light bulb is essential to avoid heating the egg yolk when using either incandescent or fluorescent bulbs (Gold and Kalb, 1976). To date, there are no reports of what should be the minimum distance of the egg to LED lamps during in-ovo lighting to minimize heat effects on the embryo during incubation.

2.4.1.2 Incubation time

Photoperiod used in-ovo may impact the time required for hatching to occur. Evidence in the literature suggested that use of an in-ovo photoperiod accelerated embryo development and shortened incubation time of fertile eggs exposed to light during incubation and hatching period compared to those eggs incubated and hatched under dark (Siegel et al., 1969; Copper et al., 2011). The exposure of the embryos to light on the 1st and 2nd week of incubation, or on the 1st and on the 3rd week of incubation accelerated embryo maturation and reduced incubation time (Siegel et al., 1969). This suggests that the exposure of the embryos to light on the first week of incubation is crucial to acceleration of the embryo development (Siegel et al., 1969). However, the embryos exposed to light on 3rd, 10th , and 15th day, or 5th, 12th , and 19th day for a 5 minutes/hour lighting cycle developed faster and reduced incubation time. But when the embryos were exposed to light on 1st, 8th , and 15th day of incubation there were no reduction in incubation time (Adam and Diamond, 1971). These data suggested a timing dependency of the embryo developmental stage and light exposure to speed embryo maturation.

The incubation photoperiod may also impact the speed of hatch. Cooper et al. (2011) studied incubation of House Sparrow (*Passer domesticus*) eggs and noted a decrease in 10% of incubation time when they were given an 18L:6D photoperiod length over a 12L:12D using fluorescent UVB lighting. A 12L:12D photoperiod under incandescent light, however, reduced incubation time for turkey poults in relation to incubation under 0L:24D (Fairchild and Christensen, 2000). Walter and Voilte (1972) reported that a photoperiod of 24L:0D under incandescent light

reduced broiler eggs incubation time over an incubation under a 12L:12D or 0L:24D photoperiod. This evidence suggested that photoperiod length can reduce incubation period in birds (Siegel et al., 1969; Walter and Voilte, 1972; Fairchild and Christensen, 2000; Cooper et al., 2011). The acceleration in development stimulated by light might be related to the increase in metabolism as the metabolism of the embryo during an in-ovo lighting incubation increases during the photophase compared to the scotophase (Cooper et al., 2011). Additionally, the literature suggests that light stimulates the divisions of cells that gives formation to body structures (Siegel et al., 1969) and proliferation of muscle cells (Halevy et al., 2006). Özkan et al. (2012a), however, did not find differences for incubation time when incubating broiler eggs under a 16L:8D photoperiod illuminated by white (fluorescent) light for 21 days or exposing the eggs to light only at the last week of incubation in comparison to incubation of embryos under dark. In general, most of the literature compares only two photoperiods, so the determination of an optimal in-ovo photoperiod is difficult based on published research.

2.4.1.3 Spread of hatch

No information was found in the literature up to this date regarding the effects of in-ovo photoperiod on the spread of hatch.

2.4.1.4 Embryo and chick Mortality

Embryo mortality during incubation could be affected by several factors such as egg storage time, hen age, hen health, eggshell conductance, and factors affecting incubator environment such as rotation of the eggs, temperature, humidity, and ventilation. Another potential could be photostimulation length, as daylength impacts mortality of live birds (Schwean-Lardner et al., 2013; Vermette et al., 2016a). The currently available data to date does not support this, and in-ovo lighting photoperiod length does not seem to impact embryo mortality (Archer et al., 2009; Özkan et al., 2012a; Archer and Mench, 2014b; Archer, 2015a, 2015b; Huth and Archer, 2015b). For example, a 16L:8D photoperiod illuminated by cool fluorescent light for 21 days or only during the last week of incubation of broiler embryos did not affect embryo mortality in relation to embryos incubated under a 0L:24D photoperiod (Özkan et al., 2012a). A photostimulation provided by fluorescent light under a photoperiod of 24L:0D or 12L:12D during incubation also did not affect broiler chick mortality compared to broiler chicks from 0L:24D incubation (Archer et al., 2009). The photoperiods 1L:23D, 6L:18D, or 12L:12D illuminated by white (fluorescent)

light used during 21 days of incubation of broiler egg did not affect broiler chicks' mortality (Archer and Mench, 2014b). Additionally, a 12L:12D photoperiod under white (LED) light did not affect early, mid, and late White Leghorn and broilers embryos' mortality compared to mortality in those embryos from 0L:24D incubation (Huth and Archer, 2015b).

2.4.1.5 Hatchability

Hatchability is one of the most critical parameters of fertile eggs' incubation and several studies reported, however, that in-ovo lighting photoperiod length does not affect the percentage of chicks' hatch. For example, photoperiods 24L:0D or 12L:12D under incandescent light did not affect the hatchability of set and fertile broiler eggs compared to a 0L:24D incubation (Walter and Voitle, 1972; Archer et al., 2009). Additionally, a 12L:12D photoperiod used during incubation of turkeys eggs did not affect the percentage of hatched poults when compared to the percentage of poults from a 0L:24D incubation (Fairchild and Christensen, 2000). Not even White Leghorn eggs under a 12L:12D in-ovo lighting photoperiod illuminated by white (LED) light was effective to change the percentage of chicks hatched in relation to a 0L:24D photoperiod (Huth and Archer, 2015b). The timing of the provision of light has also been considered in some studies. But the majority of studies still showed no effects of in-ovo photoperiod on hatchability of birds. For example, the use of light under a 16L:8D photoperiod in different stages of incubation either from day 14 to 21 or from day 0 to 21 did not affect hatchability of broiler chicks (Özkan et al., 2012a). Archer and Mench (2014b) observed that broiler eggs incubated under a 12L:12D light photoperiod provided from day 0 to 21, 7 to 21, or day 14 to 21 of incubation or incubated under a 1L:23D, or 6L:18D photoperiod had no effect on hatchability when compared to the percentage of hatched broiler chicks from a 0L:24D incubation. Adam and Diamond (1971), however, found that the addition of 5 minutes of light per hour during embryogeneses at specific days such as 3rd, 10th, and 17th day, or a 5 minutes exposure to light per hour on days 5, 12 and 19 of incubation improved hatchability of the eggs. These authors also reported that exposure of the eggs to light on days 1, 8, and day 15 of incubation was not enough to impact the eggs hatchability. The authors concluded that embryos might only be stimulated by light after the development of the visual functions, which occur after the 17th day of incubation.

2.4.2 Chick quality

2.4.2.1 Navel healing

The in-ovo lighting photoperiod length could influence health parameters of chicks. This influence on health parameters could improve navel condition at hatch. As an example, a 12L:12D (white light) photoperiod during the entire incubation of White Leghorn fertile eggs, resulted in a tendency for a lower percentage of hatchlings with unhealed navel at hatch compared to hatchings from 0L:24D incubation (Huth and Archer, 2015b). The use of a 12L:12D photoperiod under white light decreased the percentage of White Leghorn chicks hatched with unhealed navels in comparison to the hatchlings incubated under a 12L:12D photoperiod under red light, and hatchlings incubated under 0L:24D photoperiod (Archer, 2015b). Additionally, broiler chicks showed a higher percentage of navel healed when incubated under a 12L:12D photoperiod photostimulated by either white or red light in comparison to hatchlings from a 0L:24D incubation (Archer, 2015b). A photoperiod of 12L:12D under white light with high red wavelength output also decreased unhealed navels in broilers and White Leghorns at hatch in relation to those chicks from 0L:24D incubation (Archer et al., 2017). The authors, however, reported that the same effects were not observed in ducklings incubated under the same conditions as mentioned for broilers and White Leghorns. In addition, a study by Walter and Voitle (1972) found that exposure of broiler embryos during the entire incubation process to a 12L:12D or a 24L:0D photoperiod illuminated by an incandescent bulb did not affect navel quality at hatch when compared to a 0L:24D incubation (Walter and Voitle, 1972).

2.4.2.2 Chick weight

The timing of exposure of the fertile eggs to light during incubation appears to be an essential aspect of embryonic development. The exposure of broiler eggs to cool white (fluorescent) light under a 16L:8D photoperiod from day 0 to 21 of incubation increased the relative embryo weight to egg weight (Özkan et al., 2012a). A 24L:0D or a 12L:12D photoperiod illuminated by incandescent light also increased broiler embryos weight on the 5^{th} day of incubation, further the broiler embryos incubated under a 24L:0D photoperiod had a higher body weight on days 12 and 18 of incubation than broiler embryos from a 0L:24D photoperiod (Walter and Voitle,1972). However, embryos under a 12L:12D photoperiod at the age of 12 and 18 days did not differ in weight from either the embryos incubated under 0L:24D or 24L:0D photoperiod

(Walter and Voitle,1972). Although these authors observed an effect of in-ovo lighting photoperiod on embryo weight during incubation, chicks hatched from a 24L:0D or 12L:12D photoperiod did not differ in body weight from those chicks incubated under a 0L:24D photoperiod. Rozenboim et al. (2004) observed the same pattern of heavier embryo weight during incubation. In their study the authors incubated broiler eggs under green (560 nm, LED) light on a 15 min L:15 min D (INT) photoperiod from day 5 to 21 of incubation. Their results showed that the embryos had a higher relative body weight to egg weight compared to embryos incubated in the dark. The mechanisms regarding the acceleration of embryonic development and increased body weight are still not very clear. However, these results showed increased embryo weight, which might be a response from the increased plasma growth hormone found in embryos from the age 14 to 18 days of incubation (Dishon et al., 2017). Growth hormone has been linked to an increased deposition of fat in young broilers (Moellers and Cogburn, 1995). It appears, however, that the chicks' absolute body weight at hatch does not show the same effects of increased body weight as observed on embryos in response to a photoperiod length stimulation (Rozenboim et al.,2004; Özkan et al., 2012a). As an example, turkey poults weight at hatch was not affected by a 12L:12D in-ovo photoperiod photostimulated by an incandescent light compared to those poults incubated under a 0L:24 photoperiod (Fairchild and Christensen, 2000). These findings are supported by Huth and Archer (2015b) and Archer's (2015a) findings, whom observed that a 12L:12D photoperiod illuminated by white (LED) light from day 0 to 18 or from day 0 to 21 of incubation did not influence chick weight at hatch.

2.4.2.3 Chick length

Light during incubation has the potential to stimulate bone development, as it was reported that broiler chickens incubated under a 12L:12D photoperiod illuminated by cool white (LED) light had increased femur and tibia length in contrast to the femur length of broilers incubated under 0L:24D or 24L:0D photoperiods (van der Pol, nd). Additionally, bone mineral characteristics might be affected by in-ovo photoperiod with a carry-over effect lasting up to the 35 days of age in broilers, as those chicken hatched from a 24L:0D photoperiod incubation showed a higher mineral density on the femurs at 35 days of age compared to those femurs of broilers hatched from a 0L:24D incubation (van der Pol et al., 2017). Those broiler femurs hatched from a 16L:8D photoperiod, however, did not differ from the femurs of birds hatched from a 24L:0D, nor a 0L:24D incubation, but the broilers hatched from a 16L:8D showed a higher mineral density in the tibia compared to

the tibias of broilers hatched from 24L:0D or 0L:24D photoperiod (van der Pol et al., 2017). However, at the age of 35 days, broilers hatched from a 24L:0D, 16L:8D, or a 0L:24D incubation program neither differed in femur nor tibia length (van der Pol et al., 2017).

2.4.2.4 Organ absolute and relative weights

Results from the literature showed that a 12L:12D in-ovo photoperiod had no impact on organ development, as turkey poults' absolute liver and heart weights did not differ from the organs absolute weight of poults hatched from a 0L:24D incubation (Fairchild and Christensen, 2000). A 16L:8D photoperiod under white (fluorescent) light also did not affect broiler embryos' relative liver and heart weights at days 13 and 18 of incubation in comparison to 0L:24D photoperiod (Özkan et al., 2012a). The exposure of broiler embryos to an intermittent photoperiod (15 min L:15 min D) under green light throughout the incubation was also innefective in affecting the embryos absolute liver weights on days 10 to 20 of incubation compared to a 0L:24D photoperiod (Dishon et al., 2017).

2.4.2.5 Growth post-hatch

The use of different photoperiod lengths during incubation does not appear to affect growth post-hatch (Walter and Voitle, 1972; Archer et al., 2009). Commercial fertile broiler eggs incubated under a 24L:0D or a 12L:12D photoperiod throughout incubation with lighting provided by an incandescent bulb had similar body weights at 4 and 8-weeks post-hatch compared to broiler chick weights from a control 0L:24D incubation (Walter and Voitle, 1972). Additionally, incubation of fertile broiler eggs under a 0L:24D, 12L:12D, or a 24L:0D (fluorescent light) photoperiod did not affect chicks feed consumption, growth, and gain to feed over 42 days of the growth period post-hatch when those birds were reared under a 12L:12D photoperiod (Archer et al., 2009). A 16L:8D or 24L:0D photoperiod, provided by a cool white (LED) light, or a 0L:24D incubation did not impact broilers' body weight from day 7 to 35 post-hatch, when reared under a similar or a mismatched incubation photoperiod indicating no interactions between in-ovo lighting photoperiod and post-hatch brood photoperiod (van der Pol et al., 2017). If not considering post-hatch photoperiod, however, at week 4, broilers hatched from a 24L:0D incubation were heavier than broilers hatched from 16L:0D or 0L:24D incubation (van der Pol et al., 2017). Not all research is consistent in these findings, however. Despite no effects of photoperiod during incubation on

growth post-hatch, an interaction of in-ovo photoperiod and rearing photoperiod have been suggested in the literature.

Özkan et al. (2012b) observed that broilers hatched from a 16L:8D photoperiod incubation photostimulated from day 0 to 21 or day 14 to 21 by cool white (fluorescent) light , showed higher body weight at 35 days post-hatch when reared under a similar photoperiod as the one used during incubation compared to broilers reared under a mismatched lighting schedule such as incubated under dark and raised under 16L:8D photoperiod. However, when these chicks were reared under 1L:23D photoperiod post-hatch, the body weight gain from hatch to 35 days did not differ regardless of incubation treatment. It is not clear how and why a photoperiod during incubation would affect the development of the broilers reared under a matched or mismatched photoperiod post-hatch. But behavioural change could be a factor as photoperiod during incubation can change early feeding activity of broilers post-hatch (Archer et al., 2009).

2.4.3 Health and welfare

2.4.3.1 Heterophil to lymphocyte ratio

Lighting photoperiod can increase the chicken immune system response through activation of lymphocyte B and T cells from lymphoid organs (Kliger et al., 2000; Abbas et al., 2008). The relation between photoperiod and immunity is linked to melatonin hormone regulation (Markowska et al., 2017). For example, the photoperiod impact on the immune system was observed in broilers hatched from a 12L:12D photoperiod incubation program, with lower levels of stress expressed, monitored via reduction in corticosterone levels, and a decrease in the heterophil to lymphocyte (H:L) ratio tested before and post crating the broilers and compared to broilers hatched from continuous dark incubation (Archer et al., 2009; Archer and Mench, 2013, 2014b). The ratio of H:L is a good indicator of bird' stress (Gross and Siegel, 1983) as the H:L ratio can increase due to an increase in the corticosterone hormones circulating in the bloodstream (Gross and Siegel, 1983; Scanes, 2016).

Further evidence of an improvement in stress resilience as impacted by in-ovo lighting was reported by Özkan et al. (2012a). These authors observed a lower concentration of malondialdehyde (an organic by-product derived from lipid peroxidation) in the chicken brain tissues, indicating a decrease in oxidative stress on chickens incubated under a 16L:8D compared to chicks from dark incubation. The oxidative stress can occur when the amount of toxic and

reactive substances accumulated in the body is higher than the body`s capacity to release it (Halliwell and Whiteman, 2004) which can lead to a decrease in growth, production and meat quality (Fellenber and Speisky, 2006).

2.4.3.2 Composite asymmetry

Morphological asymmetry can also be indicative of stress during development. Broiler chickens hatched from in-ovo lighting 12L:12D photoperiod incubation showed lower levels of physical asymmetry than broilers incubated under a 0L:24D photoperiod (Bradley et al., 1994; Huth and Archer, 2015b). These results could indicate that incubation of fertile eggs under lighting might improve the environment for the embryo during incubation, consequently increasing embryo welfare. Özkan et al. (2012a), however, did not find any differences for physical body asymmetry resulting from the use of a 16L: 8D photoperiod when eggs were exposed to light either from day 0 to 21 or day 14 to 21 of incubation compared to physical body asymmetry in broilers from 0L:24D incubation. Additionally, the exposure of broiler embryos to a 12L:12D photoperiod under cool white (LED) light from day 0 to 18 or day 0 to 21 of incubation did not affect chicken physical asymmetry at 45 days of age post-hatch in relation to hatchlings from 0L:24D incubation (Archer, 2015a).

2.4.4 Behaviour

Broiler chicks hatched from an in-ovo lighting incubation (12L:12D photoperiod) showed lower fear response at 35 and 42 days of age, when measured through an inversion and tonic immobility test compared to broiler chicks hatched from in-ovo lighting under a 24L:0D, 6L:18D, 1L:23D, or a 0L:24D photoperiod incubation (Archer and Mench, 2017). This result indicates that a longer photoperiod in addition to a dark period are needed to impact behaviour, which might be a response to corticosterone hormone level changes due to the length of exposure to light and dark. For example, Özkan et al. (2012a) observed a decrease in corticosterone levels at hatch in broiler chicks incubated for 21 days under a 16L:8D photoperiod compared to incubation under a 0L:24D, or a 16L:8D (exposure to light from day 14 to 21) photoperiod.

2.5 Diurnal rhythm

A diurnal rhythm is a synchronized circadian rhythm that follows a day/night pattern, (Csernus and Mess, 2004a). Diurnal rhythms are triggered by factors including photoperiod length

(Schwean-Lardner et al., 2014), light wavelength (Di Rosa et al., 2015), social interaction (Mistlberger and Skene, 2004), and temperature (Kadono et al., 1981). A diurnal rhythm can affect syntheses of hormones such as growth hormone, corticosterone, and melatonin (Karatsoreos et al., 2011; Kronfeld-Schor et al., 2013). Regulate body temperature (Kadono et al., 1981), behaviour (Fonken et al., 2013), metabolic, and neural functions (Karatsoreos et al., 2011; Fonken et al., 2013).

The pineal gland functions as a biological pacemaker that controls the diurnal rhythms in chickens (Bailey et al., 2003; Piesiewicz et al., 2012). The synthesis of the melatonin hormone in the pineal gland is controlled primarily by photoperiod length (Zawilska et al., 2006). During incubation, the effects of photoperiod length on melatonin synthesis rhythm are already present at pipping and hatching in broiler embryos incubated under 16L:8D photoperiod (cool white fluorescent light) from day 0 to 21 of incubation compared to chicks from 0L:24D incubation (Özkan et al., 2012a). The lighting program used in in-ovo can impact the diurnal rhythms in chicks' behaviour. For example, chicks incubated under 12L:12D, 6L:18D, or a 1L:23D photoperiod expressed a higher level of feeding behaviour, and consumed more feed during the first three hours after the lights were switched on in comparison to chicks incubated under dark environment (Archer and Mench, 2014b). A second trial conducted by the same group of authors compared incubation under 0L:24D or 12L:12D during three different phases of incubation (days; 0-21, 7-21 and 14-21). The authors found similar behavioural patterns in feeding. Regardless of weather the chicks were exposed to light from day 0 to 21 or day 7 to 21 the chicks under the 12L:12D cycle consumed more feed on the first hour of the day compared to chicks hatched from dark incubation (Archer and Mench, 2014b). In both trials, the chicks from all treatments were raised under the same photoperiod (12L:12D), suggesting entrainment of circadian rhythm from incubation which remained for at least a short time past hatch. Interesting evidence of melatonin synthesis resulting from an in-ovo lighting photoperiod was observed. At day 19 of incubation the embryos were tested for melatonin hormone levels, and the results showed a higher plasma melatonin rhythm in embryos from 12L:12D (day 0-21) and 6L:18D (day 0-21) in comparison to embryos from 0L:24D (day 0-21) and 1L:23D (day 0-21) incubation. However, when tested again at 5 weeks past hatch, no melatonin rhythm was found (Archer and Mench, 2014b).

Although photoperiod appears to influence embryos and birds in general, the change in light does not evoke an immediate response in chickens. For example, in in-vitro White Leghorn

chicken pineal glands under natural and monochromatic wavelengths, a complete shift of the melatonin rhythm from one photoperiod to another took up to three days (Csernus et al., 1999). However, the speed at which the shift occurs is wavelength dependent. When comparing red (690-800 nm), blue (420-560 nm), or green (480-620 nm) wavelengths, the red wavelength presented the fastest melatonin rhythm reversal shift, while green and blue light delayed the melatonin rhythm shift under the new photoperiod (Csernus et al.,1999).

2.6 Impact of photoperiod on chicken during the brooding period

2.6.1 Stress indicators

Photoperiod length during brooding and rearing period may also affect fear and stress levels. Broilers and laying hens reared under continuous or near continuous photoperiods had higher H:L ratios and higher response levels to fear than birds raised under a 12L:12D or 14L:10D photoperiods (Zulkifli et al., 1998; Campo and Dávila, 2002). Bayram and Özkan (2010) reported that broilers reared under a 24L:0D photoperiod remained in a tonic state for a longer period, took a longer time to emerge from a box and spent more time isolated from other birds than broilers from 16L:8D photoperiod (Bayram and Özkan, 2010). Suggesting that broilers reared under long daylengths were more fearful than broilers reared under a 16L:8D photoperiod. Broilers also showed a longer tonic immobility duration when reared under a 16L:8D photoperiod than broilers reared under an INT lighting program (Møller et al., 1999).

Additionally, broilers reared under a 24L:0D, had higher adrenal gland absolute and relative weights to body weight at 35 days of age than broilers reared under a 12L:12D photoperiod. However, these authors did not observe changes in the plasma levels of corticosterone and glucose (Freeman et al., 1981).

2.6.2 Health

Photoperiod during brooding and rearing has been identified as playing an important part in bird health, particularly for metabolic and skeletal health. A shorter 6L:18D photoperiod during the first two weeks of brooding reduced the incidence of leg abnormalities, and mortality caused by sudden death syndrome (SDS) later in life compared to broilers reared under a near-continuous lighting schedule (NC) (23L:1D) (Classen and Riddel, 1989). Lewis et al. (2009) observed that deaths caused by SDS decreased as photoperiod increased between 2 to 10 hours of light, but SDS

increased if the photoperiod was extended to 24L:0D. Schwean-Lardner et al. (2012, 2013) also reported increased skeletal and metabolic abnormalities, increased mortality and eye weight, and a decrease in mobility in broilers reared under a NC (23L:1D) photoperiod relative to broilers reared under a 20L:4D, 17L:7D, or 14L:10D photoperiods.

These findings supported the conclusions of Brickett et al. (2007) who observed lower mortality caused by SDS and other infectious causes in birds reared under a 12L:12D compared to those reared under a longer photoperiod (20L:4D). This appears to be related to cellular immune stimulation in the body. For example, INT (2L:2D) lighting programs were found to stimulate the production of lymphocytes, T cells, B cells and antibodies compared to 23L:1D and 12L:12D photoperiods (Abbas et al., 2008). Moreover, under an INT (2L:2D) photoperiod, broilers had a higher T3 plasma level and white blood cell count than broilers under an NC (23L:1D) photoperiod. These results suggest an improvement in the immune system of broilers. The improvement in the immune system was also observed by the reduced mortality and improved body weight and feed conversion in broilers reared under an INT (2L:2D) photoperiod relative to broilers raised under an NC (23L:1D) photoperiod (Abbas et al., 2008). Furthermore, broilers improved feed conversion when reared under an INT (2L:2D) photoperiod in relation to broilers under an NC (23L:1D) photoperiod (Abbas et al., 2008).

2.6.3 Behaviour

The use of artificial lights in poultry production can stimulate physiological and behavioural changes. Weaver and Siegel (1968) studied feeding behaviour in live commercial male broilers and observed a shift in the behaviour affected by the length of the day. Birds reared under a continuous photoperiod spent less time feeding throughout the day than broilers raised under an 8L:16D photoperiod. Those findings agree with observations reported by Schwean-Lardner et al. (2012) who found that broilers reared under a 23L:1D spent less time at the feeder than broilers under shorter photoperiod length (14L, 17L, 20L). To compensate for the shorter day length available for feeding behaviour, birds might learn to eat in the dark. However, the amount of feed consumed at night when under photoperiods equal to or over 18L is minimal (Weaver and Siegel, 1968). Feeding behaviour at night is correlated to the length of the photoperiod, when broilers are reared under a 2L:22D or 21L:3D photoperiod, the feeding is higher at night relative to broilers given an 18L:6D photoperiod (Lewis et al., 2009). The increase in feeding in broilers under a 2L:22D photoperiod might be related to the absence of enough daytime to do all primary

maintenance activities, while an increase in feeding at night under a 21L:3D photoperiod could be correlated to the absence in diurnal rhythm. As reported by Schwean-Lardner et al. (2014) broilers reared under a 20L:4D or a 23L:1D photoperiod did not present diurnal rhythm for feeding behaviour at the age of 27 and 42 days.

The highest feeding before dark in birds reared under shorter photoperiods is related to the need to fill the crop for the long nights (Weaver and Siegel, 1968; Duve et al., 2011; Shynkaruk et al., 2019). Providing a day-night cycle for birds also alters morning feeding behaviour. A 16L:8D photoperiod increased broiler feeding activity in the morning but reduced the performance of other behaviours such, as stretching, compared to broilers reared under a 24L:0D photoperiod (Bayram and Özkan, 2010). Under a 16L:8D photoperiod, broilers increased pecking and foraging behaviour in the afternoon compared to those reared under a 24L:0D photoperiod (Bayram and Özkan, 2010). A 16L:8D lighting program also increased activity in broilers such as standing, walking, drinking, pecking, preening, and wing flapping while resting and sleeping behaviour decreased compared to broilers reared under a 24L:0D photoperiod (Bayram and Özkan, 2010). These effects of longer and shorter photoperiods on broiler behaviour agree with observations reported by Schwean-Lardner et al. (2012). These authors observed reduced standing, walking, running, litter pecking, preening and stretching behaviour in broilers reared under an NC (23L:1D) photoperiod than in broilers raised under shorter photoperiods (14L, 17L, 20L). A decrease in activities might increase locomotor problems. A 24L:0D photoperiod increased tibial dyschondroplasia and gait score in 35-day old broilers chicken (Møller et al., 1999), which could be indicative of poorer mobility.

Broilers at 48 days of age that were fearful (high levels of tonic immobility) and in pain (high levels of tibial dyschondroplasia and a high degree of lameness) performed less dustbathing than broilers that did not present the same locomotory and fear abnormalities (Vestergaard and Sanotra, 1999). Dustbathing is a behaviour performed when all the other needs have been met, therefore, it is categorized as a comfort behaviour (Duncan, 1998). Photoperiod length can impact the incidence of expression of comfort behaviours. For example, in 27-day old broilers, the percentage of time spent performing dustbathing behaviour decreased as photoperiod length increased up to 23L:1D (Schwean-Lardner et al., 2012). However, at 42 days of age for those broilers under the 23L:1D photoperiod the dustbathing behaviour was absent, but broilers reared under 14L:10D, 17L:7D, or 20L:4D still showed a reduction in dustbathing behaviour as photoperiod length increased (Schwean-Lardner et al., 2012). Other studies have different results.

Birds reared under a 16L:8D or 24L:0D photoperiod did not differ in dustbathing behaviour at 11 or 35 days old (Bayram and Özkan, 2010).

2.7 Welfare or stress indicators in poultry

There are many objective and subjective methods for measurements of stress and the evaluation of welfare in poultry. In the current thesis, three of these measures were used: the proportion of heterophils to lymphocytes, physical asymmetries of paired traits, and bird behaviour.

2.7.1 Heterophil to Lymphocyte ratio

Heterophil to lymphocyte ratio (H: L) is used as an index measure in poultry to evaluate levels of stress. This ratio can increase during stressful situations including environmental changes such as social stress (Gross and Siegel, 1983) or even lighting programs (Huth and Archer, 2015b). Increased levels of corticosterone, which occurs under stressful periods, influence this ratio (Scanes, 2016). A primary regulator of stress in birds is the hypothalamic pituitary adrenal axis (HPA) (Scanes, 2016). In a stressful situation, the production of corticotropin-releasing-hormone is stimulated, and glucocorticoids are released into the bloodstream to prepare the birds for the fight or flight reaction. High levels of glucocorticoids in the body can cause depression of the immune system and can increase the proportion of heterophils to lymphocytes (Gross and Siegel, 1983; Scanes, 2016).

2.7.2 Morphological asymmetry

2.7.2.1 Fluctuating asymmetry

Fluctuating asymmetry is the variability of the difference in size between the right and the left side of a bilateral symmetrical trait in an individual, caused by an environmental stressor.
The fluctuating asymmetry can occur due to severe environment disturbances such as food deprivation (Swaddle and Witter, 1994), high stocking density (Møller et al., 1995), polluted environment (Jentzsch et al., 2003) lighting schedule (Møller et al., 1999), bird selection for higher production (Nestor et al., 2000), or thermal stress (Grell, 1978; Parsons, 1992; Yalçin et al., 2001; Yalçin and Siegel, 2003). These stressors can cause physiological instability at molecular and cellular levels (Parsons, 1990). For example, genetic modification based on the selection of birds for fast growth, high production and higher body weight can be very demanding for the body and

increase relative fluctuating asymmetry (Nestor et al., 2000). Another example includes the impact of heat stress. Changes in the organism at a molecular and cellular level can be found for heat stress which is very common in poultry production. Heat stress can alter the concentration of electrolytes causing a destabilization of homeostasis and cellular malfunction (Han et al., 2010; Akbarian et al., 2016). All these reactions are very costly to the organism and are examples of how the organism can deviate energy from one function to another. This could potentially lead the organism to develop asymmetric features in symmetric parts of the body due to developmental instability caused by overwhelming the energy requirement in the body with reactions that are abnormal, thus reducing the availability of energy for normal body development and growth.

2.7.2.2 Directional Asymmetry

When directional asymmetry occurs, one side of the bilateral trait develops larger than the other side, and the occurrences are not random as all members of the population develop asymmetry. Directional asymmetry is thought to be caused by genetic origin (Van, 1962; Leamy et al., 2000). An example of directional asymmetry of genetic origin is homozygosis (Leamy et al., 2000). For example, crossing a wild and a domestic strain of mice and cross crossing the progenies back with the domestic strain increased the mandibula phenotypic differences in the second generation of mice (Leamy et al., 2000). Additionally, some speculate that behavioural lateralization also triggers directional asymmetry (Galatius, 2006). Behaviour lateralization is the tendency to continually choose one side over the other during the performance of a task (Rogers and Krebs, 1996; Galatius, 2006). In the case of behavioural lateralization, it was noted with chicks exposed to light on the last three days of incubation, where at this stage the right eye is exposed to the light. These chicks post-hatch tended to use the right eye over the left during a pecking choice test (Rogers and Krebs, 1996). Another example of directional asymmetry is the tendency in a population of white-beaked dolphins to use the right flippers more than the left ones, leading to a larger and broader humerus and larger radius on the right side than the left side (Galatius, 2006).

2.7.2.3 Antisymmetry

Antisymmetry is also related to genetic factors, but it differs from directional asymmetry because the organism develops a tendency to grow one side of a bilateral trait towards one direction more than the other by chance. This trait occurs randomly within a population (Van, 1962; Graham

et al., 1993). An example of an antisymmetric feature is lobster claws, where one claw is over developed. In that case, the tendency to develop one side larger than the other is caused by neural pathways triggered by exercising the claw at a specific stage of the development (Govind and Pearce, 1992). The lobster is stimulated to exercise the claws when substrates are available, however, if substrates are available but the sensory mechanoreceptors and tendons fail to function, the lobster fails to respond to the presence of a substrate and does not exercise one claw more than the other, leading to symmetrical development of the right and left claws (Govind and Pearce, 1992).

2.7.2.4 Composite Asymmetry

Composite asymmetry is the total of all asymmetries combined. A significant number of articles published to date present the fluctuating asymmetry results as a combination of all asymmetries, because of the difficulties in differentiating each asymmetry at a low level (Archer et al., 2009; Archer and Mench, 2013; Archer and Mench, 2014 ab).

2.7.3 Behavioural expression

2.7.3.1 Junglefowl Behaviour

Red Junglefowl are the ancestral species of the domestic chicken. As in many wild species, domestic birds such as the chicken perform similar behavioural patterns as do the wild conspecifics, although because of different circumstances (commercial chickens do not need to move far to find food sources for example), the time budgets of the behaviour output might differ (Ericsson et al., 2014). Feral Red Junglefowl (*Gallus Gallus*) birds spent most of their time performing walking, pecking, vigilant behaviour and ground scratching and allocate a lower amount of time for preening, sitting, and roosting, while the least amount of time is spent standing (Dawkins, 1989).

Behaviour is often used as an assessment tool for understanding poultry welfare. Some of the most common behaviours that can be helpful to evaluate the state of an animal include exploratory (object pecking, foraging, gentle feather pecking), aggressive (aggressive feather pecking), nutritive (at the feeder, at the drinker), active (walking, standing, running), resting (inactive), and comfort behaviours (dustbathing, preening). Literature regarding the behaviour of chickens from in-ovo lighting wavelength or day length is scarce, since the majority of literature

exploring the effects of light (wavelength or day length) on behaviour is based on live birds reared under the specific wavelength or day length.

2.7.3.2 Exploratory behaviour

Object pecking. Object pecking is an action of repeatedly pecking at things other than food. When performed repeatedly without any apparent function, this can be considered a stereotypical behaviour (review by Mellor et al., 2018). However, pecking at anything that contrasts in colour to the environment at a young age is a form of learning to aid in distinguishing objects (Rogers and Krebs, 1996). Light wavelength during rearing may impact pecking behaviour (Huber-Eicher et al., 2013). These authors observed brown hens from 18 to 22 weeks of age under green (520 nm, LED) lighting environment, and found that they performed more pecking behaviour than brown hens reared under red (640 nm) or white LED lighting environments. However, Prayitno et al. (1997a) observed higher pecking behaviour in broilers reared from 7 to 28 days under red lighting wavelength than broilers reared under green or blue lighting wavelengths and intermediate pecking behaviour when those birds were reared under the white wavelength. This suggests there may be species differences.

Foraging. Foraging behaviour consists of the chicken scratching the floor and moving the feet backwards and forward, then pecking at the scratched area (Appleby et al., 2004). Foraging is a behaviour that precedes feeding; however, if a cost is imposed to perform foraging, the chicken might not perform this behaviour (Bubier, 1996). Therefore, the expression of foraging behaviour might be positive and indicate that all basic needs are met. Light wavelength appears to affect the performance of this behaviour. Brown hens reared under green (520 nm, LED) light from the age of 18 to 20 weeks spent more time performing foraging behaviour than hens reared under red (640 nm, LED) light, whereas hens reared under white (LED) lights did not differ in foraging behaviour from hens raised under green or red lights (Huber-Eicher et al., 2013).

Gentle feather pecking. Gentle feather pecking occurs when the bird gently pecks the other bird as if it was performing allopreening, and the pecks are directed to specific parts of the body such as the frontal area (Vestergaard et al., 1993). Gentle feather pecking is an exploratory, social type of behaviour and can already be observed in one-day-old chicks (Riedstra and Groothuis, 2002).

Chickens will dedicate time in this behaviour once all the other needs are met, but the incidence might decrease if an effort to perform the behaviour is required (Bubier, 1996). An increase in gentle feather pecking post-expression of specific behaviour's such as dustbathing was suggested, as a result of particles left on the feathers from dustbathing, motivating other birds to gently peck counterpart's feathers (Savory, 1995). The light wavelength can alter this behavioural expression. Brown laying hens exposed to green (520 nm, LED) light from 18 to 22 weeks of age spent more time gently pecking cage mates than chicks raised under white or red (640 nm, LED) light (Huber-Eicher et al., 2013)

2.7.3.3 Aggressive behaviour

Aggressive feather pecking. Aggressive feather pecking is a redirected behaviour motivated at least in part by the bird`s state of frustration when unable to perform behaviour such as foraging (Huber-Eicher and Wechsler, 1997; Dixon et al., 2008). It can also be motivated by the process of hierarchy establishment or maintenance (Daigle et al., 2015). During aggressive feather pecking, the bird aggressively pecks at a cage mate, possibly resulting in damaged feathers (Huber-Eicher and Wechsler, 1997) and injury to the skin (Klein et al., 2000). The occurrences of aggressive feather pecking increase as chicks' age (Huber-Eicher and Wechsler, 1997), and lighting conditions can alter these levels. Brown hens exposed to white (LED) light from week 18 to 22 of age performed more vigorous pecks against conspecifics than hens reared under red (640 nm, LED) lights. However, hens exposed to green (520 nm, LED) light over the same period do not differ in aggressive pecking behaviour from the hens in the white or red environment (Huber-Eicher et al., 2013). These results are not consistent in all studies. Prayitno et al. (1997a) reported that broilers reared under red light from 7 to 28 days of age performed more aggressive interactions than broilers reared under white, blue, or green (incandescent filtered) light. The difference in results between Hubber-Eicher et al. (2013) and Prayitno et al. (1997a) might be related to bird genotype, as the contrast of the brown and white feathers in relation to the red lighting environment might be perceived differently by the birds and stimulate pecking when the contrast in colour is high.

2.7.3.4 Nutritive behaviours

Feeding behaviour. Feeding behaviour is stimulated by physiological processes leading to acquiring energy for maintenance, growth and reproduction (De Ruiter, 2015). Feeding behaviour

can be affected by many factors, including bird genotype (Savory, 1980), reproductive state (Savory, 1977), age (Asahida and Mimura, 1972), type of diet (Fujita, 1973), photoperiod (Schwean-Lardner et al., 2014) and light wavelength (Sultana et al., 2013ab). The photoperiod length can affect feeding behaviour rhythm in chicks. For example, when the broiler is reared under a continuous lighting schedule, the peak in feeding behaviour occurs in the morning, while when raised under dark and light cycle the peak occurs in the morning and before the dark period (Weaver and Siegel, 1968). These findings are supported by Schwean-Lardner et al. (2014), as the authors noted an increase in broiler feeding activity in the morning and before dark when the birds were reared under a photoperiod of either 14L:10D or 17L:7D. However, when those birds were under a near continuous or a 20L:4D lighting photoperiod, a diurnal behaviour rhythm for feeding activity was lacking at 27 and 42 days of age (Schwean-Lardner et al., 2014).

Light wavelength also affects feeding behaviour. Brown layer hens observed from 18 to 22 weeks of age under the red (640 nm) or white lighting spent more time at the feeder, and the least while reared under a green (520 nm) lighting (Huber-Eicher et al., 2013). For broiler birds, feeding behaviour seems to be affected differently depending on gender. Male birds spent the highest amount of time feeding when reared under green (550 nm) or blue (450 nm) lights, while female birds spent the highest percentage of time feeding when reared under white or red (650 nm) lighting colours (Prayitno et al., 1997b).

Drinking behaviour. Drinking behaviour can differ depending on the type of drinker system used. It is an essential behaviour expressed even when a cost is imposed (Bubier, 1996). In open water situations (bell waterers for example), the bird approaches the water (open drinker), lowers its head and inserts the beak into the water. It then raises its head vertically to swallow the water (Ross and Hurnik, 1983). Drinking water from nipples consists of the chick raising their head in an angled vertical position and drinking from a droplet of water that shines from the nipple. As the chick ages, it learns that by applying pressure on the nipple, water is released. Chicks do not hatch with the ability to drink - they must learn (Hunt and Smith, 1967), and they do this by pecking surfaces that contrast in colour to the environment (Appleby et al., 2004). For that reason, open drinkers are supplemented on the first week post-hatch in commercial barns to facilitate chicks learning to drink.

However, it appears that wavelength does not affect drinking ability in adult birds. Rearing brown hens for 4 weeks under green (520 nm), red (640 nm), or white (LEDs) light did not result in alterations of drinking behaviour among hens in any of the lighting treatments (Huber-Eicher et al., 2013). This study does not mention however, if wavelength impacts the ability to drink at a very young age.

2.7.3.5 Active behaviours

Walking. Walking is a behaviour where the bird makes forward movements with one foot in front of the other (Hurnik et al., 1995). It is important to move the birds from place to place but can also be important in bone and muscle development. The time spent walking can be affected by many factors as well, including photoperiod (Schwean-Lardner et al., 2012, 2014), light intensity (Newberry et al., 1988), the colour of light (wavelength) (Prayitno et al., 1997a) and combinations of these and other factors (Prayitno et al., 1997b). Regarding light wavelength, broiler chicks at the 2nd and 3rd week of age spent more time performing walking behaviour when reared under white light and less time when reared under green (550 nm) light (Prayitno et al., 1997a). Ducks reared under blue (460 nm) light decreased walking activities compared to ducks under green (560 nm) and yellow (600 nm) light (Sultana et al., 2013b). On the other hand, Huber-Eicher et al. (2013) did not find a difference regarding walking behaviour in laying hens reared under red (640 nm) green (520 nm), or white (LED) light at 18 to 22 weeks of age.

Standing. Standing is a behaviour where the bird stands on its feet in a still position (Hurnik et al., 1995). Broiler chickens observed at the age of 7 to 28 days showed the highest percentage of time allocated to standing behaviour when reared in a blue (415 nm) light environment compared to broilers reared in a red (635 nm) light environment. A light cofactor might also exist as those birds performed most of the standing behaviour when raised under high-intensity blue light as compared to low and medium light intensity blue light (Prayitno et al., 1997b). However, Huber-Eicher et al. (2013) did not find differences in standing behaviour for brown layer chickens reared under red (640 nm), green (520 nm), or white lighting at weeks 18 to 22.

Running. A bird runs when it performs movements forward with one foot in front of the other at high speed (Hurnik et al., 1995). Running behaviour can be restricted in cages and when the

stocking density is high due to space restriction. However, as chicks post-hatch are housed in floor pens or brooding cages with more space than conventional laying cages, a short distance of running behaviour can still be performed. An animal can run for different reasons, including during playing behaviour which could indicate good welfare. For example, piglets increased running when excited due to a novelty in the rearing environment (Wood-Gush and Vestergaard, 1991; Reimert et al., 2013). Running could also occur for other reasons such as a fear response to escape from fights or predators (Burghardt, 2005).

2.7.3.6 Resting behaviour

Resting. Resting behaviour is an extended period of inactivity, where movements are stopped or reduced to lower energy expenditure and prevent energy depletion or regain strength (Hurnik et al., 1995). The lighting wavelengths can affect energy requirements during resting. Chickens reared under green lighting spent more energy while resting during the light period than chickens under red, blue, or white lighting, which was measured through oxygen consumption and carbon dioxide production (Kim et al., 2014). Prayitno et al. (1997a) observed broiler chickens from day 7 to 28 under incandescent light with wavelength filters that output various spectrums of light. The authors reported that the chicken spent more time in a sleeping state when raised under red light or white light, those raised under green light spent more time sitting, while birds reared under blue light spent more time in a dozing state (‘‘neck reclining with the eyes half closed’’). Sultana et al. (2013b) reported that when ducks were raised under various wavelengths of light for 42 days, most of the ducks performing inactive behaviour were the ones raised under blue light as compared to those reared under white, green, or yellow lights (fluorescent lamps). However, Huber-Eicher et al. (2013) did not observe changes in resting behaviour among brown hens reared from week 18 to 22 under green (520 nm), red (640 nm), or white LEDs lighting.

2.7.3.7 Comfort behaviours

Dustbathing. Dustbathing is a behaviour performed by the bird to aid in the maintenance of feathers conditions and to reduce parasite infestation (Borchelt and Duncan, 1973). It can be expressed when the birds are provided with a proper substrate as litter (Colson et al., 2007), but also can occur in more barren environments such as cages (sham-dustbathing). However, birds in cages have a lower incidence of this behaviour due to space and substrate availability (Appleby et

al., 1993). Lighting wavelength during rearing resulted in no effects on dustbathing behaviour of brown hens over week 18 to 22 when exposed to green (520 nm), red (640 nm), or white (LED) lights (Huber-Eicher et al., 2013).

Preening. Preening is a self-maintenance behaviour performed with the beak to spread oil from the uropygial gland into the feathers (Sandilands et al., 2004). It is used to aid in removing parasites from the body (Ostfeld and Lewis, 1999). Additionally, preening behaviour could be also a signal of frustration (Duncan and Wood-Gush, 1972). Preening is a behaviour which the bird would perform even when an effort is required (Bubier, 1996). Indeed, a decrease in preening behaviour might indicate poor welfare, as the essential behaviour needs are not met. However, lighting wavelength does not seem to affect this behaviour as brown hens did not differ in preening behaviour when exposed to green (520 nm), red (640 nm), or white lighting at the age of 18 to 22 weeks (Huber-Eicher et al., 2013).

2.8 Conclusion

Most of the in-ovo wavelength studies focus only on production traits; the information regarding behaviour post hatch is very limited. In addition to wavelength, a study using graded levels of daylength in-ovo has not been examined. Work published to date often compared two photoperiods only. Graded photoperiod helps to understand the variability of results between studies. Brood lighting photoperiod length can affect a bird`s behaviour with consequences on production and health variables. However it has not been evaluated in chicks hatched from in-ovo lighting. Finally, it is of interest to understand if various genotypes react post-hatch differently due to exposure of various light wavelength during incubation. This is particularly true of in-ovo lighting, as various shell characteristics, such as colour, could certainly affect the amount of light penetrating through to the embryo. In conclusion, the movement towards using light or light/dark cycles in commercial incubation systems appears to be growing. Therefore, understanding the characteristics of that light, as well as the effect of lighting programs post-hatch on various genotypes, could improve both production and welfare traits in commercial poultry species.

2.9 Objectives

2.9.1 Experiment I

The objective of the first experiment was to determine the behaviours of Lohmann White and Brown pullets hatched from various in-ovo light (wavelengths) incubation and brooded under either an intermittent (INT) or an near continuous (NC) lighting schedule.

2.9.2 Experiment II

The second study was designed to investigate the impact of a range of photoperiod lengths using red (644 nm, LED) lighting systems on White Leghorn fertile eggs during incubation. Measures included hatch traits, growth, behaviour and presence or absence of behavioural rhythm over the photophase post-hatch.

2.10 Hypothesis

2.10.1 Experiment I

1. Behaviour output from white in-ovo lighting will differ from pullets incubated under red, blue, or dark conditions because of the stimulus of light on functional lateralization of the forebrain (Rogers and Krebs, 1996).
2. The provision of white light will improve adaptation of the hatchlings to a new environment, resulting in higher expression of comfort behaviours. This will result from a decrease in fear response and a reduction of stress levels in a new environment (Archer and Mench, 2013; Archer and Mench, 2014a), and possibly as a result of brain lateralization (Rogers and Krebs, 1996).
3. Lohmann Brown pullets will be less affected by incubation lighting than Lohmann White birds. This is due to the pigmentation of brown shelled eggs and its effects on light transmission through the eggshell (Maurer et al., 2015 and Shafey et al., 2002, 2004, 2005).

2.10.2 Experiment II

1. A reduction in incubation time possibly related to the increase in metabolism in the diurnal phase (Copper et al., 2011) speeding up embryo development (Isakson et al., 1970; Lauber., 1975; Copper et al., 2011).
2. An increase in chick quality at hatch, as it is possible that physiological rhythm exists under day/night in-ovo photoperiod that do not occur under constant dark. The photoperiod length can affect the rhythm of melatonin hormone syntheses (Zawilska et al., 2006) and the melatonin hormone can have beneficial effects on the immune system.
3. Behavioural changes, including decreased aggressive and increased comfort behavioural expression. These changes may be a result of a reduction in fear responses, resulting in a decrease in stress levels. (Archer and Mench, 2013; Archer and Mench, 2014a).
4. All L:D incubated flocks (6L:18D, 12L:12D and 18L:6D) will show the presence of a diurnal behaviour rhythm over the photophase while a behaviour rhythm over the day for pullets from 0L:24D will be lacking.

3.0 Chapter 3: Effects of light wavelength during incubation on early life behavioural expression of egg production chicks

This thesis focuses on the impact that lighting during incubation has on embryonic development, chick health, and bird behaviour. This chapter in particular focuses on the effects of incubating Lohmann White and Lohmann Brown fertile eggs under monochromatic wavelengths of light that provide red, white, or blue light in comparison to incubation without light (dark), on early behavioural output over the photophase of hatchlings reared under a NC and an INT lighting program. This project was conducted in co-operation with the poultry group at Dalhousie University, where another MSc. student focused on the production and health impacts from these incubation systems

3.1 Abstract

This study investigated the impact of in-ovo lighting wavelength on early behaviour of two Leghorn strains reared under an Intermittent (INT) or a Near Continuous (NC) photoperiod. A total of 144 Lohmann LSL (LSL) and 144 Lohmann Brown (LB) pullets hatched from one of four treatments: white, blue or red lighting under a 12L(Light):12D (Dark) photoperiod or a dark incubation (control), were reared under either an INT photoperiod from day 1 to 3 (17L:2D:0.5L:2D:0.5L:2D) and day 4 (16L:3D:0.5L:2D:0.5L:2D) or a NC photoperiod 23L:1D from day 0 to 3 and day 4 a 20L:4D. Behaviour was video recorded on days 0, 2 and 4, and the behaviour over the photophase data analysed through a 20 minutes scan sampling technique, followed by a statistical analyses using a nested factorial design on Glimmix SAS 9.4. Significance was declared when $P<0.05$ and a trend when $0.10>P>0.05$. Post-hatch, no interactions were observed between incubation lighting wavelengths, pullet genotypes and brooding lighting. The chicks hatched from dark incubation tended to spend a higher percentage of time standing ($P=0.059$) than chicks from incubators provided lighting. Bird strain affected the behaviour output, as LSL pullets spent a higher percentage of time in nutritive behaviours; at the drinker ($P=0.012$) on day 0 and day 2 ($P=0.031$), at the feeder on day 2 ($P<0.001$) and 4 ($P=0.005$) and lower percentage of time expressing walking on day 0 ($P=0.001$), and increased resting on day 0 ($P<0.001$) than LB pullets. The brooding lighting also impacted the pullet's behaviour, pullets reared under an INT brooding photoperiod spent more time at the feeder on day 0 ($P=0.036$) and 2 ($P=0.022$) and performing more walking behaviour on day 2 ($P=0.039$) and 4 ($P=0.01$) than those from NC photoperiod. Overall, these results indicate that in-ovo light wavelength does not impact hatchlings behaviour post-hatch. However, post-hatch behaviour is affected by bird strain and brood lighting program. Additionally, no benefit or detrimental effects were observed on the behaviour of Lohmann White and Brown chicks hatched from a red, white, or blue in-ovo lighting wavelength incubations. However, an INT photoperiod during brooding increased chicks mobility behaviours, which might be beneficial in encouraging the chicks to find feed and water on the first days post-hatch.

Keywords: In-ovo lighting, wavelength, brooding photoperiod, behaviour, Leghorn.

3.2 Introduction

Animal behaviour can be influenced by internal factors, such as age (Bizeray et al., 2000), strain (Lewis and Hurnik, 1990), hormones and external environmental factors including temperature, food availability, stocking density and lighting program (Blokhuis, 1986). Artificial lighting programs are widely used in poultry production to control a birds' growth and reproduction. The effects of the lighting program can vary depending on the source of light, wavelength, output intensity and daytime length. Birds have a distinct capability to perceive light wavelengths (Prescott and Wathes, 1999; Lewis and Morris, 2006) due to their diversity in photoreceptors (Maier, 1992; Osorio et al., 1999).

The light can also penetrate the eggshell and affect the developing embryo. The amount of light that gets through the eggshell can be affected by its pigmentation (Shafey et al., 2004; Maurer et al., 2015) and thickness (Maurer et al., 2015). Although the transmission of light to the embryo is low (Shafey et al., 2002), it can still impact the embryo during incubation. Development of birds during incubation utilizing light programs can be affected by white (Copper et al., 2011) or monochromatic colours (Ghatepande et al., 1995). The white light is a mixture of all the colours commonly used in poultry units because of previously available sources such as incandescent light bulbs. However, light emitting diode (LED) lights have now allowed the use of specific monochromatic colours in place of white light. Light during the last three days of incubation can affect lateralization of the brain, affecting a bird's cognitive performance and selective pecking when exposed to specific wavelengths during the hatcher stage (Rogers and Krebs, 1996). White light during incubation reduces chicks response to fear post-hatch compared to incubation under darkness (Özkan et al., 2012b; Archer and Mench, 2013). But it also increases competitive behaviour among counterparts compared to chicks from dark incubation (Rogers and Workman, 1989). White light increases feeding behaviour in broiler chicks during the first few hours of the lights on period compared to chicks from dark incubation (Archer et al., 2009).

Despite some evidence of in-ovo light effects on birds behaviour post-hatch, the impact of light wavelength on multiple behaviours post-hatch is limited, and there is no information about the possible interaction of in-ovo lighting wavelength and photoperiods during brooding in both brown and white feathered birds. Therefore, the objective of this study was to investigate the impact of three different lighting wavelengths used during incubation and compared to a incubation

without light (dark) on the behavioural output of the hatchlings of two egg production strains reared under NC and INT lighting programs. The general experimental hypothesis was that wavelength and strain impact on the time the birds spend performing specific behaviours which may be related to the eggshell pigmentation and capacity of light to reach the embryo during incubation:

1. Behaviour output from white in-ovo lighting will differ from pullets incubated under red, blue, or dark conditions because of the stimulus of light on functional lateralization of the forebrain (Rogers and Krebs, 1996).
2. The provision of white light will improve adaptation of the hatchlings to a new environment, resulting in higher expression of comfort behaviours. This will result from a decrease in fear response and a reduction of stress levels in a new environment (Archer and Mench, 2013; Archer and Mench, 2014a), and possibly as a result of brain lateralization (Rogers and Krebs, 1996).
3. Lohmann Brown pullets will be less affected by incubation lighting than Lohmann White birds. This is due to the pigmentation of brown shelled eggs and its effects on light transmission through the eggshell (Maurer et al., 2015 and Shafey et al., 2002, 2004, 2005).

3.3 Materials and methods

The research and experimental procedures were approved by the Dalhousie University Animal Care and Use Committee (ACUC) on the Truro Campus and the University of Saskatchewan's Animal Care Committee in accordance with the Canadian Council of Animal Care guidelines (2009). This portion of the experiment included the early behavioural responses of hatched pullets as affected by incubation wavelength lighting, genotype and early brooding photoperiod. All production data, including incubation mortality, pullet growth and productivity traits, were analyzed by the Dalhousie group and will form a second thesis.

This experiment was designed using a nested factorial structure. Eight Master® G09 incubators located at the Atlantic Poultry Research Centre in Truro, Nova Scotia were used. Six of the eight incubators were equipped with four light emitting diodes (LEDs) light strips each, with the lights attached to the left side of the incubator wall in a vertical position.

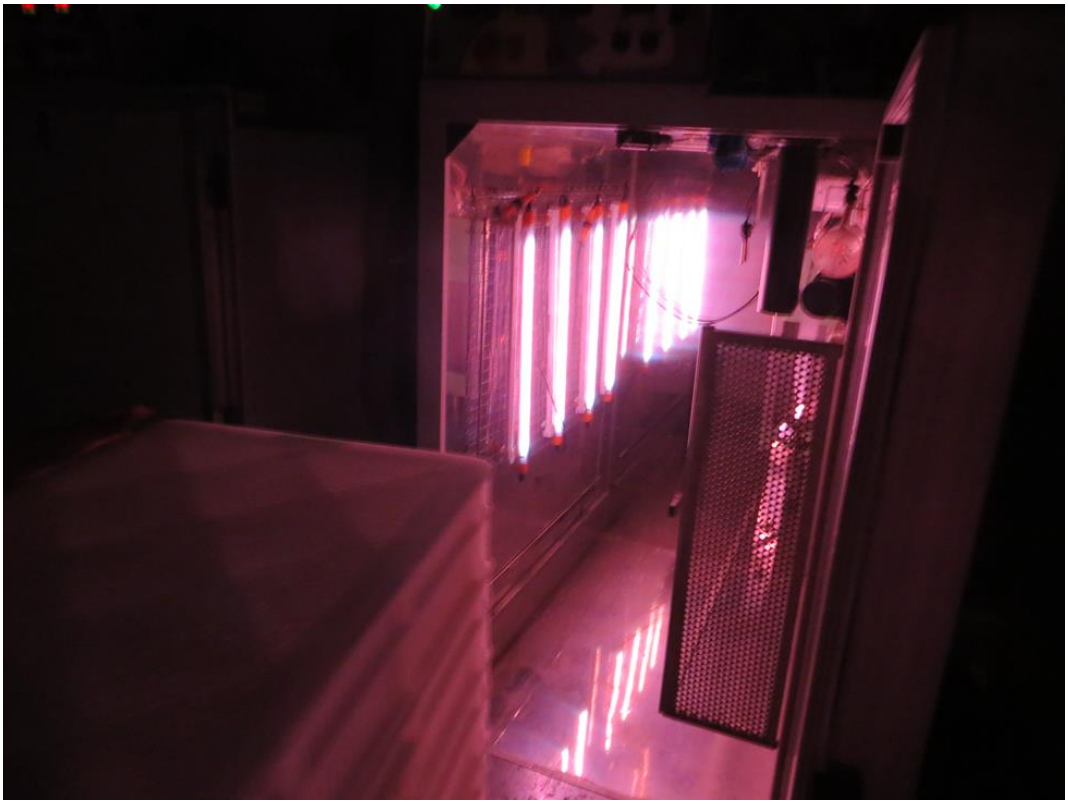

Figure 3.1. LED lights outfitted into a Master G09 incubator.

Two incubators were outfitted with white lights (Canarm®,4100K), two incubators with blue (AgriShift® TLP, 120 V) and two incubators with red (LED) light strips (AgriShift® TLL, 120 V). The photoperiod throughout the incubation for white, blue and red lighting treatments was 12 hours light on and twelve hours light off (12L:12D) with 220-260 lux intensity at egg level (measured using light data loggers (OMYL-M62, Omega® Engineering, Quebec, Canada)). The remaining two incubators were left without lights for a control treatment (0L:24D).

A total of 640 Lohmann LSL Lite (LSL) and 640 Lohmann Brown (LB) fertile eggs, originating from parent stocks at 48 weeks of age, were purchased from a commercial hatchery. The eggs were randomly assigned to one of the eight incubators (160 eggs per incubator). The temperature of the incubator was maintained at 37.5°C and relative humidity at 85.0% from time of set until 18 days of incubation. Egg trays were turned at a 90-degree arc every 15 minutes from 0 to 18 days of incubation as recommended by the incubator's manufacturer. Eggs were candled at 18 days of incubation, and infertile eggs and eggs with dead embryos were removed before transferring to individual labelled hatch boxes and placed inside the hatch baskets. The incubator temperature was dropped to 37.0°C and relative humidity increased to 94.5%. At 512 hours post

set, the relative humidity was reduced to 55.0%. After hatch, chicks were feather sexed. The female chicks 144 Lohmann LSL Lite and 144 Lohmann Brown were housed in groups of 6 birds per in-ovo lighting treatment (each cage measuring 60 x 48 cm), with 48 cages in total in 6 separate rooms (12.57 m^2 each room). Pullets were reared under one of two photoperiods, with three rooms per brooding light treatment: a near-continuous lighting program (NC) (based on common husbandry practices used on farms which received a photoperiod of 23L:1D from day 0 to 3 and at day 4 reduced to 20L:4D), or intermittent lighting program (INT) (chosen to stimulate chicks' early feed consumption), chicks received 18L:6D (17L:2D:0.5L:2D:0.5L:2D) for day 0 to 3, then 17L:7D (16L:3D:0.5L:2D:0.5L:2D), on day 4. Illumination for all the rooms was produced by blue (LED) light strips (Once Innovations, Plymouth, MN – USA). The light strips were installed on the top front of the cages above the feeders. The light intensity ranged from 20 to 40 lux (from front to back of cage) at bird level post-hatch from day 0 to 3, and 20 - 30 lux from day 4.

The pullets were provided ad libitum water through nipple drinkers (2 nipples per cage) and ad libitum feed (Table 3.1) in metal troughs (56.52 cm length x 12.7 cm width) installed at the front of each cage. Supplemental water in ice cube trays and supplemental feed on paper was provided for the first four days. Environmental temperature was adjusted as recommended by the breeder's growers management guide (Tierzucht, nd).

3.3.1 Data collection

The behaviour of the pullets during the early brooding period was video recorded continuously (24 h periods) for days 0, 2 and 4 post-hatch with the use of 32 infrared video cameras (Speco Technologies® 4-Channel 4MP HD-TVI DVR, Amityville NY-USA) (one camera per two cages). The cameras were placed above the cages, supported by a metal stick positioned to capture a 360° view of the birds. One camera recorded two cages. After recording, the recordings were downloaded onto eight storage drives (WD My Passport™ Ultra 259D USB 2TB Portable Device - San Jose, CA) at the end of the recording period for further viewing and analyses.

3.3.2 Behaviour analyses

The behaviour data was video recorded in 24 h periods, however only the photophase data were analyzed. The behaviour data from days 0, 2 and 4 during the photophase were assessed using scan sampling techniques at 20 min intervals. This allowed the calculation of the percentage of time spent performing behaviours outlined in a pre-defined ethogram (Table 3.2). If a behaviour

could not be determined on a still screen, the video was reversed for 3 seconds then allowed to run so the activity could be identified. The behaviour output was determined by the averaged data for the photophase period (daily percentage of behaviour over time) analyzed from the 20 minutes scan samples. The presence or absence of a diurnal rhythm over the photophase in specific behaviours was determined from all representative data from the 20 minutes scan samples, and the determination of a presence or absence of a diurnal rhythm analyzed through an ANOVA followed by a regression analysis.

3.3.3 Statistical analyses

The statistical software SAS 9.4 was used to analyze the behaviour data. Data normality was checked using the univariate procedure (Shapiro Wilk test) prior to analyses. The behaviour output data were analyzed using a nested factorial structure. Strain, in-ovo wavelength and brood lighting were set as fixed effects, and room within brood lighting as a random effect, the statistical analyses were performed with a 3 room (experimental unit) replications per brood lighting treatment. Proc Glimmix, using a negative binomial distribution, link log and the degrees of freedom obtained from a Contain method was used to determine significance of the main effects and their interactions. Significance was declared when $P<0.05$ and a trend was noted when $0.10>P>0.05$.

3.4 Results

There were no interaction between in-ovo lighting wavelength, pullet genotype, nor brooding lighting program for the data collected.

Behaviour

Percent of time spent walking

In-ovo lighting wavelength treatment had no impact on the percentage of time pullets spent walking at day 0, 2 or 4 (Tables 3.3 to 3.5) post-hatch. At 0 day of age, LB pullets spent more time walking ($P=0.001$, Table 3.6) than did LSL pullets, but no differences were noted past that time. Brooding lighting program had no effect on the percentage of time pullets spent walking at 0 day of age (Table 3.9), but at 2 ($P=0.039$, Table 3.10) and 4 ($P=0.041$, Table 3.11) days of age, pullets

reared under an INT programs walked a greater percentage of time than those reared on the NC program.

Percentage of time spent running

In-ovo lighting program (Table 3.3-3.5) did not impact running behaviour at days 0, 2 and 4. Genotype did affect the percentage of time pullets spent running on days 0 and 4 only, as higher values noted for the LB pullets for day 0 (P=0.003, Table 3.6) and for day 4 (P=0.040, Table 3.8). Brooding lighting program (Table 3.9, 3.10, or 3.11) did not impact the percentage of time running in pullets for days 0, 2 or 4.

Percentage of time spent standing

In-ovo lighting wavelength treatment had little impact on the percent of time spent standing, with only a tendency noted at day 0 (P=0.059, Table 3.3), where pullets from dark incubation showed a higher percentage of time standing than pullets from white, blue, or red incubation. No effects were noted on days 2 (Table 3.4) and 4 (Table 3.5). Bird genotype affected the percentage of time pullets spent standing (P<0.001, Table 3.6) on day 0 post-hatch, LSL pullets spent a higher percentage of time standing compared to LB pullets. No genotype influences were found for standing behaviour on day 2 (Table 3.7) and day 4 (Table 3.8). Brooding photoperiod showed a trend for a greater percentage of time spent standing on day 0 post-hatch (P=0.093, Table 3.9) when pullets from INT photoperiod stood more. On day 2 and 4 there were no differences in standing behaviour (Table 3.10-3.11).

Percentage of time spent resting

Resting behaviour post-hatch was not affected by incubation wavelength in any of the observation periods (Tables 3.3, 3.4 and 3.5). Resting behaviour was not affected by pullet genotype at day 0 and 4, but at day 2 (P<0.001, Table 3.7), LB pullets rested more than LSL pullets. Brooding lighting schedule consistently affected the percentage of time pullets spend resting for days 0 (P=0.005, Table 3.9) , 2 (P<0.001, Table 3.10) and 4 (P=0.004, Table 3.11) post-hatch, with pullets from NC brooding photoperiod spending a higher percentage of their time resting compared to pullets from the INT program.

Percentage of time spent at the drinker

In-ovo lighting wavelength did not affect time pullets spent at the drinker at days 0, 2 and 4 (Table 3.3-3.5). The percentage of time spent at the drinker was affected by pullet genotype at day 0 (P=0.012, Table 3.6), day 2 (P=0.001, Table 3.7) and showed a trend on day 4 (P=0.060,

Table 3.8) with LSL pullets spending more time at the drinker than LB pullets. Brooding lighting program did not affect the time pullets spent at the drinker on day 0, 2 and 4 (Table 3.10).

Percentage of time spent at the feeder

In-ovo lighting wavelength did not affect the percentage of time pullets spent at the feeder on days 0, 2 and 4 post-hatch. The percentage of time pullets spent at the feeder was not affected by pullet`s strain at day 0 (Table 3.6), but at day 2 (P<0.001, Table 3.7) and 4 (P=0.005, Table 3.8), LSL pullets spent a greater percentage of time at the feeder than did LB pullets.

Pullets from INT brooding photoperiod spent a higher percentage of the photophase at the feeder at day 0 (P=0.036, Table 3.9), day 2 (P=0.022, Table 3.10); and at day 4 a trend (P=0.052, Table 3.11) only was found.

Percentage of time spent preening

In-ovo lighting wavelength did not affect the percentage of time pullets spent performing preening behaviour on day 0, 2 and 4 (Tables 3.3, 3.4 and 3.5). The percentage of time spent performing preening behaviour was not affected by pullets genotype at days 0, and day 2, but showed a tendency on day 4 (P=0.065, Table 3.8), LB preened for a greater period than LSL pullets. No impact of brooding lighting program was noted at days 0, 2, and 4 (Tables 3.9, 3.10 and 3.11).

Percentage of time spent in exploratory behaviours

Incubation lighting treatment did not affect exploratory behaviours at day 0, 2 and 4 which include gentle feather pecking, ground scratching, object pecking, and ground pecking while sitting. Exploratory behaviour tended to occur for a greater percentage of time at day 0 (P=0.074, Table 3.6), with LSL pullets being more active. At day 2 and 4 no effects were observed. Brood photoperiod did not have any impact on exploratory behaviour in any day.

Percentage of time spent in comfort behaviours

Comfort behaviours which included stretching, dustbathing and wing flapping was not affected by in-ovo lighting wavelength, the bird strain or brood lighting post-hatch for any of the observed days.

Percentage of time spent in low incidence behaviours

Low incidence behaviour is a category that includes all low-performance behaviours such as unknown, expelling excrements, head shaking, beak wiping, and vigorous feather pecking observed at day 0, 2 and 4. No effects of in-ovo lighting wavelength, bird strain or brooding lighting program were found.

3.5 Discussion

Behaviour can be a good indicator of a birds' welfare in their environment (Broom, 1986). Expression of comfort behaviours, including dustbathing, preening, wing flapping and stretching might indicate that the basic needs of the birds have been met (Bubier, 1996; Albentosa and Cooper, 2004). As bird's motivation to perform a behaviour might be affected due to environment space availability (Albentosa and Cooper, 2004), photoperiod (Schwean-Lardner et al., 2012, 2014) or even effort required to perform the behaviour (Bubier, 1996). The birds might reduce comfort behaviour expressions (luxury behaviours), to favour behaviours with a more critical function such as essential behaviours or so-called need behaviours that are behaviours necessary for survival of the species (Dawkins, 1983; Hughes and Duncan, 1988; Bubier, 1996). However, comfort behaviour can also have a more practical function, as an example, dustbathing and preening, which are behaviours performed to help with feather maintenance, have also been indicated as essential behaviours (Weeks and Nicol, 2006).

Preening behaviour could also be a signal of frustration (Duncan and Wood-Gush, 1972). The current study hypothesized that light wavelengths during incubation of brown and white feathered egg production chicks would affect the early behaviour expression differently, due to eggshell colours and light wavelength factors. Further, the hatchlings would be more adaptable to the new environment independent of brooding day length, therefore, presenting a higher expression of comfort behaviours and lower aggressive responses than those hatchlings from dark incubation.

Previous research showed that light wavelength could be a factor that affects the behaviour of live birds due to the bird`s sensitivity to specific wavelengths (Prescott and Wathes, 1999). In the current study, the fertile brown and white shelled eggs were photostimulated with the use of red, white, or blue light during incubation. Although temperature in this work was not tested, other works indicate that some wavelengths may alter incubator temperature. For example, Xujie et al. (unpublished data) noted that red light used during incubation reduced air cell temperature in broiler eggs, compared to white, blue, or dark incubation. Changes in the environment temperature during incubation can affect chick's behaviour (Bertin et al., 2018; Belnap et al., 2019), and exposure to low temperature during incubation has been associated with an increase in corticotropin-releasing factor receptor expression in the amygdala, resulting in increased fear response in chicks post-hatch (Bertin et al., 2018). An incubation environment temperature higher

than the standard (37.5˚C) can reduce bone development and affect mobility in quail chicks (Belnap et al., 2019). The addition of light, however, did not impact the behaviour of the pullets for the short-term post-hatch. Standing behaviour only tended to lower with the addition of any light wavelength applied to the incubator as compared to dark incubation, but this result was just noted for the initial 0-day period. This lack of different outcomes is similar to what is found in the literature focusing on post-hatch birds, where the percentage of time brown feathered laying hens spent performing standing behaviour was not affected when those birds were reared under red, white, green, or blue light for four weeks (age 18 - 22 weeks old) (Huber-Eicher et al., 2013). These findings may indicate the wavelength of light not being as important in influencing the bird's behaviour as factors such as bird strain and photoperiod. Despite indication in the literature that light used during incubation affects cognitive functions in chicken, causing changes in behaviour performance (Rogers and Krebs, 1996; Johnston et al., 1997) the current study did not observe in-ovo lighting wavelength effects on active and inactive behaviours post-hatch. These results concur with results Sultana et al. (2013c) reported on behaviour of live brown hens reared under red, white, or green light. Rearing brown hens under blue lighting, however, decreased the hen`s active behaviour (Sultana et al., 2013c).

In addition to the lack of impact of light wavelength during incubation on mobility behaviours, effects on early nutritive behaviour post-hatch were also absent. The effect of light wavelength on nutritive behaviour, however, is very clear in live birds. The results from the current in-ovo lighting wavelength study are similar to the findings reported by Campbell et al. (2015) on the nutritive behaviour of Pekin ducks reared under white, red, or blue lighting. These findings support Praytino et al.'s (1997a) results which showed an absence of light wavelengths (white, green, red, or blue) effects in nutritive behaviour of live broilers reared under those specific light wavelengths for four weeks. However, as mentioned earlier, contrasting results in the literature on light wavelength impact on live birds can be found. For example, Sultana et al. (2013a) noted that green, red, or altering red/yellow light increased broiler' feeding behaviour whereas Huber-Eicher et al. (2013) observed that green light decreased feeding behaviour of brown laying hens (Huber-Eicher et al., 2013). The contrasting results of light wavelength effects on birds' feeding behaviour observed in the cited literature might be due to the different lamps used to provide the illumination. Light sources can output different wavelength spectrum range in addition to the differences in illumination intensity. Hence the birds might perceive the light wavelength output differently

depending on the light source. This information is often not reported. Furthermore, the mechanisms behind the wavelength effects on live birds' behaviour are not very clear. However, the mechanisms behind light effects during incubation are thought to be caused by exposure of the embryo's right eye to light during the development of visual functions around the 17^{th} to 19^{th} day of incubation. This exposure is believed to increase the densities of glutamatergic and GABAergic neurotransmitter receptors in the forebrain (Johnston et al., 1997), which might be the mechanism behind the physical and functional asymmetry changes of the brain resulting in changes in cognitive functions and behaviour (Rogers and Krebs, 1996). However, the changes in brain asymmetry observed when the right eye is photostimulated (Rogers and Krebs, 1996; Johnston et al. 1997) are dependent on light wavelength, with the white light or an alternation of red and green light having the most significant impact on asymmetrical and functional changes in the brain (Rogers and Krebs, 1996).

Additionally, as stated earlier the transmission of light to the embryo during incubation can be affected by the pigmentation of the eggshell (Shafey et al., 2002), and this might suggest we should find an interaction between genotype and in-ovo lighting. However, the interaction was not present in the current study. But, within each chicken strain, behaviour differed significantly, as LSL pullets at an early age engaged more in nutritive related behaviours (feeding and drinking) and less in locomotor (walking, running) and resting behaviours than LB pullets. The differences in nutritive and active behaviours between strains might relate to genetic selection for growth and production. Although the percentage of time spent at the feeder and drinker at an early age for LSL pullets was higher, it does not mean they were consuming feed, as the observed behaviour was simply the percentage of time the birds were present at the feeder and drinker manipulating the feed or water independent of consumption. These findings agree with the results from another research group related to the same in-ovo wavelength study and birds. It showed that the LSL pullets had higher weight gain than LB pullets six hours post first access to feed, which supports our findings, indicating that those LSL pullets were more active feeding (Abeysinghe et al., data unpublished). Although LSL pullets showed the highest weight gain, LB pullets were heavier than LSL pullets at 7 days post-hatch (Abeysinghe et al., data unpublished). Drinking and feeding behaviour is essential for the maintenance of homeostasis and development of the organism. Maintenance behaviour such as feeding and drinking can be dependent on locomotor behaviour as the minimum mobility performed by the chicken is to reach feed and water (Lewis and Hurnik,

1990). In the current study, LSL pullets were less mobile (walking, running), but spent a higher percentage of time standing than LB pullets on day zero, LSL pullets spent more time resting on day two, and decreased mobility (running) again on day four. Other research has also reported genotype differences in behaviour. Kozak et al. (2016) observed that LSL leghorns performed more inactive behaviours than LB and Dekalb White strains, while nutritive behaviours were performed more by LB than the LSL strain. In the current study, the opposite effect was observed, as LSL pullets spent a higher percentage of time at the drinker and the feeder than LB pullets. Comfort and exploratory behaviours did not differ among pullet strains at an early age on the days observed under the environment studied, which might indicate the selection for production among the studied strains had no impact on those behaviours at an early age. However, at day two post-hatch LB pullets spent a greater percentage of time preening than LSL pullets. In the context of this study it is difficult to say if that represents a comfort/displacement behaviour or just an isolated event as it only occurred at day two during the observed period and no other comfort or aggression related behaviour differences were observed to support a conclusion about distress or comfort among the two chicken lines. However, at an older age differences in temperament have been found among white and brown laying hens (Duncan and Wood-Gush, 1972), which could be related to genetic selection and differences in hormonal production during the laying phase (Navara and Pinson, 2010).

The most significant impact on pullet behaviour post-hatch was from the brooding lighting program. Pullets reared under the INT lighting schedule spent a higher percentage of time at the feeder than birds from the NC lighting program. These findings agree with the studies by Schwean-Lardner et al. (2012, 2014) in broilers and by Vermette et al. (2016b) in turkeys, where the authors reported birds reared under a NC photoperiod spent a lower percentage of time at the feeder during the photophase period than birds reared under a shorter photoperiod. However, in the current study when determining the absolute time spent at the feeder, it was observed that the chicks under the NC lighting schedule on day 0 spent a similar amount of absolute time (239.29 minutes) at the feeder than birds under an INT lighting program (230.90 minutes). The same tendency was observed on day 2 and 4 when pullets under NC photoperiod spent 319.61 and 306.24 minutes at the feeder and those under INT lighting spent 307.37 and 301.72 minutes there. The percentage of time the birds spent at the drinker did not differ statistically, however, chicks under the NC lighting

schedule spent 39.88 (d0), 27.88 (d2), and 40.8 (d3) minutes at the drinker over the observed period while chicks under the INT spent 34.56 (d0), 31.10 (d2), and 36.82 (4) minutes at the drinker.

The program involving darkness also impacted other activity behaviours. An INT lighting program post-hatch increased the percentage of time pullets spent walking and standing, reducing the percentage of time spent resting over the photophase. This result suggests that INT lighting programs could stimulate chicks to increase mobility behaviour, as it encourages the birds to stand and move when the light is switched on. Under a NC brooding lighting program, pullet spent a higher percentage of the photophase time resting. These findings agree with Malleau et al. (2007) who compared an NC and a periodic lighting schedule for broilers and leghorn chicks. These authors found similar results. It is possible that the reason for greater resting behaviour over the photophase for chicks under longer photoperiods might be related to the desynchronization in diurnal rhythms due to an absence of a minimum scotoperiod required for the birds to adequately rest in synchrony (Schwean-Lardner et al., 2014).

There is a negative aspect of a very long period of inactivity, laying or sitting in the same position, which could reduce birds' welfare, particularly in older birds, due to increased contact of the skin with the litter leading to an increase in dermatitis lesions (Vermette at al., 2016b). Mobility behaviours (standing, walking, running) are important for many reasons, as they may positively impact bone and muscle strength (Lewis and Hurnik, 1990). Therefore, at an early age, the higher mobility of chicks reared under an INT lighting schedule observed in the current study might help to stimulate and strengthen skeletal development that is crucial for laying hen strains. It was hypothesized that the hatchlings in the current study would increase expression of comfort behaviours independent of rearing environment; but the lighting wavelengths used during incubation in this study did not affect the expression of comfort related behaviours post-hatch. However, the lights were outfitted on the incubator wall on a vertical position parallel to the egg trays. Therefore, the embryo might not have received direct light on the right eye, which would have resulted in the absence of light wavelength effects on brain functional lateralization (Rogers and Krebs, 1996) impacting the hatchlings early behaviour. Additionally, the embryos might not have received light equally (intensity) during incubation as the light bulbs parallel to the eggs on one side of the incubator might have had a greater impact on those closer to the light bulbs than on the far side of the tray. This may have an impact by reducing the transmission of light through the eggshell when further from the light source (reduced intensity) (Shafey et al., 2002). The

distribution of light within the incubator and position of the eggs in relation to the light bulbs could have increased variability resulting in the low impact of light wavelength observed in the current study. A future study using the light bulbs placed above the eggs might show a different outcome for behavioural expression of pullets hatched from specific in-ovo lighting (wavelength) incubation.

3.6 Conclusion

The wavelength of light during incubation tested in this study did not define hatchling early behavioural output over the early brooding photophase. In contrast, the brooding photoperiod, independently of incubation lighting wavelength and bird genotype, did. Therefore an INT lighting schedule post-hatch might be beneficial for the pullets to stimulate feeding and drinking as the pullets increased the percentage of time spent in mobility behaviours and the percentage of time spent at the feeder and drinker under that brooding photoperiod.

3.7 Tables

Table 3.1. Lohmann LSL diet starter composition with calculated nutrient levels

Ingredients (%)	Starter
Corn	59.92
Soybean meal	23.03
Canola meal	11.72
Limestone	1.79
Dicalcium phosphate	1.41
Animal/Vegetable fat	1.00
Mineral and vitamin premix[1]	0.50
Salt	0.40
Methionine premix	0.20
Lysine HCL	0.02
Nutrients	
Metabolizable energy (kcal/kg)	2900.03
Crude protein (%)	20.00
Calcium (%)	1.05
Digestible lysine (%)	0.98
Digestible threonine (%)	0.75
Digestible methionine and cysteine (%)	0.68
Available phosphorus (%)	0.48
Digestible tryptophan (%)	0.21
Sodium (%)	0.18

[1]**Supplied per kilogram of feed:** vitamin A, 10000 IU; vitamin D_3, 2000 IU; vitamin E, mg 20-30; menadione, mg 3; thiamine, mg 1; riboflavin, mg 6; niacin, mg 30; pyridoxine, mg 3; vitamin B_{12}, mcg 20; pantothenic acid, mg 8; folic acid, mg 1.0; and biotin, mcg 50; choline, mg 300; iron, mg 25; zinc, mg 60; manganese, mg 100; copper, mg 5; iodine, mg 0.5; and selenium, mg 0.2.

Table 3.2. Ethogram description of behaviours for measurement in egg production pullets

Behaviour	Behaviour	Description
Active	Walking	Moving around the cage at a slow pace by putting one foot in front of the other
	Running	Moving around the cage at a fast speed by putting one foot in front of the other
	Standing	Still position and not performing any other behaviour
Resting	Resting	Sitting position with the breast touching the ground in an inactive state
Aggressive	Forceful feather pecking	Forceful pecking feathers from conspecifics directed to any part of the body
Comfort	Wing flapping	Running or just standing with both wings flapping
	Dustbathing	Lying on the side with the feathers fluffed while making movements with the body against the litter and shaking wings, moving the head and scratching the ground
Preening	Preening	Grooming the feathers with the beak
Exploratory	Gentle feather pecking	Gently pecking gently at plumage of a cage-mate
	Object pecking	Pecking at physical objects including cage walls, water lines with the exception of the nipples and water cups.
	Ground scratching	Scratching the ground with the feet and making movements forward and backward
Nutritive	Eating	Head extended into the feeder, manipulating or ingesting feed
	Drinking	Head extended to the water line, manipulating water nipple or water cups
Other	Beak wiping	Head lowered and in movements from side to side with the beak touching the ground
	Head shaking	Movements of the head from side to side in the air
	Expelling excrements	Expulsing wastes from the body
	Unknown	Chick out of the field of view

Adaptated from Hurnik et al., (1995).

Table 3.3. Main effects of in-ovo lighting wavelength on the behaviour of egg production pullets at day 0 post-hatch

	Percentage of time over the photophase				*P* value				
Behaviour	**Dark**	**Blue**	**White**	**Red**	**H**	**H[5] x G[6]**	**HxB[7]**	**GxB**	**HxGxB**
Walking	[1]6.69±0.869	6.91±0.850	7.24±0.869	7.90±0.922	0.632	0.44	0.88	0.56	0.93
Running	1.04±0.293	1.55±0.357	2.38±0.446	2.35±0.444	0.130	0.98	0.85	0.73	0.60
Standing	25.86±1.468	21.58±1.342	21.69±1.345	21.13±1.327	0.059	0.49	0.97	0.21	0.85
Resting	41.36±1.857	41.45±1.858	39.03±1.803	38.35±1.787	0.378	0.91	0.17	0.52	0.90
At the drinker	2.49±0.460	2.76±0.487	3.22±0.525	3.69±0.562	0.421	0.20	0.32	0.78	0.82
At the feeder	16.69±1.180	19.69±1.281	20.48±1.307	20.57±1.309	0.164	0.58	0.26	0.66	0.57
Preening	3.69±0.582	3.97±0.598	3.87±0.594	3.39±0.554	0.948	0.59	0.41	0.48	0.78
Exploratory[2]	0.82±0.277	0.64±0.248	1.10±0.305	0.72±0.247	0.551	0.94	0.36	0.65	0.78
Comfort[3]	0.64±0.236	0.48±0.202	0.33±0.165	0.59±0.223	0.704	0.62	0.92	0.90	0.76
Low incidence[4]	0.69±0.239	0.81±0.260	0.67±0.238	1.31±0.329	0.300	0.78	0.76	0.96	0.93

[ab]Means within a row with different letters differ significantly (P<0.05). [1]Mean ± Standard error.

[2]Exploratory behaviours = Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours = Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

[5]H = Hatch (in-ovo wavelength).

[6]G = Genotype.

[7]B = Brooding lighting program.

Table 3.4. Main effects of in-ovo lighting wavelength on the behaviour of egg production pullets at day 2 post-hatch

	Percentage of time over the photophase				*P* value				
Behaviour	**Dark**	**Blue**	**White**	**Red**	**H**	**H[5]**	**HxB[7]**	**GxB**	**HxGxB**
Walking	[1]8.68±0.851	7.40±0.786	7.95±0.813	8.14±0.824	0.747	0.93	0.89	0.76	0.52
Running	1.73±0.379	2.16±0.423	2.40±0.447	2.41±0.448	0.650	0.34	0.75	0.42	0.96
Standing	17.40±1.365	17.04±1.350	17.36±1.366	15.51±1.270	0.610	0.26	0.73	0.13	0.59
Resting	39.47±1.814	38.61±1.793	37.47±1.767	38.39±1.788	0.909	0.40	0.75	0.79	0.84
At the drinker	2.49±0.455	2.22±0.430	2.42±0.456	2.69±0.479	0.956	0.73	0.79	0.38	0.92
At the feeder	24.35±1.424	27.28±1.509	25.51±1.459	26.10±1.474	0.416	0.29	0.85	0.61	0.53
Preening	4.18±0.592	3.58±0.547	4.65±0.622	4.66±0.624	0.593	0.90	0.85	0.20	0.78
Exploratory[2]	0.52±0.431	0.57±0.529	0.68±0.618	0.84±0.756	0.796	0.45	0.87	0.50	0.98
Comfort[3]	0.48±0.200	0.53±0.210	0.72±0.245	0.82±0.262	0.786	0.86	0.91	0.44	0.86
Low incidence[4]	0.70±0.260	0.60±0.238	0.86±0.302	0.44±0.200	0.708	0.70	0.82	0.28	0.95

[1]Mean ± Standard error.

[2]Exploratory behaviours = Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

[5]H= Hatch (in-ovo lighting wavelength).

[6]G= Genotype.

[7]B= Brooding photoperiod.

Table 3.5. Main effects of in-ovo lighting wavelength on the behaviour of egg production pullets at day 4 post-hatch

	Percentage of time over the photophase				*P* value				
Behaviour	**Dark**	**Blue**	**White**	**Red**	**H**	**H[5] xG[6]**	**Hx**	**GxB**	**HxGxB**
Walking	[1]9.80±0.923	8.10±0.837	9.23±0.894	9.37±0.902	0.564	0.61	0.94	0.14	0.55
Running	2.45±0.451	2.40±0.447	2.26±0.439	2.98±0.501	0.670	0.59	0.87	0.94	0.41
Standing	17.11±1.195	18.06±1.227	18.13±1.229	15.37±1.131	0.295	0.27	0.73	0.81	0.23
Resting	33.07±1.725	31.92±1.692	32.25±1.701	32.50±1.709	0.981	0.70	0.94	0.85	0.42
At the drinker	3.88±0.574	3.23±0.519	3.66±0.552	3.25±0.520	0.827	0.46	0.28	0.69	0.64
At the feeder	26.33±1.481	28.76±1.548	26.56±1.489	28.55±1.542	0.452	0.18	0.96	0.64	0.77
Preening	4.55±0.616	5.31±0.665	5.45±0.673	4.99±0.645	0.833	0.94	0.74	0.48	0.99
Exploratory[2]	1.13±0.307	0.83±0.263	0.65±0.230	1.35±0.335	0.304	0.62	0.66	0.83	0.99
Comfort[3]	0.90±0.273	0.96±0.284	0.87±0.270	0.94±0.280	0.991	0.73	0.93	0.43	0.99
Low	0.77±0.258	0.44±0.192	0.94±0.298	0.70±0.243	0.782	0.86	0.38	0.58	0.35

[ab]Means within a row with different letters differ significantly (P<0.05). [1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

[5]H= Hatch (in-ovo lighting wavelength).

[6]G= Genotype.

[7]B= Brooding photoperiod.

Table 3.6. Main effects of the genotype on the behaviour of egg production pullets at day 0 (n=3)

	Percentage of time over the photophase		
Behaviour	**Lohmann LSL**	**Lohmann Brown**	***P* value**
Walking	5.73±0.583[b]	8.64±0.773[a]	0.001
Running	1.10±0.215[b]	2.56±0.329[a]	0.003
Standing	25.29±1.027[a]	19.84±0.909[b]	<0.001
Resting	38.98±1.275	41.11±1.309	0.281
At the drinker	3.73±0.405[a]	2.36±0.320[b]	0.012
At the feeder	19.02±0.890[1]	19.70±0.906	0.600
Preening	3.50±0.415	3.96±0.444	0.183
Exploratory[2]	1.07±0.216	0.57±0.163	0.074
Comfort[3]	0.53±0.157	0.49±0.145	0.923
Low incidence[4]	1.04±0.208	0.69±0.170	0.169

[ab]Means within a row with different letters differ significantly (P<0.05)

[1]Mean ± Standard error.

[2]Exploratory behaviours=Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

Table 3.7. Main effects of the genotype on the behaviour of egg production pullets at day 2 (n=3)

	Percentage of time over the photophase		
Behaviour	**Lohmann LSL**	**Lohmann Brown**	***P* value**
Walking	8.57±598[1]	7.51±560	0.252
Running	2.03±0.291	2.32±0.312	0.661
Standing	16.20±975	17.46±1.024	0.317
Resting	34.06±1.191[b]	42.90±1.337[a]	<0.001
At the drinker	2.95±0.351[a]	1.96±0.287[b]	0.031
At the feeder	30.50±1.127[a]	21.12±0.938[b]	<0.001
Preening	3.88±0.402	4.65±0.440	0.210
Exploratory[2]	0.79±0.183	0.51±0.145	0.285
Comfort[3]	0.53±0.148	0.74±0.175	0.388
Low incidence[4]	0.48±0.166	0.82±0.232	0.316

[ab]Means within a row with different letters differ significantly (P<0.05).

[1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

Table 3.8. Main effects of the genotype on the behaviour of egg production pullets at day 4 (n=3)

	Percentage of time over the photophase		
Behaviour	**Lohmann LSL**	**Lohmann Brown**	***P* value**
Walking	[1]8.79±0.627	9.45±0.653	0.322
Running	2.01±0.290[b]	3.03±0.358[a]	0.040
Standing	16.26±0.823	18.07±0.868	0.130
Resting	32.15±1.201	32.73±1.212	0.758
At the drinker	4.01±0.409	3.00±0.354	0.060
At the feeder	29.81±1.114[a]	25.29±1.027[b]	0.005
Preening	4.42±0.429	5.73±0.488	0.065
Exploratory[2]	0.96±0.204	1.02±0.207	0.693
Comfort[3]	0.80±0.183	1.04±0.208	0.422
Low incidence[4]	0.79±0.184	0.64±0.166	0.546

[ab]Means within a row with different letters differ significantly (P<0.05).

[1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

Table 3.9. Main effects of brooding photoperiod on the behaviour of egg production pullets at day 0 (n=3)

	Percentage of time over the photophase		
Behaviour	**NC**	**INT**	***P* value**
Walking	[1]6.60±0.734	7.77±0.832	0.345
Running	1.65±0.267	2.01±0.286	0.677
Standing	21.14±0.939	23.99±1.000	0.093
Resting	45.06±1.370[a]	35.03±1.208[b]	0.005
At the drinker	2.89±0.365	3.20±0.379	0.621
At the feeder	17.34±0.850[b]	21.38±0.944[a]	0.036
Preening	3.14±0.419	4.32±0.510	0.183
Exploratory[2]	0.76±0.194	0.88±0.193	0.455
Comfort[3]	0.41±0.133	0.61±0.173	0.610
Low incidence[4]	1.00±0.210	0.74±0.175	0.552

[ab]Means within a row with different letters differ significantly (P<0.05).

[1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

Table 3.10. Main effects of brooding photoperiod on the behaviour of egg production pullets at day 2 (n-3)

	Percentage of time over the photophase		
Behaviour	**NC**	**INT**	***P* value**
Walking	[1]6.80±0.532[b]	9.28±0.622[a]	0.039
Running	1.94±0.285	2.41±0.317	0.361
Standing	16.10±1.067	17.56±1.132	0.445
Resting	44.22±1.358[a]	32.75±1.168[b]	<0.001
At the drinker	2.02±0.292	2.88±0.347	0.112
At the feeder	23.16±1.089[b]	28.46±0.983[a]	0.022
Preening	4.08±0.412	4.46±0.431	0.485
Exploratory[2]	0.55±0.151	0.76±0.179	0.523
Comfort[3]	0.62±0.160	0.66±0.165	0.947
Low incidence[4]	0.52±0.210	0.79±0.257	0.720

[ab]Means within a row with different letters differ significantly (P<0.05).

[1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking.

Table 3.11. Main effects of brooding photoperiod on the behaviour of egg production pullets at day 4 (n=3)

	Percentage of time over the photophase		
Behaviour	**NC**	**INT**	***P* value**
Walking	[1]7.76±0.605[b]	10.48±0.719[a]	0.041
Running	2.13±0.298	2.92±0.349	0.141
Standing	16.23±0.822	18.10±0.869	0.191
Resting	37.65±1.309[a]	27.22±1.100[b]	0.004
At the drinker	3.40±0.377	3.61±0.389	0.715
At the feeder	25.52±1.110	29.58±1.032	0.052
Preening	4.71±0.443	5.44±0.476	0.380
Exploratory[2]	0.91±0.198	1.07±0.212	0.547
Comfort[3]	0.97±0.201	0.88±0.191	0.880
Low incidence[4]	0.72±0.179	0.70±0.177	0.834

[ab]Means within a row with different letters differ significantly (P<0.05).

[1]Mean ± Standard error.

[2]Exploratory behaviours= Gentle feather pecking, ground scratching, object pecking, ground pecking while sitting.

[3]Comfort behaviours= Stretching, dustbathing, wing flapping.

[4]Low incidence behaviours= Unknown, eliminating excretes, head shaking, beak wiping, forceful feather pecking, dustbathing, wing flapping.

4.0 Chapter 4: Effect of photoperiod length during incubation on hatch traits, growth, and behaviour of Leghorn chicks to 21 days of age

The aim of this study was to evaluate the impact of photoperiod length during incubation of Lohmann LSL-Lite eggs on embryo mortality, hatch traits including the spread of hatch, incubation time, hatchability, chick quality traits including navel closure, body length, body weight, developmental traits including weights of the heart, liver and gastrointestinal tract (GIT) segments measures, and stress indicators by evaluating the composite asymmetry and heterophil to lymphocyte ratio, and finally growth and uniformity.

4.1 Abstract

This study determined the effects of photoperiod length during incubation of Lohmann LSL eggs on hatch traits, and pullets' growth and behaviour. A total of 100 eggs were randomly assigned to one of four treatments: 0L (Light):24D(Dark)(0L), 6L:18D (6L), 12L:12D (12L) or 18L:6D (18L). Data was collected to measure embryo mortality, incubation time, spread of hatch, chick weight, heterophil to lymphocyte ratio (H: L), yolk-free body weight, yolk sac residue, organ weights ((liver, heart and gastrointestinal (GIT) segments)) and composite asymmetry. Data were analyzed using SAS 9.4 with one-way ANOVA in an RCBD. The behaviour rhythm was analyzed through repeated measures followed by REG and RSREG regressions. Significance was declared when $P<0.05$. In-ovo lighting did not affect embryo mortality, hatchability and spread of hatch. Incubation time was affected, as 10% to 75% of hatched chicks under 18L ($P=0.001$; $P<0.019$) hatched earlier than chicks from 0L, 6L, and 12L. Male chicks, from 0L ($P<0.001$) were heavier than chicks from 12L and 18L. Female chicks from 0L ($P<0.001$) were heavier than females from 6L, 12L and 18L. Incubation treatment did not affect navel closure, female body length, H: L ratio, composite asymmetry, relative yolk-free body weight and relative yolk sac residue. Chick relative liver ($P<0.001$), heart ($P=0.004$), duodenum ($P<0.001$), ileum ($P<0.001$) and cecum ($P=0.013$) weights to body weight under 18L were heaviest. Relative colon weight was not affected by photoperiod length. Pullets weight at days 7 ($P=0.001$), 14 ($P=0.017$) and 21 ($P=0.027$) differed; however, flock uniformity was not affected. No effects were observed on composite asymmetry or H:L ratio at day 21 post-hatch. Behaviour output, longer photoperiods such as 18L during incubation increased pullet's mobility behaviours walking ($P=0.029$), running ($P=0.003$), standing ($P=0.015$) on day 0; walking ($P=0.037$) and running ($P=0.023$) on day 1; and standing ($P=0.014$) on day 3 and decreased resting ($P=0.039$) on day 0; day 1 ($P=0.001$), and day 2 ($P=0.020$) post-hatch in comparison to pullets from dark incubation. Behaviour rhythms over the photophase were present in resting behaviour on day 0 ($P<0.035$), day 2 ($P=0.001$), walking day 0 ($P<0.001$) and day 1 ($P=0.023$) and foraging on day 2 ($P=0.017$) under all four treatments. In conclusion, in-ovo photoperiod impacted LSL incubation time without negatively affecting the development of the embryo or increasing the stress levels at hatch or 21 days post-hatch.

KEYWORDS: Incubation, photostimulation, stress, the spread of hatch, H:L ratio, asymmetry.

4.2 Introduction

The past decades have shown progress in understanding the benefit of using in-ovo lighting to improve bird development and health (Rogers and Krebs, 1996; Özkan et al., 2012a, 2012b; Archer and Mench 2014a). Evidence suggests that the effects of in-ovo lighting on the embryo can differ with the length of the photoperiod. For example, longer photoperiods resulted in increased multiplication of cells and accelerated embryo development and metabolism (Siegel et al., 1969; Walter and Voitle, 1972; Copper et al., 2011). Exposure of fertile eggs to a daily 18L:6D photoperiod during incubation accelerated house sparrow (*Passer domesticus*) embryonic development and metabolism and reduced incubation time (Copper et al., 2011). Accelerated development and reduced metabolism during incubation were also noted on White Leghorn embryos exposed to a 24L:0D photoperiod during the first week of incubation (Siegel et al., 1969).

Additionally, light can stimulate an increase in White Leghorn embryo weight as early as the first 4.5 days of incubation with a 24L:0D (incandescent light) photoperiod (Lauber, 1974). The exposure of broiler eggs to a 16L:8D (fluorescent light) photoperiod from day 0 to 21 also increased embryo weight relative to egg weight and reduced yolk residue weight compared to eggs exposed to light on the last week of incubation only or a 0L:24D incubation photoperiod (Özkan et al., 2012a).

A photoperiod length during incubation can also affect stress levels. Archer and Mench (2013) reported impacts on bird health. These authors exposed fertile broiler eggs to a 12L:12D photoperiod during incubation, and this resulted in a stimulated immune system showing higher production of antibodies measured 3 weeks post-hatch compared to chicken incubated under a 0L:24D or 1L:23D photoperiod. Additionally, 12L:12D (fluorescent light) photoperiod during broiler eggs incubation reduced the levels of corticosterone in 4 weeks old broilers as a post stress response (Archer and Mench., 2013). Furthermore, a lower asymmetry level was observed in seven-week old birds hatched from a 12L:12D in-ovo incubation (Archer and Mench., 2013). However, the timing of light exposure could potentially be a cofactor. For instance broiler eggs incubated under a 12L:12D photoperiod from day 0 to 21, showed reduced fear level, lowered body asymmetry, decreased corticosterone and increased antibodies in the hatched chicks compared to chicks incubated under the same illumination and photoperiod from day 7 to 21, or from day 14 to 21 or incubated without light from day 0 to 21 (Archer and Mench, 2014a). These

changes signal a reduction in stress, through actions of the body's stress system. Specifically, a primary regulator of stress in birds is the hypothalamus-pituitary-adrenal (HPA) axis (Scanes, 2016). Under stress, birds stimulate the release of glucocorticoids, adrenocorticotropic hormone, and corticotropin-releasing hormone into the bloodstream in response to a stressor. High levels of glucocorticoids in the body can result in depression of the immune system and elevate the proportion of heterophils to lymphocytes (Scanes, 2016).

The most common types of light sources in previous in-ovo lighting studies were either incandescent, fluorescent, or light emitting diode (LED). The incandescent light has become less common, while the LED light bulbs have increased in popularity due to the availability of monochromatic options and durability. Red light is among the monochromatic options for in-ovo lighting incubation, and it may potentially be a good fit for White Leghorn egg incubation (Archer, 2015b), as it may result in improvements in hatchability and chick quality compared to chicks incubated under white light (Archer, 2015b).

Despite the available evidence, there appears to be no research focusing on the determination of the optimal length of photoperiod using red-light spectrum LEDs for hatch and early brooding parameters in chicks. Therefore, the objectives of this work were to investigate the impact of a range of photoperiod lengths using red (644 nm) LED lighting systems on Lohmann LSL-Lite (LSL) fertilized eggs during 21.5 days of incubation, on hatch traits, and chick growth, and behavioural expression (in percentage of time). A further objective was to determine if the use of various photoperiods during incubation results in diurnal expression of behaviour during the photophase. It was hypothesized that as photoperiod increased under red light, the following would occur:

1) A reduction in incubation time because of increased metabolism in the diurnal phase (Copper et al., 2011) and accelerated embryo development (Isakson et al., 1970; Lauber., 1975; Copper et al., 2011).
2) An improvement in chick quality at hatch, as it is possible that physiological rhythms exist under day/night in-ovo photoperiods that do not occur under constant dark. Photoperiod length affects the rhythm of melatonin hormone syntheses (Zawilska et al., 2006) and the melatonin hormone can have beneficial effects on the immune system.
3) Behavioural changes, including decreasing aggressive behavioural performance and increasing comfort behavioural expression. The changes may be a result of a reduction in

fear responses, resulting in a decrease in stress levels. (Archer and Mench, 2013; Archer and Mench, 2014a).

4) All of the L:D incubated (6L:18D, 12L:12D and 18L:6D) flocks will show the presence of a diurnal behaviour rhythm over the photophase while a behaviour rhythm will be lacking for pullets from the 0L:24D.

4.3 Material and methods

The research and experimental procedures were approved by the University of Saskatchewan's Animal Care Committee following the guidelines set out by the Canadian Council on Animal Care (2009).

Experimental Design

Four incubators (1502 Sportsman, GQF Manufacturing Co., Savannah, GA) with windows blocked to eliminate outside light were outfitted with dim to red LED lighting tubes (1 tube 72.6 cm. 7W from AgriShift® TLL, Once Inc., Plymouth, MN) placed under the turning trays. Three lighting Dim to Red tubes (red; 641 nm at full intensity) were used per incubator.

Figure 4.1. LED lights outfitted into a 1502 Sportsman incubator.

Each incubator was outfitted with an automatic lighting timer (Noma® Outdoor Heavy-Duty Timer) to create the possibility of providing one of four in ovo-photoperiods in each machine: 0

hr of light and 24 hours of dark (0L:24D), 6L:18D, 12L:12D or 18L:6D at an intensity of 535-568 lux at eggs level. The photoperiods were in place from the day of the set until hatch. Exposing embryos to light during incubation occurred under the following photoperiod schedules 0L:24D, 6L:18D (lights on at 7 am and off at 1 pm), 12L:12D (lights on 7 am and off 7 pm), or 18L:6D (light on 7 am and off 1 am). Post-hatch, chicks from all incubation photoperiods were housed in the same room separate by pens and exposed to the following lighting schedule; day 0 - 24L:0D, day 1- 2 - 23L:1D (light off 6 am and on 7 am), and day 3-4 - 20L:3D (light off 3 am and on 7 am).

A total of 1200 Lohmann LSL-Lite fertile eggs were used for this work (Clark's Hatchery, Brandon Manitoba). Upon arrival at the University of Saskatchewan's Poultry Research Centre, the eggs were stored in a cooler at 13.3 °C, then incubated within a maximum of 4 days past day of lay. The trial included three replicated hatches, set in a completely randomized block (hatch) design. In each hatch, 400 eggs were randomly distributed between the four incubators (100 eggs per treatment per hatch). The breeder flock age variability was minimized as much as possible to reduce the effect of age; block one breeder flock age was 46 weeks, block two 35 weeks and block three 45 weeks of age.

4.3.1 Experiment 2a: Hatch traits

Incubation management

At the set day of incubation, the eggs were removed from the cooler 2 to 3 hours before setting in incubators, individually identified (pencil mark), weighed as a group and placed with the air cell end of the egg facing up in the incubator trays. One temperature and humidity miniature data logger (Hygrochron™ DS1923-F5#, Maxim Integrated, San Jose, CA), set to record every 20 minutes, was placed among the eggs on the top shelf near the back of the machine of each incubator to record temperature and humidity among the eggs.

The incubation temperature was set to 37.8°C and relative humidity (RH) of 50% to 55% from 0 to 18 days of incubation. At 18 days of incubation, the eggs were transferred to hatch trays located at the lower level of the incubator. The temperature was maintained at 37.8°C and RH was increased to 55% - 65% until the hatch endpoint, as recommended by the incubator's manufacturer. Incubator temperature and humidity were monitored daily via machine readouts in the morning and evening.

4.3.1.2 Hatch data collection

Embryo mortality - On the 9th and 18th day of incubation, all eggs were weighed and individually candled for removal of clear eggs and dead embryos. After removal, clear eggs and those with identifiable dead embryos were carefully opened through the air cell for identification of infertile eggs and classification of the age of embryo death if present. This was repeated at the hatch endpoint when all non-hatched eggs were checked for identification of dead embryo and the stage that the death occurred.

Spread of hatch, duration of incubation and hatchability - On the 20th day (480 hours) of incubation, the incubators were checked for hatchlings. Checks started from 8 pm on the 20th day until 8 am on the 21.5nd (516 hours) day. During this time, incubators were checked every 4 hours, and hatched chicks were counted, then moved to a labelled drawer in an external hatcher (without light) until the completion of 21.5 days of incubation (considered the end of the hatch). Lights in the hatchery room were switched off during the hatch check when doors needed to be opened, with light for checks provided only by a small headlamp worn by the operator. At the hatch endpoint, all hatched chicks were counted, and the numbers applied on the Equations 4.1 and 4.2 to calculate the hatching percentage of the set and fertile eggs.

$$Hatchability\ of\ set\ eggs\ \% = \frac{Number\ of\ hatched\ chicks}{Number\ of\ set\ eggs}\ x\ 100 =$$

$$Hatchability\ of\ fertile\ eggs\ \% = \frac{Number\ of\ hatched\ chicks}{Number\ of\ fertile\ eggs}\ x100 =$$

Navel score - At the end of the hatch (516 hours of incubation), all chicks were separated by gender using the feather sexing technique described in the Lohmann Tierzucht, Hatchery Management Guide (Tierzucht, 2014) and were wing banded for individual identification. Chicks (30 female and 30 male) were scored for navel quality using the technique developed by Hatchtech, (nd). In this system, the scores ranged from 1 to 3, with a score of 1 meaning the navel was closed and clean, 2 indicating the navel had a swollen or dried button to 2 mm resquicious of the umbilical cord, and 3 indicating a swollen or dried button larger than 2 mm or an open navel.

Body weight and length – Thirty male and thirty female chicks were individually weighed using a precision scale (Mettler Toledo Pj-3600 Delta Range®). The chick length (30 chicks/treatment)

was measured using a standard manual ruler, measuring from the end of beak to the end of the middle toe (not including the nail) (Hatchtech, nd).

Heterophil to lymphocyte ratio - Blood samples (0.3 ml) were taken from the jugular vein of 15 male chicks right after the hatch endpoint using one cc syringes and EDTA tubes. Blood smears were then made using the standard two slide technique (Campbell, 1988), where one slide is used to spread out the drop of blood onto the slide. The slides were left for 24 hours before staining using Hema 3 stain set (Fisher Scientific Company L.L.C. Kalamazo, MI-USA). The blood smear slides were covered with stain in sufficient quantity to cover the whole slide, left for 1 minute and rinsed, then left to dry. The slides were observed using a microscope (B-290 TB Optika Microscopies® - Italy) at a resolution of 100x /1.25 oil immersion. A total of 100 heterophil or lymphocyte cells were counted per slide. The number of heterophils was divided by the number of lymphocytes to obtain the H/ L ratio as in equation 4.3 (Vleck et al., 2000).

$$H{:}L\ ratio = \frac{Heterophils}{Lymphocytes} =$$

Yolk sac residue, yolk-free body weight - After the blood sampling, 20 male chicks were euthanized via cervical dislocation. Dissection then took place to remove yolk sac residue and to harvest organs. The yolk-free body weight and yolk sac residue were weighed separately using a precision digital scale (PB3002-S Mettler Toledo®). Equations 4.4 and 4.5 were used to calculate yolk sac residue and yolk-free body weight relative to live body weight:

$$Relative\ yolk\ free\ body\ weight = \frac{Yolk - free\ body\ weight}{Live\ body\ weight}\ x\ 100 =$$

$$Relative\ yolk\ sac\ residue = \frac{Yolk\ sac\ residue}{Live\ body\ weight}\ x\ 100 =$$

Heart, liver and GIT (Gastrointestinal tract) segment weights - The heart, liver and GIT segments were collected from the male chicks. The GIT was then separated into sections duodenum (pancreas loop without the pancreas), jejunum, (from pancreatic loop to Meckel's diverticulum) ileum (from Meckel`s diverticulum to the ileum-cecal junction), cecum and colon. The length of each tissue was measured with a ruler, and the weight of the GIT segment was

recorded using a two-decimal digital scale (PB3002-S Mettler Toledo®). Organ weight relative to live body weight was calculated using the following equation 4.6:

$$Relative\ organ\ weight = \frac{Organ\ \ weight}{Live\ body\ weight}\ x\ 100 =$$

Morphological asymmetry - Asymmetry of the left and right femur, tibiotarsus and metatarsus of the 20 male chicks were measured post-hatch using an electronic calliper (±0.1 mm) (Mastercraft®). For the femur length, the calliper was positioned from the Trochanter major to the Gondylus fibularis (Driesch, 1979). The tibiotarsus length was measured with the calliper positioned from the Processus cnemialis to the Trochlea tibiotarsi (Driesch, 1979), and the tarsometatarsus length from the proximal end to the distal end (Driesch, 1979).

4.3.2 Experiment 2b: Pullets growth, uniformity health and behaviour

4.3.2.1 Pullets housing and management

Post-hatch, females (30 per pen based on in-ovo treatment) were placed in pens (Figure 4.1) within one room at the University of Saskatchewan Poultry Research Centre (246.43 m^2) (four pens in total, 4.6 m^2 per pen). Before placement, each pen was bedded using wheat straw to approximately 10 cm in height, and a cardboard ring was used on the first week to contain the chicks for ease of video observation.

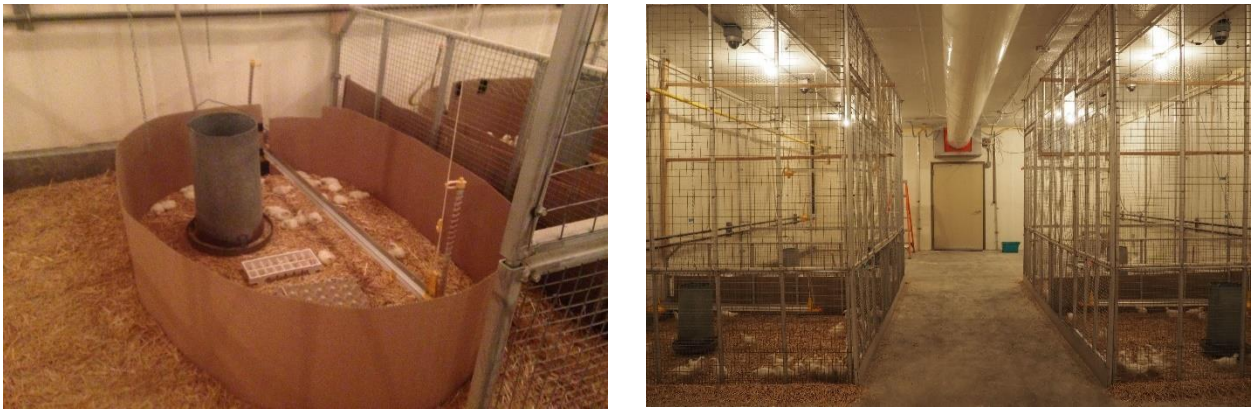

Figure 4.2. View of the room where the chicks were housed post-hatch

The temperature was maintained at 34°C from day 0 to 2 and dropped to 33°C from day 3 to 4. From day 5 to 7, the temperature was maintained at 31°C and from the second week to day

21 was kept at 28 °C. The photoperiod in the room (all treatments) during the rearing phase was 23L:1D from day 0 to 2 with a light intensity of 30 lux, 20L:4D from day 3 to 6 with a light intensity of 30 lux, 16L:8D from day 7 to 14 with a light intensity 20 lux, and 13L:11D from day 15 to 21 with a light intensity of 20 lux. The light was provided with incandescent bulbs, and light intensity measured at the bird's level with a LT-Lutron® LM-8000 (Lutron Electronic Enterprise Co., LTD, Taiwan) lux meter.

Birds were fed commercial chick starter (Table 4.1) ad libitum in tubular feeders (36 cm in diameter) with a 20 kg feed capacity (one per pen). Water was available through lubing drinking nipples (6 nipples per pen). Supplemental feed (one paper egg tray) and water (one ice cube tray) were available in each pen for the first four days.

4.3.2.2 Pullet data collection

Growth and uniformity

Weekly body weight **-** Female chicks were monitored weekly and weighed individually (to allow calculation of flock body weight uniformity) every week up to 21 days of age.

Behaviour

Behaviour **-** The behaviour of the pullets was video recorded for analyses from days 0 to 3 using infrared cameras (Panasonic WV-CF224FX; Panasonic Corporation of North America, One Panasonic Way 7D-4, Secaucus, NJ – USA) mounted on the ceiling above each pen (4 pens per block). The videos were then downloaded to storage drives and later observed using software provided by Genetec Omnicast (Genetec Inc., Montreal, Quebec, Canada). At a later date, videos were observed using a 20 minutes scan sampling technique, which measures the incidence of each behaviour (described on the ethogram on Table 4.2) at each time point in the group and is expressed as a percentage of time-frequency of each behaviour.

Health

Heterophil to lymphocyte ratio (21 days of age) **-** The procedures required to calculate heterophil to lymphocyte ratio were completed for all pullets at 21 days of age using the same technique used on the male at hatch described above.

Morphological **asymmetry** ***(21 days of age)*** **-** At 21 days of age, the females were euthanized by cervical dislocation and dissected to remove the femur, tarsometatarsus, and tibiotarsus from both

legs. The bones were identified according to bird and position of the leg (right) or (left). They were then stored in a refrigerator at a temperature of 3.33°C and measured within five days post-sampling date. The bones measurements were repeated twice, using the technique described above for the males at the hatch endpoint.

Liver, heart and GIT segments sampling (21 days of age) **-** Twenty pullets per treatment were euthanized via cervical dislocation for the collection of heart, liver and GIT segments measurements. The GIT was separated into sections including the pancreas, duodenum (pancreas loop), jejunum (from pancreatic loop to Meckel`s diverticulum) ileum (from the Meckel`s diverticulum to the end of ileum-cecal junction), cecum both sides in longitudinal length and colon from the end of ileum to the beginning of the cloaca measured with a ruler. GIT segment weights were also recorded using a two-decimal digital scale (PB3002-S Mettler Toledo®).

4.3.3 Statistical analyses

A randomized complete block design (hatch period as the block) was used in this study with photoperiod as a fixed effect and hatch as the random effect. All statistical analyses were performed using SAS® 9.4. Proc Univariate (Shapiro Wilk test) was used to check the data for normality. If the data were not normally distributed, a log (y+1) transformation was applied before analyses. The incubation duration and spread of hatch were analyzed using non-linear regression, based on a 3-parameter logistic growth model:

$$Y = \frac{\theta_1}{1+e^{\left(-\frac{(x-\theta 2)}{\theta 3}\right)}}$$

Where:

Y: Photoperiod

$\theta 1$: Maximum hatchability for the experimental unit (incubator).

$\theta 2$: Hours of incubation when reaching 50% of the hatch.

$\theta 3$: Interval from 50 to 75% of the maximum hatchability.

The parameter estimates were calculated by applying the Gauss-Newton method.

Asymmetry data were analyzed using a two-sample comparison t-test to verify differences between left and right side, then as ANOVA with Proc Mixed to test for differences between treatment means.

All other data were tested with a one-way ANOVA in Proc Mixed including the spread of hatch, duration of incubation, percentage of the hatch of set eggs, percentage of the hatch of fertile eggs, navel score, chick length, heterophil to lymphocyte ratio, yolk sac residue absolute and relative weight, yolk-free body absolute and relative weight, and organs (liver, heart and GIT segments) absolute and relative weights.

The daily behaviour means output was analyzed using Proc Glimmix with a Gaussian distribution, and Identity link function. The daily mean of behaviour output was normally distributed. Results were declared significantly different when $P<0.05$ and a trend was noted when $0.10>P>0.05$. The Tukey test was used to separate the means when the differences were significant.

Behaviour rhythm during the photophase were analyzed using two way ANOVA repeated measures (fixed effects photoperiod and time) in a RCBD design (blocked by hatch). If an interaction between photoperiod and time showed significant differences ($P<0.05$) or a trend ($0.10>P>0.05$) within a measured variable, then each treatment was analyzed separately using regression linear (REG) and quadratic (RSREG) model to check if behaviour rhythm over the photophase was present or absent.

4.4 Results

4.4.1 Experiment 2a: Hatch traits

Incubator temperature

In-ovo photoperiod had no impact on incubator ambient temperature (Table 4.3).

Embryo mortality

In-ovo photoperiod did not impact embryo mortality (Table 4.4) during the early, mid or late stages of incubation.

Duration of incubation spread of hatch and hatchability

Eggs incubated under 18L reached 5% of chicks hatched at 481.6 hours of incubation ($P=0.001$) starting hatch earlier compared to those incubated under 0L (489.2 h) or 6L (485.6 h).

Chicks hatched from 12L (484.6h) did not differ from 6L or 18L incubation, but the chicks still hatched earlier compared to incubation without light. When examining the time taken to reach 10%, 25%, 50% or 75% of eggs hatched, those incubated under 18L reached that level the soonest (P<0.05) compared to 0L, 6L, and 12L. In addition, the time to 90% of the chicks to hatch differed with in-ovo photoperiod, but only eggs incubated under 18L differed from those incubated under 0L. Photoperiod length did not impact the spread of hatch (Table 4.6), nor hatchability of set and fertile eggs (Table 4.7).

Navel score

Chicks navel scores for navel healing at hatch did not differ (Table 4.8) between photoperiods for score 1, 2 or 3 for either the male or female chicks.

Body length and weight

Body length of hatchlings was affected by photoperiod (P<0.05, Table 4.9). Photostimulation under 18L increased male chick length (P=0.023) compared to those chicks from 12L and 6L incubation but did not differ from chicks hatched from 0L incubation. Female chick length tended (P=0.091) to be longer for chicks hatched from 12L compared to chicks hatched from a 18L, 6L or 0L incubation.

A significant difference was observed for male chick weight (P<0.001, Table 4.9) with male chicks from 0L incubation being heavier at hatch endpoint than chicks from the 6L or 18L incubation photoperiods. The chicks hatched from the 12L photoperiod were heavier than 18L chicks but lower than 0L hatched chicks. Female chick weight at hatch endpoint (P<0.001, Table 4.9) were heavier for 0L hatched chicks than those hatched from the 6L, 12L, and 18L treatments. Female body weight from 18L incubation chicks were the lowest compared to 6L chicks, and 12L chicks' weight were intermediate.

Yolk sac residue and yolk-free body weight

In-ovo lighting photoperiod affected absolute yolk sac residue weight (P=0.006) and absolute yolk-free body weight (P=0.001, Table 4.10). Yolk residue weight was lower in chicks incubated under 18L compared to yolk residues of chicks from 0L incubation, while yolk sac residue from chicks incubated under 6L and 12L were intermediate. The absolute yolk-free body weight was heavier in chicks hatched from 0L incubation compared to chicks from 12 and 18L in-ovo lighting incubation, and absolute yolk-free body weight from chicks incubated under 6L was

intermediate. On the other hand, no differences were observed for relative yolk sac residue and yolk-free body weights to live body weight (P>0.05, Table 4.10).

Liver, heart and GIT segment weights

Absolute weight

No differences were observed as a result of in-ovo lighting photoperiods for male chicks' liver, heart, jejunum, ileum, cecum, and colon (P>0.062, Table 4.11). However, in-ovo photoperiod did impact duodenum weight (P=0.004), with lower duodenum weight for chicks from 18L compared to chicks hatched from a 0L, 6L, and 12L photoperiods.

Relative weight

When comparing organs and GIT segment weights relative to live body weight (Table 4.13), incubation photoperiod resulted in significant differences for percentage of liver weight (P=<0.001), with 18L photoperiod chicks having greater relative weight compared to chicks from 0L and 6L photoperiods. The chick relative liver weight from photoperiod 12L did not differ from chicks derived from the 18L photoperiod. Chick relative heart weight differed significantly (P=0.004) with a higher percentage noted in chicks from 18L in-ovo lighting photoperiod compared to chicks from the 0L and 6L in-ovo lighting photoperiods. Chicks incubated under 12L had an intermediate relative heart weight. Chick relative duodenum weight to live body weight was higher (P≤0.001) under 18L compared to those hatched from 0L, 6L and 12L treatments. Relative ileum weight to live body weight was also greater (P≤0.001) for chicks hatched from an 18L photoperiod compared to chicks from 0L. The relative ileum weight for chicks under 6L and 12L photoperiods were intermediate. Relative cecum weight differed (P=0.013) between chicks from differing in-ovo photoperiods. For chicks incubated under 18L, the relative cecum weight was heavier (P=0.013) compared to chicks incubated under 0L and 6L photoperiods. However, the relative cecum weight of hatchlings from 18L in-ovo photoperiod did not differ from chicks incubated under a 12L photoperiod. Relative jejunum weight was not affected by in-ovo graded photoperiod.

Welfare and stress indicators

Heterophil and lymphocyte ratio (male at hatch)

Incubation photoperiod did not impact chick stress as measured by heterophil to lymphocyte ratio (Table 4.12).

Morphological asymmetry (male at hatch)

Incubation photoperiod did not impact chick stress as measured by composite asymmetry (Table 4.12).

4.4.2 Experiment 2b: Pullets growth, uniformity health and behaviour

Weekly body weight and uniformity

During incubation, photoperiod did not influence female weight uniformity, as measured by the coefficient of variation of individual weights taken at 0, 7, 14 or 21 days of age (Table 4.13). It did, however, result in significant differences in body weight at day 0 (P<0.001) and day 7 (P=0.001), with pullets hatched from a 0L photoperiod having a greater body weight than those hatched from the 6L and 18L treatments. Pullets from the 12L were intermediate. At day 14, the average pullets body weight or chicks from the 0L photoperiod incubation remained heavier (P=0.013) compared to pullets hatched from a 6L and 12L photoperiod. However, pullets hatched from the 18L photoperiod incubation resulted in an intermediate body weight value. At day 21, pullets hatched from the 0L incubation photoperiod continued to have a greater body weight (P=0.018) compared to those hatched from a 12L and 18L incubation photoperiods, and pullets hatched from a 6L photoperiod incubation had intermediate body weight values.

Welfare and stress indicators

Heterophil to lymphocyte ratio (21 days old)

Incubation photoperiod did not influence stress in LSL pullets as measured by heterophil to lymphocyte ratio at 21 days of age (Table 4.14).

Morphological asymmetry (21 days old)

Morphological asymmetry, analyzed as composite asymmetry, did not significantly differ as a result of in-ovo photoperiod incubation. However, a trend (P=0.060) was noted, with 12L incubated birds having a higher score for asymmetry compared to 18L, 0L, and 6L incubation photoperiods (Table 4.14).

Liver, heart and GIT segments weights (21 days old)

Absolute weights

The absolute weight of organ and GIT segments (liver, heart, pancreas, duodenum, jejunum, ileum, cecum and colon) measured in female pullets at 21 days of age (Table 4.15) did not differ as a result of in-ovo photoperiods length.

Relative Weights

When corrected for body weight, no treatment differences were observed for the relative heart, duodenum, jejunum, cecum and colon weights (Table 4.15). However, relative liver weight to live body weight was different (P=0.036) due to incubation photoperiod, with lower relative weights resulting when incubated under 18L compared to 6L. The relative liver weights from chicks incubated under 0L and 12L were intermediate. A trend was noted for relative pancreas weight (P=0.055) with LSL pullets hatched from 0L having a lower relative weight compared to those hatched from 18L, 6L, and 12L incubation. Additionally, another tendency was observed for relative ileum weight (P=0.051), as pullets hatched from 6L photoperiod had a higher relative weight compared to pullets hatched from 0L, 12L, and 18L incubation photoperiods**.**

Behaviour output of LSL pullets hatched from various in-ovo lighting photoperiod

Day 0 – Female chick behaviour was monitored on the first four days post-hatch. No, differences (P>0.05) were noted at day 0 for the percentage of time chicks were foraging, at the feeder, at the drinker, preening, aggressive or low incidence behaviours (including beak wiping, unknown, stretching, dustbathing, ground pecking and object pecking) (Table 4.16). However, active behaviours differed among the flocks. Chicks incubated under 18L photoperiod (P=0.029) walked the most and those under 0L the least. Pullets from the 0L and 12L photoperiod were intermediate.

The flocks also differed significantly in the percentage of time spent running (P=0.003) as a result of in-ovo photoperiods, pullets from 18L in-ovo lighting spent a higher percentage of time performing running behaviour compared to chicks from 0L, 6L and 12L incubation photoperiods. The percentage of time spent standing differed (P=0.015), as pullets hatched from an 18L spent a higher percentage of their time standing when compared to 0L and 12L, while pullets hatched from a 6L photoperiod were intermediate.

Additionally, resting behaviour differed significantly among pullets from different in-ovo photoperiod length (P=0.039). Pullets hatched from an 18L incubation photoperiod spent a lower percentage of their time performing resting behaviour compared to pullets hatched from a 0L, 6L and 12L incubation photoperiod.

Day 1 - On day one post-hatch (Table 4.17) no differences were observed as a response to incubation photoperiods for a percentage of time at the feeder, at the drinker, or performing standing, preening, aggressive, or low incidence behaviours. Walking behaviour remained different (P=0.037), and pullets from an 18L incubation photoperiod spent a higher percentage of time performing walking behaviour than the pullets hatched from 0L incubation photoperiod; pullets hatched from 6L and 12L incubation photoperiod were intermediate. Furthermore, running behaviour differed (P=0.023) among pullets, with chicks hatched from an 18L incubation photoperiod spending a higher percentage time expressing running behaviour compared to those hatched from a 6L incubation photoperiod. Chicks hatched from the 0L, and 12L incubation photoperiods were intermediate. Pullets hatched from an 18L incubation photoperiod still spent a lower percentage of the time performing resting behaviour (P=0.001) than pullets from 0L, 6L and 12L treatments. Foraging behaviour was affected by in-ovo lighting (P=0.034) at day one, as pullets from 18L spent a greater percentage of time performing foraging than pullets from 0L, chicks from 6L and 12L incubation were intermediate.

Day 2 - On day two post-hatch (Table 4.18) no differences were noted among pullets hatched from the various in-ovo lighting photoperiods for the percent of time spent at the feeder or drinker, walking, running, standing, preening, and performing aggressive, and low incidence behaviours. Differences were observed for the percent of time pullets spent perform resting behaviour (P=0.020). The 0L incubation photoperiod still resulted in chicks that spent a greater percentage of time resting as compared to pullets hatched from the 18L incubation photoperiod, while the pullets hatched from 6L and 12L incubation photoperiods were intermediate. Foraging behaviour differed (P=0.016) among pullets incubated under different in-ovo lighting photoperiods, as pullets from the 0L incubation treatment spent a lower percentage of time performing foraging behaviour compared to chicks hatched from 18L photoperiod, while pullets hatched from 6L and 12L were intermediate.

Day 3 - On the third-day post-hatch (Table 4.19) no differences in behavioural performance over the diurnal period were seen for the percentage of time spent at the feeder, at the drinker, or

performing walking, running, resting, foraging, preening, aggressive, or low incidence behaviours among all treatments. However, at day three a shift in standing behaviour was observed, as flocks incubated under 6L photoperiod spent a higher percentage of the photophase time performing standing behaviour (P=0.014), compared to chicks hatched from 0L and 12L photoperiod incubation, while chicks from 18L photoperiod were intermediate. A trend was observed for flock resting behaviour (P=0.065), with the chicks hatched from a 12L incubation treatment spending the highest percentage of their time resting, and those from the 6L the least. Additionally, a trend was noted for the percentage of time spent at the feeder (P=0.087), where chicks incubated under the 6L treatment spent the greatest percentage of the time, and those from the 12L the least.

Pullet behaviour rhythm over the photophase

Behavioural data that showed significant differences in the repeated ANOVA measures were then tested for the presence or absence of a diurnal rhythm during the photoperiod. The results are shown in Figures 4.2 – 4.7.

Resting

On the day of the hatch (day 0), the resting behaviour of chicks housed in floor pens over the brooding photophase followed a quadratic fashion for pullets from all four-incubation treatments (P<0.001) regardless of the in-ovo lighting duration) (Figure 4.2).

On day one post-hatch resting behaviour over the diurnal photophase period still followed a quadratic model (P<0.001), for 0L, 6L, 12L, and 18L (Figure 4.3).

On day two, the resting behaviour over the photophase still followed a quadratic mode for 0L (P<0.001), 6L (P<0.001), 12L (P=0.003), and 18L (P<0.001) (Figure 4.4), with highest resting behaviour performance at the end and beginning of the day for all four groups.

Walking

On day zero pullets from all in-ovo treatments performed walking behaviour in a quadratic fashion, 0L (P=0.001), and 6L, 12L and 18L (P<0.001) over the photophase (Figure 4.6). On day one walking activity still followed a quadratic model (P<0.001) for all four treatments (0L, 6L, 12L, and 18L) Figure 4.7. The graphs suggest that timing of the behaviours differs, with birds incubated under 0L walked most for a short period in the late afternoon, while those from 6L treatments showed a higher level of walking through the major part of the day.

Foraging

On day two post-hatch 0L hatched pullets did not perform foraging in a diurnal rhythm during the photophase, but chicks from all other incubation treatments did (P<0.001 for all treatments) Figure 4.13. Visual interpretation of the graphs suggests that pullets hatched from a 6L and 12L incubation photoperiods expressed foraging behaviour over the day in a wider spread distribution over the photophase, while pullets hatched from an 18L incubation photoperiod showed an increase in foraging behaviour at the end of the day, with the highest peak in the percentage of the behaviour performed between 10 pm and 4 am just one hour before the lights off period. The 12L highest percentage of behaviour was performed between 7 pm and 3 am, which decrease 3 hours before the lights off period. On the other hand, pullets from 6L and 12L showed lowest foraging activity about three hours before the lights went off.

At the feeder

Times spent at the feeder during the photophase on day two showed a trend (P=0.061) between in-ovo photoperiod and time of day post-hatch. The behaviour rhythm over the photophase was performed in a quadratic mode for all four groups 0L, 6L, 12L (P<0.001), and 18L (P=0.001).

4.5 Discussion

Previously researchers have published evidence of in-ovo lighting photostimulation impacts on embryo development (Siegel et al., 1969; Copper et al., 2011). The majority of that work that examined photoperiod during incubation focused on white light, with a comparison of only two photoperiods. The current research differs, as it has compared four photoperiods, using red light when provided, to allow an understanding of the relationships between light duration and the measured parameters.

In the current research, photoperiod length primarily affected the duration of incubation, when comparing standard dark incubation to in-ovo lighting photoperiods, longer daylength resulted in faster hatching, and the addition of dark (i.e. from 18 to 12 or 6) slowed the hatch. Copper et al. (2011) reported similar data in their study of House Sparrows (*Passer domesticus*). Their experimental design incubated these eggs under an 18L and a 12L photoperiod and found that the incubation time was reduced under 18L. Additionally, Walter and Voitle (1972) observed

that broiler eggs incubated (incandescent light) under a 24L photoperiod hatched earlier than those eggs incubated under a 0L or 12L photoperiod. It appears this is not species specific, as Fairchild and Christensen (2000) observed acceleration in turkey embryo development, which resulted in a reduced incubation time when those turkey eggs were incubated under a 12L (incandescent light) in comparison to a 0L photoperiod. Shafey and Al-mohsen (2002) also reported reduced incubation time for fertile broiler eggs incubated under a 24L photoperiod, however these authors used green fluorescent light as illumination source. The mechanism(s) whereby longer daylength increases the speed of embryonic development has not been confirmed. Cooper et al. (2011) reported higher metabolism in embryos during the light phase, which may contribute to acceleration in the development of the embryo and an earlier hatch. Another possible reason for an earlier hatch initiation could be an increase in incubator temperature as a result of an addition of lighting systems to the incubation equipment. This is more likely to occur with the use of incandescent lighting, which produce heat, LED bulbs are reported to produce minimal heat. Rozenboim et al. (2004) observed that the use of continuous lighting (green 560 nm LED) during incubation increased broiler egg yolk temperature by 0.01% for every 1 minute of eggs exposure to the light and suggested that an intermittent lighting program would suppress the addition of heat caused by lighting. These authors did not report if this heat was a response to the bulb, or if a higher metabolism from eggs incubated under lighting, as suggested by Cooper et al. (2011) could be the reason. Walter and Voitle (1972) and Fairchild and Christensen (2000), using incandescent light bulbs, incubated fertile broiler and turkeys' eggs and did not observe the increase in heat from in-ovo lighting. Not all studies agree, however, Özkan et al. (2012a) did not find differences for an incubation duration of broiler eggs under a 16L photoperiod (cool white fluorescent) to a 0L photoperiod incubation. The incubation of broiler eggs under a 15 min L:15 min D (INT) photoperiod using green LED light as illumination source also did not affect the incubation duration and hatch time (Rozenboim et al., 2004). The majority of in-ovo lighting studies do not include incubation time in their reports, or temperature of the incubators other than the temperature on the machine readouts, resulting in a limited number of studies reporting effects of light on incubation time.

In the current study, our results mimicked those found by Walter and Voitle (1972) and Fairchild and Christensen (2000), in that the temperature among the incubating eggs did not differ statistically between the photoperiods. Although photoperiod length under red (LED) light in the

current research affected incubation length, it did not affect the spread of hatch, meaning that variability in time from start to end of hatch did not differ.

The literature suggests that the use of in-ovo lighting impacts the hatchability of set and fertile eggs. Adam and Diamond (1971), reported an increase in hatchability of broiler eggs by providing peaks of light (incandescent light) at critical stages of development (17 or 19 days of incubation). Archer (2015b), using red (LED) light on a 12L schedule noted an improvement in hatchability of fertile White Leghorn eggs, and the use of white (LED) light under a 12L photoperiod improved the hatchability of fertile broiler eggs (Archer, 2015b; Huth and Archer, 2015). In the current trial, using a red (LED) light, hatchability of either fertile or set eggs were not affected by the photoperiod tested. The differences in studies could be a result of the timing of light exposure or could be related to the minimal number of replicates used in the current study (3).

Despite the earlier hatch with the use of a light-dark cycle, particularly the 18L photoperiod, chick health did not suffer. No effect on mortality or navel healing was noted in the current research. This agrees with the data reported by Huth and Archer (2015), who tested two photoperiods (0L or 12L, LED light) and found no differences in embryonic death. Other studies reported an increase in navel healing when chicks hatched from a 12L photoperiod compared to a 0L photoperiod (Walter and Voitle, 1972; Archer, 2015b). Walter and Voitle (1972) observed increased development and maturation of embryos as a result of longer day length during incubation, thereby reducing incubation time and as consequence possibly increasing navel healing.

It is thought that the length of the chick at hatch can impact meat yield (Molenaar et al., 2008; Petek et al., 2010). However, female chicks hatched from 18L photoperiod showed a tendency in having a longer length at hatch in photoperiods >12L but showed a lower body weight at hatch and 21 days old. If chicks hatch earlier, does it impact the body weight of the hatchling? Walter and Voile, (1972) did not observe any differences in broiler chicks body weight at hatch when embryos were exposed to 0L, 12L, or 24L (incandescent bulb) during the incubation process. Huth and Archer (2015) also did not find a difference in body weight at hatch for chicks incubated under 0L and 12L (white LED light) photoperiods. In the current work, however, the body weight of chicks was measured at hatch endpoint (516 hours post start of incubation period) for all treatments independent of hatching time, and chicks that hatched sooner were lighter. These

differences could be attributed to experimental methodology, and it can be speculated that the reduction in body weight was likely related to the length waiting time without feed and water from hatch to processing resulting in dehydration and weight loss. However, Shafey and Al-mohsen (2002) did observe lower chick weight in hatchling`s exposed to a 24L green light for the first 18 days of incubation. Their study design differed from the current research as these authors weighed the hatchlings at the same time they checked for hatched chicks, whereas in the current study we waited to weigh all the chicks at the hatch endpoint. Future work should consider this and weigh all chicks as they hatch.

Along with the reduction in incubation time, and the decrease in absolute body weight at hatch, a reduction in absolute yolk sac residue at the hatch endpoint was observed with increased day length in this study. Chicks incubated under 18L had lower yolk sac residue and lower yolk-free body weight compared to those exposed to a 0L incubation treatment. In contrast, the relative yolk sac residue and relative yolk-free body weight at hatch endpoint were not different among photoperiods. Özkan et al. (2012a) reported similar results, and these authors observed a lower yolk sac residue weight in broiler embryos at 18 days of incubation when under a 16L photoperiod. They did not mention yolk sac residue at hatch. The relative weights of the duodenum, jejunum and cecum (in relation to live body weight) at the end of hatching process for chicks incubated under ≥12L photoperiods were heavier than those chicks from in-ovo lighting photoperiods ≤6L. Heavier relative intestine weight at hatch has been linked to an improvement in bird performance through improvements in weight gain and feed conversion in broiler chicks (Ipek and Sozcu, 2015; Villanueva et al., 2016). Increasing in-ovo lighting day length in the current study increased the relative weights (to body weight) of the heart, liver and some segments of the GIT at hatch. Liver and heart weight relative to body weight was also heavier in chicks from in-ovo lighting photoperiods ≥12L. The increase in relative weight to body weight in some of the variables measured might be due to the reduction in absolute yolk sac weight in chicks that hatched earlier in addition to dehydration as those chicks that hatched earlier spent longer waiting time without feed and water to be processed, which could affect the proportional weights. The in-ovo photoperiod incubation had minimal impact for absolute weights; as only the duodenum weight was affected by in-ovo lighting photoperiod. This differs from the findings of Fairchild and Christensen (2000), who observed effects on absolute liver and heart weights for chicks hatched from 0L and 12L photoperiods. These authors noted a hatching time effect on liver and heart

weights, and despite hatching later, the chick`s body weight did not differ when compared to chicks that hatched earlier. It was noted that absolute heart and liver weights of the chicks, increased as the timing of hatch increased (Fairchild and Christensen, 2000), which is opposite to the results from the current study, where an increase in relative heart and liver weights was observed in hatchlings. The artificial light in the incubator might accelerate these physiological processes as the requirements for maintenance energy increases under the light. As an example, an increase in the embryo's metabolism during the photophase of the L:D cycle was observed in embryos incubated under an 18L photoperiod (Copper et al., 2011). Additionally, the energy expenditure in live broiler birds is also higher during the photophase when reared under an L:D cycle (Kim et al., 2014). However, Özkan et al. (2012a) found that a 16L photoperiod incubation illuminated by white (fluorescent) light does not affect broiler chick`s relative liver and heart weights at the embryonic age 13 and 18 compared to embryos incubated under dark. However, the current research observed increased in relative liver and heart weights in hatchlings when embryos were exposed to 12 > photoperiod during 21 days of incubation. These findings suggest that if a change in relative liver weight to live body weight occurs, it could be during the last three days of incubation, when the yolk sac absorption is increased due to changes in metabolism processes, increased energy requirements and increased need for mobilization of glucose. The chicks from 12L and 18L photoperiods started the hatching process earlier and had lower yolk free body weight at hatch. This suggests that a different metabolic state in the chicks may have been due to the photoperiod length impact on embryo development.

During the grow-out period up to day 21 post-hatch, pullets hatched from a 0L incubation were heavier than those incubated under 18L. Of note, difference in body weight was already present at the hatch. It would be of interest to use a longer research study period to observe the development of pullets until they reach a mature age. Other published studies have not observed body weight effects on birds hatched from various in-ovo photoperiods. For example, broilers hatched from 0L, 12L, and 24L (fluorescent light) photoperiods and reared under a 12L schedule did not differ in body weight from hatch to 42 days of age (Archer et al., 2009). In addition, no differences in body weight were seen in broiler chicks hatched from 0L and 12L (cool white LED) incubation photoperiods and reared under a 12L lighting schedule (Huth and Archer, 2015)

In the current study at 21 days of age, only relative liver weight was affected by in-ovo photoperiod length, with the percentage weight lower for livers from pullets hatched from 18L incubation.

Stress does not seem to be impacted by in-ovo photoperiod, as no effect was noted on H:L ratio or composite asymmetry levels in males at hatch or pullets at 21 days of age. However, at 21 days old, pullets from 12L showed a tendency for higher asymmetry level which would indicate an increased stress level. This is contrary to Huth and Archer (2015) who observed a reduction in physical asymmetry scores and H:L ratio in 14 days old leghorn chicks compared to those chicks from dark incubation. In agreement with Archer et al. (2009), Archer and Mench (2013) also observed reduced composite asymmetry in 42 day of age chickens hatched from a 12L incubation compared to chicks incubated under 6L, 1L, or 0L, and Archer and Mench (2014a) observed reduced composite asymmetry in broilers hatched from a 12L photoperiod incubation compared to 0L incubation. However, when the eggs were exposed to light just on the first or last week of incubation, the chicks showed intermediate values for composite asymmetry, suggesting that an exposure to light during the entire incubation could be beneficial in minimizing environmental stress during incubation. Low asymmetry levels and a lower H: L ratio might indicate good welfare. However, in the current study, the tendency for increase of composite asymmetry in 21 days of age pullets hatched from a 12L incubation appears random rather than a strong indicator of stress.

It is known that photoperiod length provided to birds can cause significant changes to their behaviour (Schwean-Lardner et al., 2012). With evidence that light can reach embryos through the shell (Shafey et al., 2002), it is of interest to know whether adjusting daylengths during incubation can alter chick behaviour soon after hatch. In this study, there was little impact on nutritive and comfort behaviour of pullets from the day of hatch to three days of age. For example, no effect was found on the percentage of time the pullets spent at the feeder, at the drinker, preening and other low occurrence behaviours post-hatch.

However, the impact of in-ovo photoperiod on hatchlings active and inactive behaviours is similar to the effects seen on birds reared under different photoperiods. Furthermore, the most significant effects on pullet's locomotor activities were observed from photoperiods ≥ 12L which increased active behaviours, and the use of shorter ≤ 6L photoperiods during incubation decreased active behaviours on the first-day post-hatch. Visually, it appears that post-hatch, the time pullets

began resting on days 0, 1 and 2 under a NC brooding photoperiod coincides with the in-ovo lighting scotoperiod time. Pullets showed lower resting in the middle of the day, with resting post-hatch decreasing as in-ovo lighting photoperiod increased. As those longer photoperiods ≥ 12L delayed pullets resting behaviour, those pullets spent more time performing other behaviours such as standing, running, walking and foraging when compared to pullets hatched from photoperiods 6L or 0L (standard dark incubation). The higher percentage of time spent in foraging behaviour observed from pullets hatched from an 18L incubation could be a result of the earlier hatch and higher consumption of yolk sac that was observed at the hatch endpoint. Those pullets that expressed higher percentage of active behaviours could possibly be hungrier, however no alterations in at the feeder or at the drinker were observed. These results are similar to what has been observed in broilers and turkeys reared under various photoperiod length where locomotor activities decrease and inactive behaviour increase as day length increased (Schwean-Lardner et al., 2012; Vermette et al., 2016b). The increase in locomotor behaviour might be positive for bird development and wellbeing as an increase in physical activities can increase bone health and strength. As an example, Hy-line pullets reared in an environment that allows more activities demonstrated an increase in bone strength, due to an increase in the bone cortical and medullary area, trabecular fitness and bone volume compared to hens reared in more restricted environment cages with limited opportunities for locomotor behaviours (Shipov et al., 2010).

Photoperiod also impacts diurnal rhythms of many behaviours in broiler birds, such as feeding, resting etc. (Schwean-Lardner et al., 2014). In the current study, however, the expression of a behaviour rhythm over the photophase was noted across all incubation treatments for specific behaviour up to an age of 2 days, and only at 2 days old did birds incubated under 0L not demonstrated a rhythm for one behaviour (foraging). It is unclear why this occurred. All chicks were housed under one lighting program. It could be a chance occurrence that these rhythms were not found on day 2 for foraging behaviour in the 0L incubation treatment. It could be possible that the rhythm in the 0L birds was not as strong and reached free running status sooner. Further studies are needed to evaluate diurnal rhythm over a 24 hour period, in the current study behaviour over the scotophase was recorded, however, as the infrared cameras were installed at the room ceiling, the distance from the ceiling to the bird level was too high (height 2.90 meters) and clear visualization of the chicks become difficult at night due to the small size of the birds on top of a wheat straw bedding, even though videos were recorded using an infrared camera.

4.6 Conclusion

This research indicates that the use of longer photoperiods (18L in this case) can alter the time to hatch in White Leghorn chicks, by initiating hatch sooner and ending sooner. This did not appear to have detrimental impacts on embryonic or chick health. Although chick weight was lower in these hatchlings, it is possible that this was a result of experimental design rather than a true change in weight. Behaviourally, chicks from the longer photoperiods are more active, which could be beneficial in finding feed and water and stimulate bone development and strength in commercial situations.

Interestingly, no effects on measurements of stress were noted, suggesting in-ovo photoperiod has little impact. All flocks demonstrated rhythms in many behaviours post-hatch up to day 2, but the behaviour rhythm over the photophase in pullets from dark-incubated flocks started disappearing earlier. To conclude, using a longer day-night cycle could have beneficial impacts for hatcheries and young pullets due to reduction in incubation time without affecting stress measures, but would require earlier processing time for the chicks at the hatchery due to potential reduction in body weight possible caused by over waiting time without feed and water at the hatchery.

4.7 Tables

Table 4.1. Ingredients and nutrients composition of diets diet fed to Lohmann LSL from placement to 21 days old

Ingredients	**Starter**
Wheat	42.90
Corn	12.0
Barley	10.0
Peas/lentils	10.0
Meat meal	9.50
Canola meal	7.00
Corn distillers' dried grains with soluble	5.52
Tallow	1.00
Limestone	0.79
Choline chloride	0.08
DL-Methionine	0.08
L-Lysine HCL	0.16
Mono calcium carbonate	0.28
Potassium chloride	0.08
Biotin	0.02
Enzyme[1]	0.02
Amprolium 25%[2]	0.05
DG-200 mg selenium	0.04
Vitamin premix[3]	0.08
Mineral premix[4]	0.07
Nutrients	
Metabolizable energy (kcal/kg)	2738.00
Crude protein (%)	19.20
Arginine (%)	1.13
Lysine (%)	0.99
Calcium (%)	0.96
Methionine and cysteine (%)	0.73
Chloride (mg/kg)	0.70
Isoleucine (%)	0.66
Threonine (%)	0.64
Non-phytate P (%)	0.43
Methionine (%)	0.39
Tryptophan (%)	0.18
Sodium %	0.17

[1]1β-glucanase, 700 activity units/g and xylanase enzymes 2,250 activity units/g (GNC Bioferm Inc., Bradwell, Canada). [2]Coccodiostat. [3] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11,750 IU; vitamin d, 3,000 IU; vitamin E (dl-α-topheryl vitamin D_3 2200 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60 mg;

pyridoxine, 4 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; and biotin, 0.15 mg. [4] Supplied per kilogram of feed: iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

Table 4.2. Ethogram description of behaviours for measurement in egg production pullets

Category	Behaviour	Description
	Walking	Movement of the foot one in front of the other towards any direction
Active	Running	Accelerated motion of movements of one foot in front of the other in any direction.
	Standing	Still position
Resting	Resting	Sitting with both feet covered
	Wing flapping	The action of fast-moving both wings at the same time
	Dustbathing	Lying on the side while head rubbing, bill raking, wing
Comfort		shaking and scratching on the floor
	Preening	Using the beak to groom the feathers
	Stretching	Stretching legs or wings or both at the same time
Nutritive	At the feeder	Head extended into the feeder, manipulating or ingesting feed
	At the drinker	Head extended to the water line towards the nipple, manipulating or not water nipple
	Ground pecking	Movement of the head downwards with the beak touching the ground
Exploratory	Foraging	Scratching the ground with the foot and at the same time making movements forward and backwards
	Object pecking	Pecking pen walls, feeder walls, top of the drinker's line
	Gentle feather pecking	Pecking at plumage of a cage-mate
	Fighting	Engaged in an aggressive act with a cage mate with both birds in a frontal position to each other and investing
Aggressive		aggressively towards one another.
	Forceful feather pecking	Forceful pecked directed to another bird
Others	Beak wiping	Movement with the head side to side with the beak touching the ground
	Head shaking	Fast movements of the head from side to side without any clear reason.
	Unknown	Behaviour not able to be identified by the observers because the bird is out of the field of view

Adapted from Hurnik et al., (1995).

Table 4.3. Effects of in-ovo photoperiod on average (0-21.5 days of incubation) overall incubator temperature (n=3)

Photoperiod	Temperature °C
0L:24D	37.86
6L:18D	38.16
12L:12D	37.49
18L:6D	38.08
SEM[1]	0.052
P value	0.440

[1]Standard Error of the Mean.

Table 4.4. Effects of in-ovo photoperiod on Lohmann LSL embryo mortality (n-3)

	Mortality (%)		
Photoperiod	Early[1]	Mid[2]	Late[3]
0L:24D	4.84	0.69	2.76
6L:18D	4.49	0.68	2.76
12L:12D	5.29	0.00	2.80
18L:6D	5.60	1.78	3.16
SEM[4]	1.445	0.752	0.866
P value	0.912	0.447	0.958

[1]Early=0 to 9 days of incubation.
[2]Mid=10 to 18 days of incubation.
[3]Late=19 to 21.5 days of incubation.
[4]SEM=Standard Error of the Mean.

Table 4.5. Effects of in-ovo photoperiod on time in hours to reach a specific percentage of Lohmann LSL eggs hatching (set time to hatch time) (n=3)

	% Chicks hatched						
Photoperiod	5%	10%	25%	50%	75%	90%	95%
0L:24D	489.2[a]	491.5[a]	494.9[a]	498.7[a]	502.3[a]	505.1[a]	510.1
6L:18D	485.6[b]	487.7[b]	490.8[b]	494.1[b]	498.5[b]	501.2[ab]	507.3
12L:12D	484.6[bc]	487.0[b]	490.4[b]	494.2[b]	499.7[b]	503.5[ab]	508.1
18L:6D	481.6[c]	483.8[c]	487.1[c]	490.6[c]	495.2[c]	498.2[b]	503.5
SEM[1]	0.71	0.68	0.66	0.71	0.56	1.10	2.19
P value	0.001	<0.001	<0.001	<0.001	<0.001	<0.019	0.256

[1]Standard Error of the Mean

[a-c] Means within a column with different letters differ significantly (P<0.05).

Table 4.6. Effects of in-ovo photoperiod on the spread of hatch (time to hatch a specific percentage of Lohmann LSL chicks in hours) (n=3)

	% Chicks hatched			
Photoperiod	5-95%	10-90%	25-75%	50-75%
0L:24D	20.9	13.6	7.4	3.6
6L:18D	21.7	13.5	7.7	4.4
12L:12D	23.5	16.6	9.2	5.4
18L:6D	21.9	14.4	8.1	4.6
SEM	2.72	1.36	0.70	0.68
P value	0.921	0.406	0.354	0.361

[1]Standard Error of the Mean.

Table 4.7. Effects of in-ovo photoperiod on the percentage hatch of set and fertile Lohmann LSL eggs (n=3)

Photoperiod	Hatch of the set (%)	Hatch of fertile (%)
0L:24D	84.33	87.25
6L:18D	89.33	92.09
12L:12D	84.00	88.41
18L:6D	86.33	90.57
SEM[1]	2.614	2.566
P value	0.191	0.522

[1]Standard error of the Mean.

Table 4.8. Effects of in-ovo photoperiod on navel scores of male and female Lohmann LSL chicks at hatch (n=3)

	Male (%)			Female (%)		
Photoperiod	1[1]	2[2]	3[3]	1[1]	2[2]	3[3]
0L:24D	76.81	19.85	3.33	86.66	12.22	1.11
6L:18D	78.89	15.56	5.55	88.89	11.11	0.00
12L:12D	77.78	21.11	1.11	83.33	14.44	2.22
18L:6D	85.29	11.34	3.37	88.66	11.34	0.00
SEM[4]	0.096	0.552	0.580	0.065	0.726	0.345
P value	0.639	0.449	0.413	0.892	0.972	0.189

[1]Score 1 = Button is closed and clean.
[2]Score 2 = Black button up to 2 mm or black string.
[3]Score 3 = Black button over 2 mm or an open navel.
[4] Standard Error of the Mean.

Table 4.9. Effects of in-ovo photoperiod on body weight and length[1] of female and male Lohmann LSL chicks at hatch endpoint (n=3)

	Live body weight (g)		Length (cm)	
Photoperiod	Female	Male	Female	Male
0L:24D	41.14[a]	41.62[a]	16.95	17.16[ab]
6L:18D	40.07[b]	41.13[ab]	16.95	17.04[b]
12L:12D	39.97[bc]	40.31[bc]	17.10	17.03[b]
18L:6D	39.03[c]	39.38[c]	17.03	17.20[a]
SEM[2]	0.456	0.378	0.436	0.431
P value	<0.001	<0.001	0.091	0.023

[a-c] Means within a column with different letters differ significantly (P<0.05).
[1]Length measured from the tip of beak to end of the mid toe.
[2]Standard Error of the Mean.

Table 4.10. Effects of in-ovo photoperiod on Lohmann LSL chick yolk-sac and yolk-free body absolute weights and weights relative to live body weight at hatch endpoint (n=3)

Photoperiod	Yolk sac (g.)	Yolk sac (%)	Yolk-free body weight	Yolk-free body weight (%)
0L:24D	4.92[a]	11.70	36.90[a]	88.30
6L:18D	4.68[ab]	11.34	36.36[ab]	88.66
12L:12D	4.55[ab]	11.32	35.59[bc]	88.68
18L:6D	4.24[b]	10.75	34.96[c]	89.25
SEM[1]	0.218	0.565	0.514	0.565
P value	0.006	0.127	0.001	0.127

[a-c] Means within a column with different letters differ significantly (P<0.05).
[1]Standard Error of the Mean.

Table 4.11. Effects of in-ovo photoperiod on liver, heart and gastrointestinal tract segments weight of male Lohmann LSL hatchlings (n=3)

Photoperiod	Liver	Heart	Duo	Jejunum	Ileum	Ceca	Colon
			Absolute organs and GIT segment weights (g)				
0L:24D	0.89	0.29	0.35[b]	0.31	0.27	0.17	0.10
6L:18D	0.92	0.28	0.35[b]	0.32	0.27	0.17	0.10
12L:12D	0.91	0.29	0.35[b]	0.32	0.28	0.17	0.10
18L:6D	0.92	0.29	0.37[a]	0.31	0.29	0.18	0.10
SEM[1]	0.018	0.007	0.007	0.006	0.007	0.007	0.006
P value	0.062	0.426	0.004	0.799	0.131	0.338	0.955
			Relative organs and GI tract segment weights to live body weight (%)				
0L:24D	2.13[c]	0.70[b]	0.83[b]	0.75	0.66[b]	0.42[b]	0.24
6L:18D	2.25[b]	0.70[b]	0.85[b]	0.78	0.67[b]	0.42[b]	0.25
12L:12D	2.27[ab]	0.71[ab]	0.87[b]	0.79	0.70[ab]	0.43[ab]	0.24
18L:6D	2.35[a]	0.75[a]	0.95[a]	0.79	0.74[a]	0.46[a]	0.25
SEM[1]	0.033	0.020	0.018	0.014	0.014	0.014	0.013
P value	<0.001	0.004	<0.001	0.154	<0.001	0.013	0.798

[a-b] Means within a column with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

Table 4.12. Effects of in-ovo photoperiod on stress indicators (Heterophil: Lymphocyte (H: L) ratio and composite asymmetry of male Lohmann LSL on the day of hatch (n=3)

Photoperiod	H:L ratio	Composite asymmetry[1]
0L:24D	0.36	0.90
6L:18D	0.42	0.99
12L:12D	0.38	0.94
18L:6D	0.41	0.85
SEM[2]	0.086	0.105
P value	0.367	0.117

[1]Composite asymmetry length and width=Femur + Tibiotarsus + Metatarsus.
[2]Standard Error of the Mean.

Table 4.13. Effects of in-ovo photoperiod during incubation on Lohmann LSL pullets body weight (Wt.) and body weight uniformity at day 0, 7, 14 and 21 (n=3)

	Wt. (g)							
Day	Wt0	CV%[2]	Wt7	CV%	Wt14	CV%	Wt21	CV%
0L:24D	[1]41.1±0.470[a]	6.13	73.5±3.735[a]	6.81	128.0±4.538[a]	5.94	205.1±3.518[a]	6.57
6L:18D	40.1±0.470[b]	6.24	70.6±3.735[b]	8.04	124.5±4.538[b]	7.66	200.1±3.511[ab]	6.99
12L:12D	40.0±0.471[b]	6.24	71.9±3.735[ab]	7.68	124.2±4.540[b]	7.50	200.1±3.521[ab]	7.11
18L:6D	39.0±0.470[c]	6.34	70.7±3.736[b]	7.14	125.0±4.539[ab]	7.24	199.6±3.518[b]	6.50
P-value	<0.001	0.985	0.001	0.539	0.017	0.200	0.027	0.755

[a-e] Means with different letters differ significantly (P<0.05).
[1]Mean ± Standard Error of the Mean.
[2]Coefficient of variation.

Table 4.14. Effects of in-ovo photoperiod on Lohmann LSL pullets stress indicators Heterophil: Lymphocyte ratio (H: L) and composite asymmetry at day 21 (n=3)

Photoperiod	H:L ratio (%)	[1]Composite asymmetry (mm)
0L:24D	0.44	1.21
6L:18D	0.39	1.11
12L:12D	0.45	1.27
18L:6D	0.40	1.21
SEM[2]	0.062	0.065
P value	0.536	0.060

[1]Composite asymmetry length and width=Femur + Tibiotarsus + Metatarsus.
[2]Standard Error of Mean.

Table 4.15. Effects of in-ovo photoperiod on Lohmann LSL pullet's organ and GIT segment weights at 21 days of age (n=3)

Photoperiod	Liver	Heart	Pancreas	Duodenum	Jejunum	Ileum	Ceca	Colon
Absolute organs and GI tract segment weights (g)								
0L:24D	6.26	1.30	1.11	2.40	3.40	2.65	1.21	0.62
6L:18D	6.31	1.27	1.14	2.38	3.40	2.68	1.17	0.62
12L:12D	6.10	1.26	1.13	2.34	3.38	2.60	1.16	0.58
18L:6D	6.13	1.24	1.12	2.39	3.49	2.57	1.19	0.61
SEM[1]	0.332	0.095	0.028	0.086	0.076	0.039	0.042	0.059
P value	0.141	0.105	0.647	0.701	0.444	0.135	0.634	0.316
Relative organs and GI tract segment weights to live body weight (%)								
0L:24D	3.08[ab]	0.64	0.55	1.18	1.68	1.30	0.59	0.30
6L:18D	3.17[a]	0.64	0.57	1.20	1.71	1.34	0.59	0.31
12L:12D	3.07[ab]	0.64	0.57	1.19	1.71	1.31	0.58	0.29
18L:6D	3.05[b]	0.62	0.56	1.19	1.74	1.29	0.59	0.30
SEM[1]	0.127	0.057	0.014	0.064	0.066	0.037	0.015	0.033
P value	0.036	0.284	0.055	0.905	0.300	0.051	0.937	0.418

[a-b]Means within a column with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

Table 4.16. Effects of In-ovo photoperiod during incubation on LSL pullet's behaviour post-hatch at day 0 (n=3)

	Photoperiod					
Behaviour	0L	6L	12L	18L	SEM[1]	*P* value
Walking	3.62[ab]	3.19[b]	4.74[ab]	4.78[a]	0.346	0.029
Running	0.81[b]	0.85[b]	1.20[b]	2.63[a]	0.406	0.003
Standing	14.89[b]	16.46[ab]	14.34[b]	20.20[a]	1.033	0.015
Resting	59.01[a]	60.3[a]	56.59[a]	44.99[b]	3.117	0.039
Foraging	2.95	5.28	4.06	6.30	0.986	0.168
At the feeder	12.68	9.95	12.18	13.23	2.265	0.651
At the drinker	1.39	1.06	1.78	2.38	0.339	0.110
Preening	1.41	1.56	1.45	1.86	0.493	0.913
Aggressive[2]	0.39	0.44	0.31	0.67	0.112	0.209
Low incidence[3]	2.84	2.61	3.35	2.97	0.668	0.843

[a-b]Means within a row with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

[2]Aggressive = Fighting + forceful feather pecking.

[3]Low incidence = Beak wiping + unknown + stretching + dust bathing + ground pecking + object pecking.

Table 4.17. Effects of In-ovo photoperiod during incubation on LSL pullet's behaviour post-hatch at day 1 (n=3)

	Photoperiod					
Behaviour	0L	6L	12L	18L	SEM[1]	*P* value
Walking	6.35[b]	6.87[ab]	6.95[ab]	8.85[a]	0.761	0.037
Running	3.86[ab]	3.02[b]	3.93[ab]	4.30[a]	0.363	0.023
Standing	16.19	19.51	17.16	19.79	1.313	0.224
Resting	48.50[a]	43.74[a]	43.57[a]	35.76[b]	1.182	0.001
Foraging	4.35[b]	5.19[ab]	6.13[ab]	7.72[a]	0.939	0.034
At the feeder	12.23	12.49	11.78	13.79	0.723	0.184
At the drinker	2.03	2.46	2.25	2.30	0.359	0.559
Preening	2.35	2.46	2.57	3.25	0.544	0.657
Aggressive[2]	0.37	0.31	0.43	0.29	0.078	0.600
Low incidence[3]	3.76	3.95	5.23	3.95	1.257	0.748

[a-b]Means within a row with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

[2]Aggressive = Fighting + forceful feather pecking.

[3]Low incidence = Beak wiping + unknown + stretching + dust bathing + ground pecking + object pecking.

Table 4.18. Effects of in-ovo photoperiod during incubation on LSL pullet's behaviour post-hatch at day 2 (n=3)

	Photoperiod					
Behaviour	0L	6L	12L	18L	SEM[1]	*P* value
Walking	7.89	7.38	8.48	8.77	0.390	0.130
Running	3.37	3.81	3.93	4.30	0.770	0.859
Standing	11.00	13.15	12.20	10.46	1.189	0.329
Resting	47.52[a]	42.55[ab]	42.52[ab]	40.25[b]	1.437	0.020
Foraging	5.31[b]	7.41[ab]	8.18[ab]	11.79[a]	1.067	0.016
At the feeder	16.07	17.33	15.26	15.79	0.898	0.457
At the drinker	1.84	2.09	1.84	2.21	0.297	0.373
Preening	2.85	2.22	2.82	2.63	0.325	0.526
Aggressive[2]	0.31	0.44	0.16	0.28	0.075	0.154
Low	3.83	3.63	4.61	3.53	1.150	0.837

[a-b]Means within a row with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

[2]Aggressive = Fighting + forceful feather pecking.

[3]Low incidence = Beak wiping + unknown + stretching + dust bathing + ground pecking + object pecking.

Table 4.19. Effects of in-ovo photoperiod during incubation on LSL pullet's behaviour post-hatch at day 3 (n=3)

	Photoperiod					
Behaviour	0L	6L	12L	18L	SEM[1]	*P* value
Walking	8.55	7.41	7.31	7.28	0.542	0.350
Running	2.98	2.58	2.77	2.14	0.642	0.819
Standing	10.81[b]	13.55[a]	9.87[b]	10.90[ab]	0.779	0.014
Resting	42.82	38.58	45.21	44.26	2.199	0.065
Foraging	6.87	7.87	8.27	7.93	0.870	0.706
At the feeder	20.55	22.80	18.27	19.69	1.452	0.087
At the drinker	1.58	2.11	2.10	1.70	0.283	0.472
Preening	3.05	2.31	2.99	2.89	0.368	0.286
Aggressive[2]	0.24	0.13	0.17	0.21	0.090	0.829
Low incidence[3]	2.54	2.66	3.04	3.10	0.637	0.890

[a-b]Means within a row with different letters differ significantly (P<0.05).

[1]Standard Error of the Mean.

[2]Aggressive = Fighting + forceful feather pecking.

[3]Low incidence = Beak wiping + unknown + stretching + dust bathing + ground pecking + object pecking.

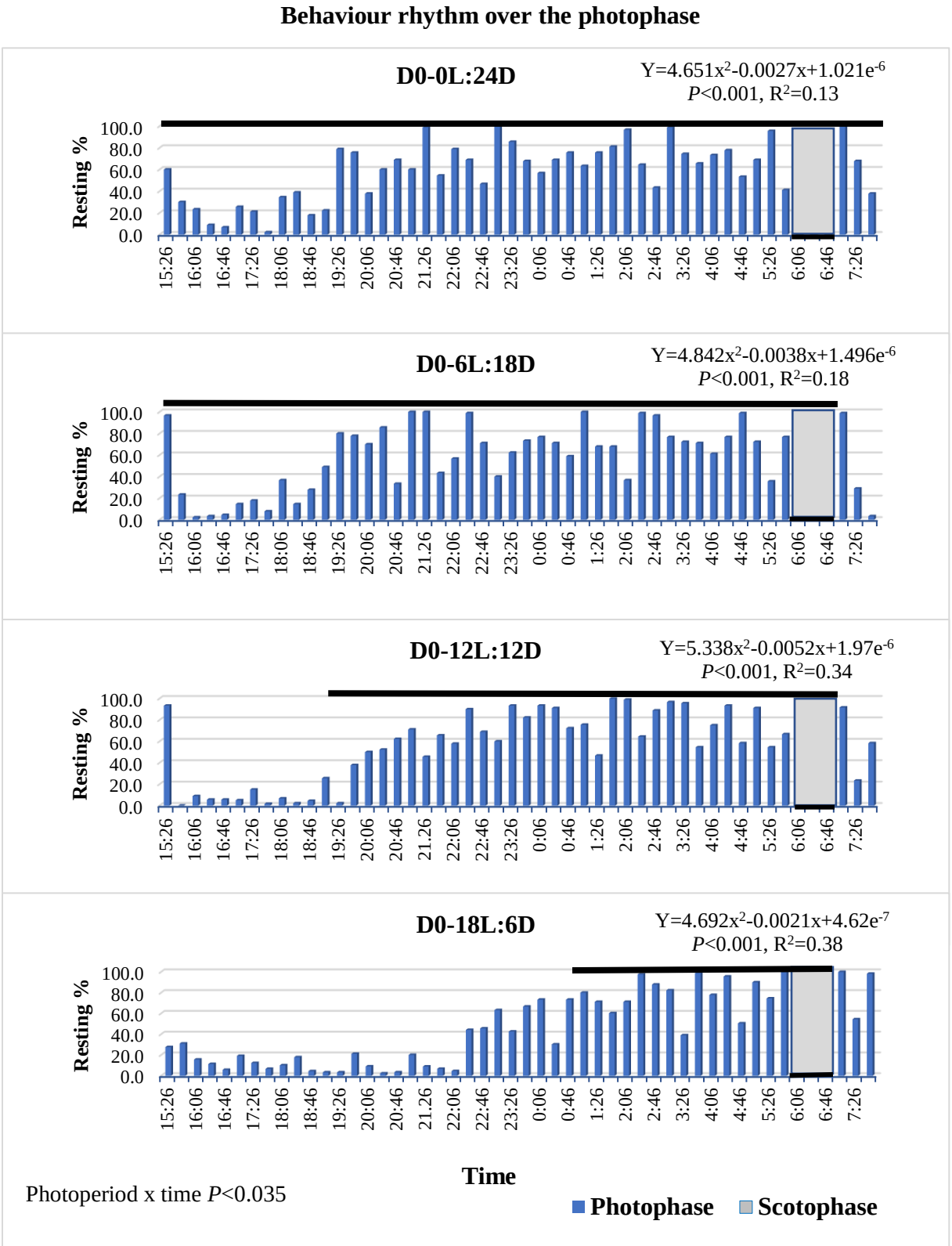

Figure 4.3. Resting behaviour over the photophase at the age 0 post-hatch from LSL pullets exposed to various in-ovo lighting photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

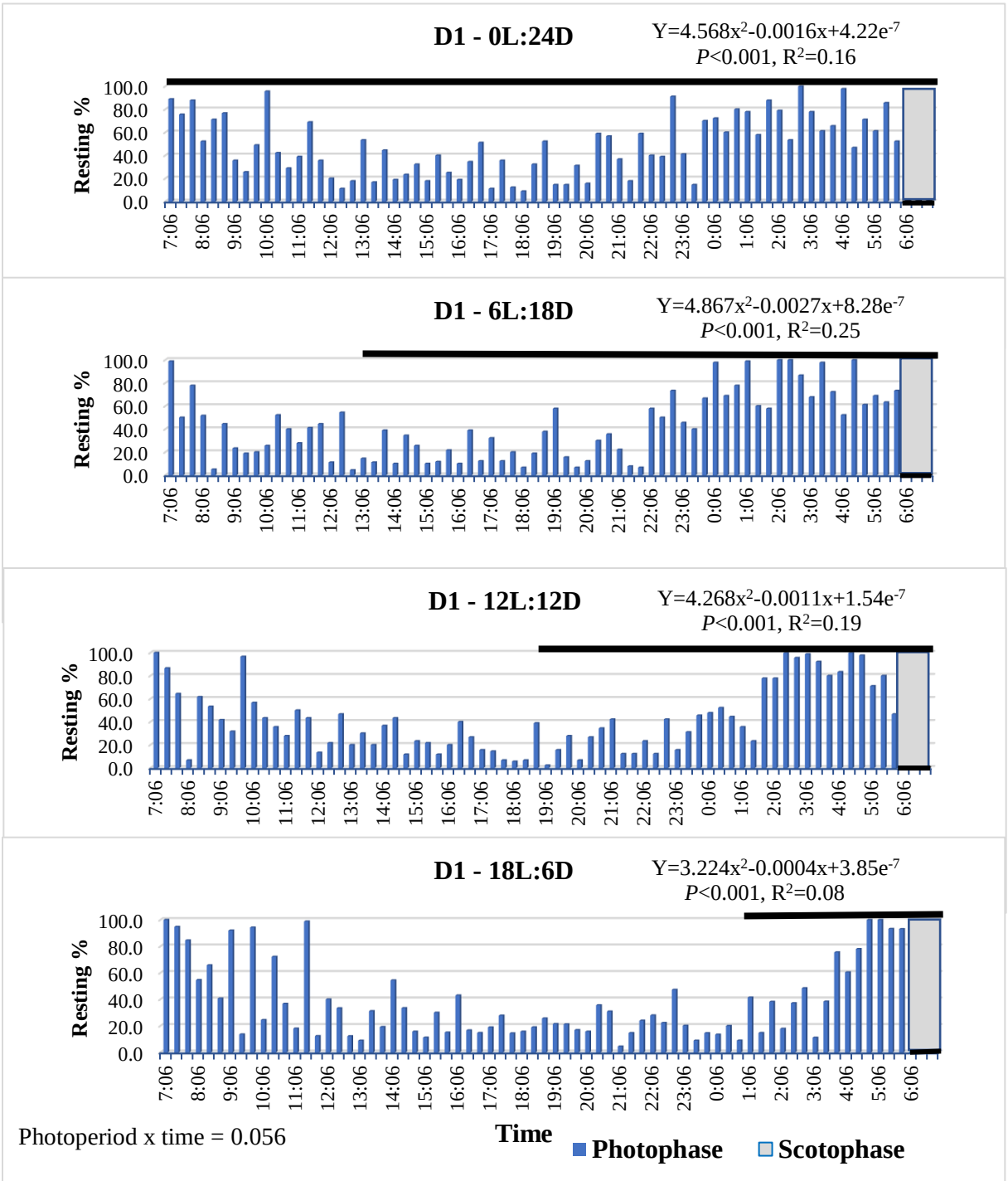

Figure 4.4. Resting behaviour over the photophase at the age 1 from LSL pullets exposed to various in-ovo lighting photoperiod. The horizontal bar on top of the graphs represents the scotophase period during incubation.

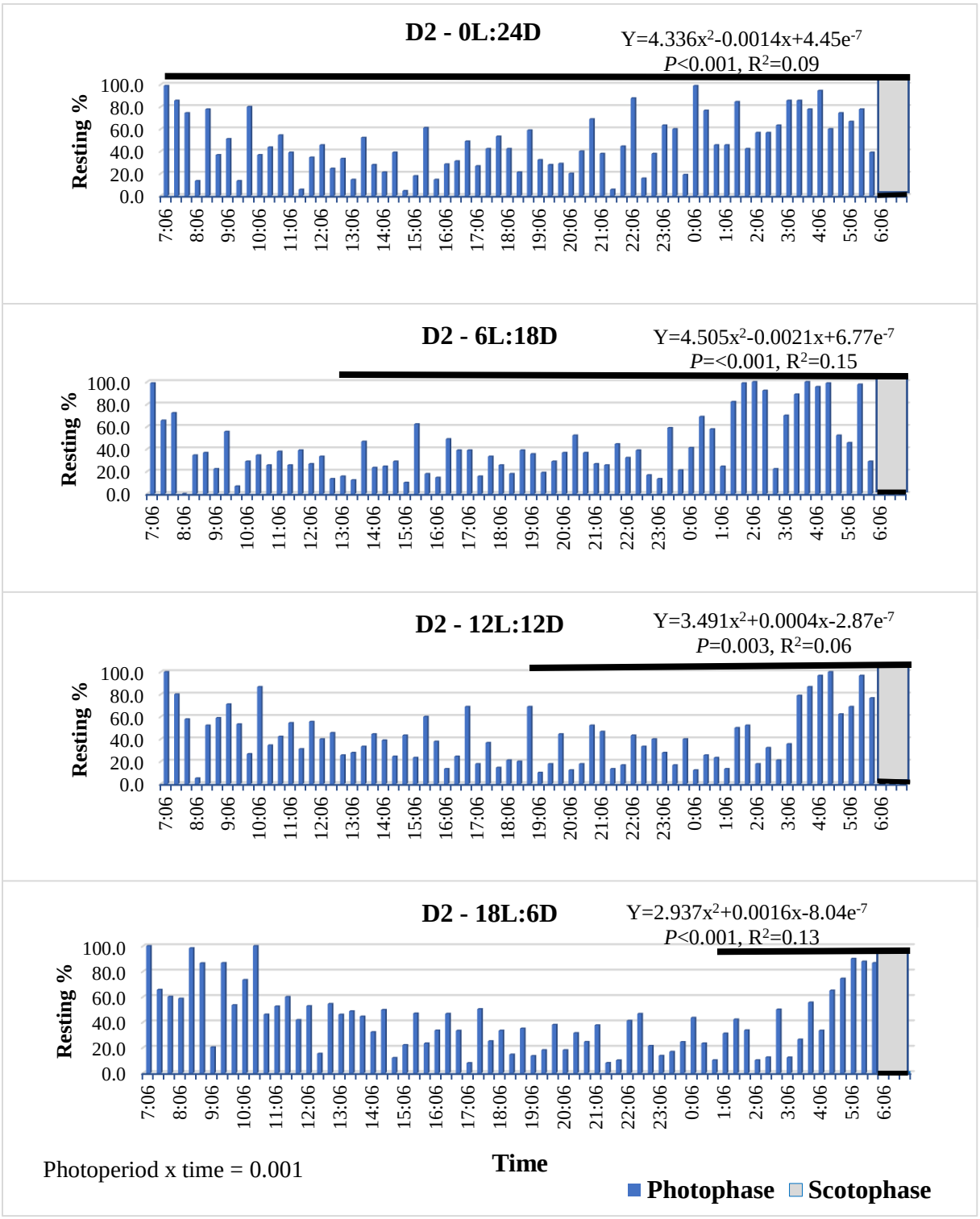

Figure 4.5. Resting behaviour over the photophase at the age of 2 post-hatch from LSL pullets hatched from various photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

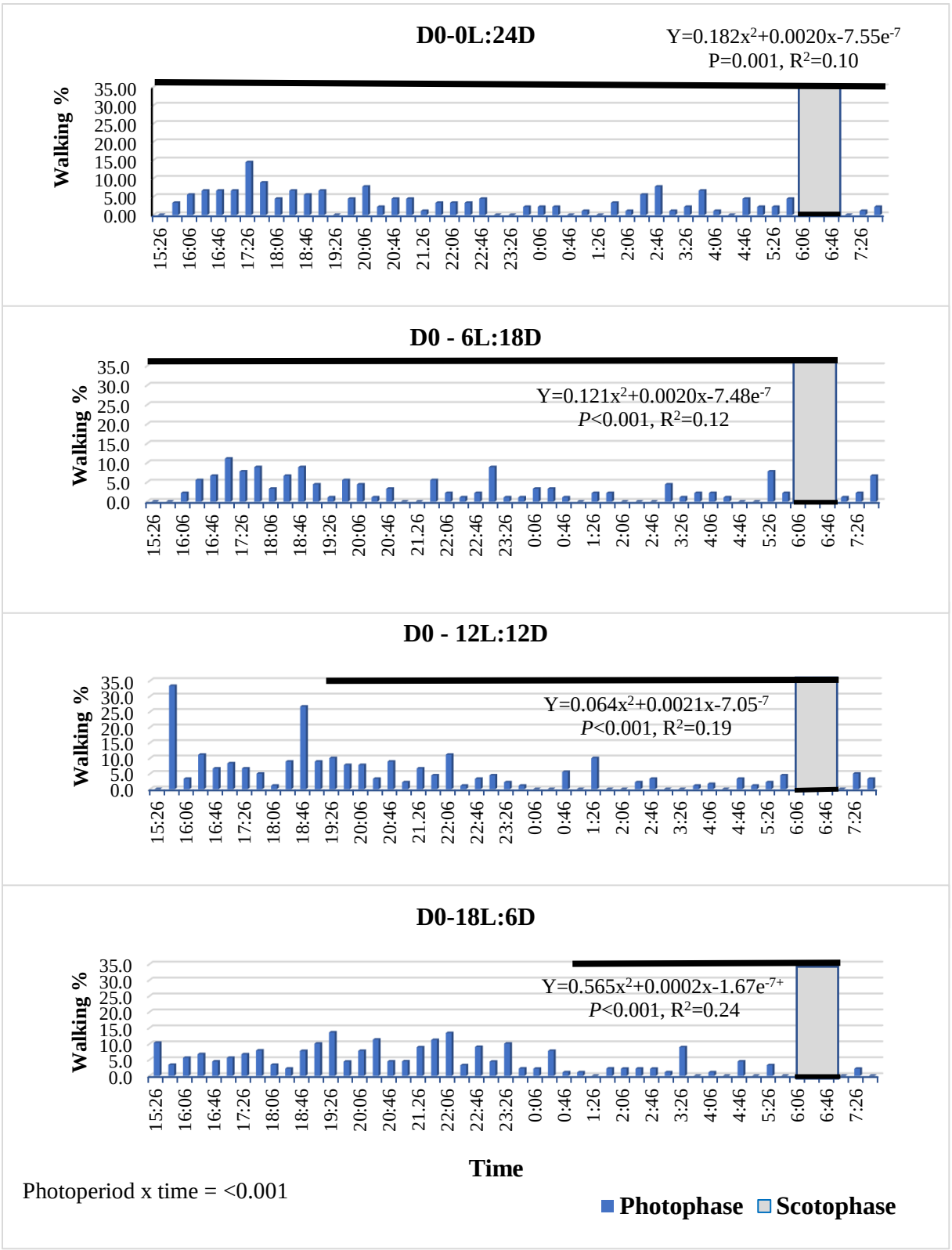

Figure 4.6. Walking behaviour over the photophase at the age 0 post-hatch from LSL pullets exposed to various in-ovo lighting photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

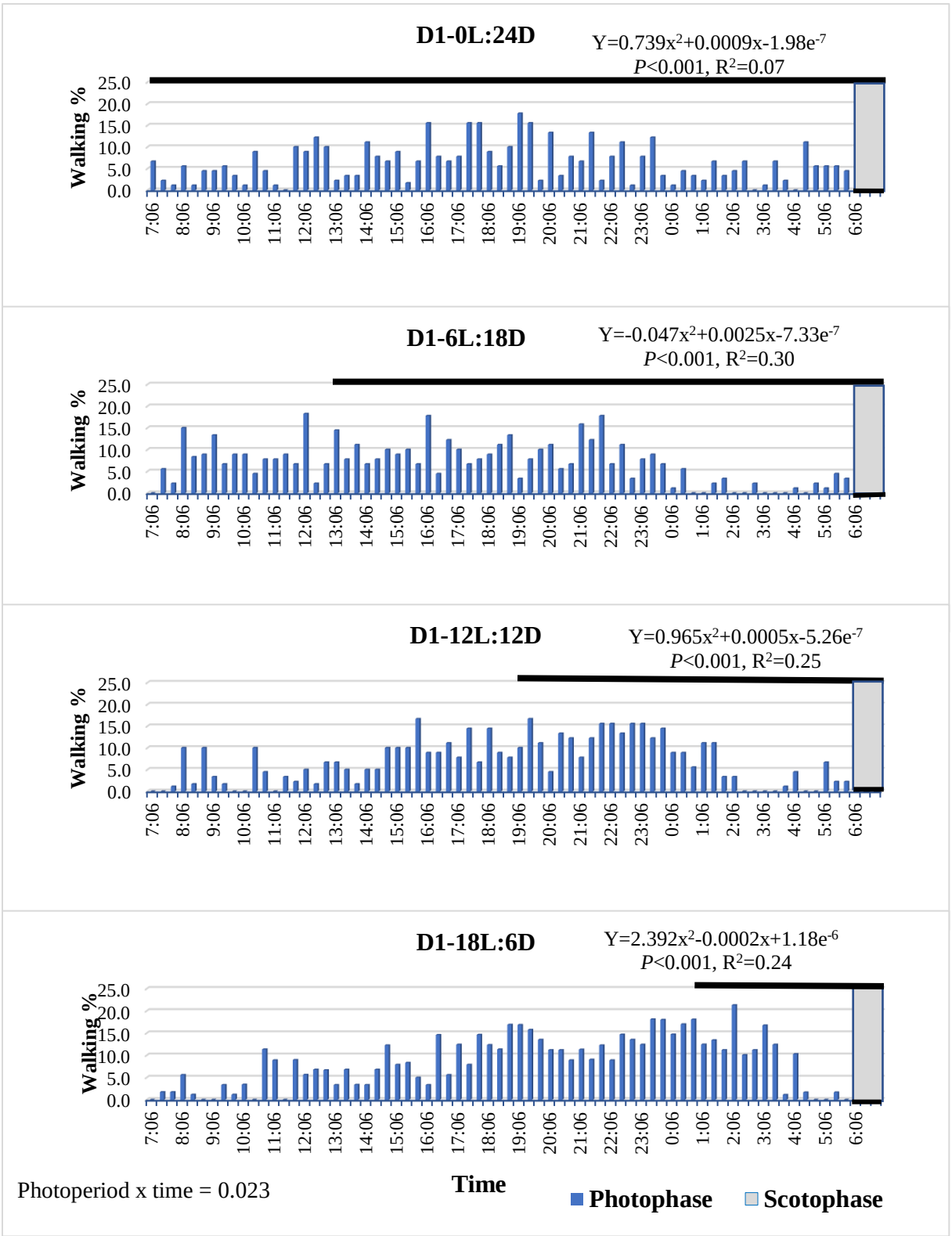

Figure 4.7. Walking behaviour over the photophase at the age of 1 post-hatch from LSL pullets exposed to various in-ovo lighting photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

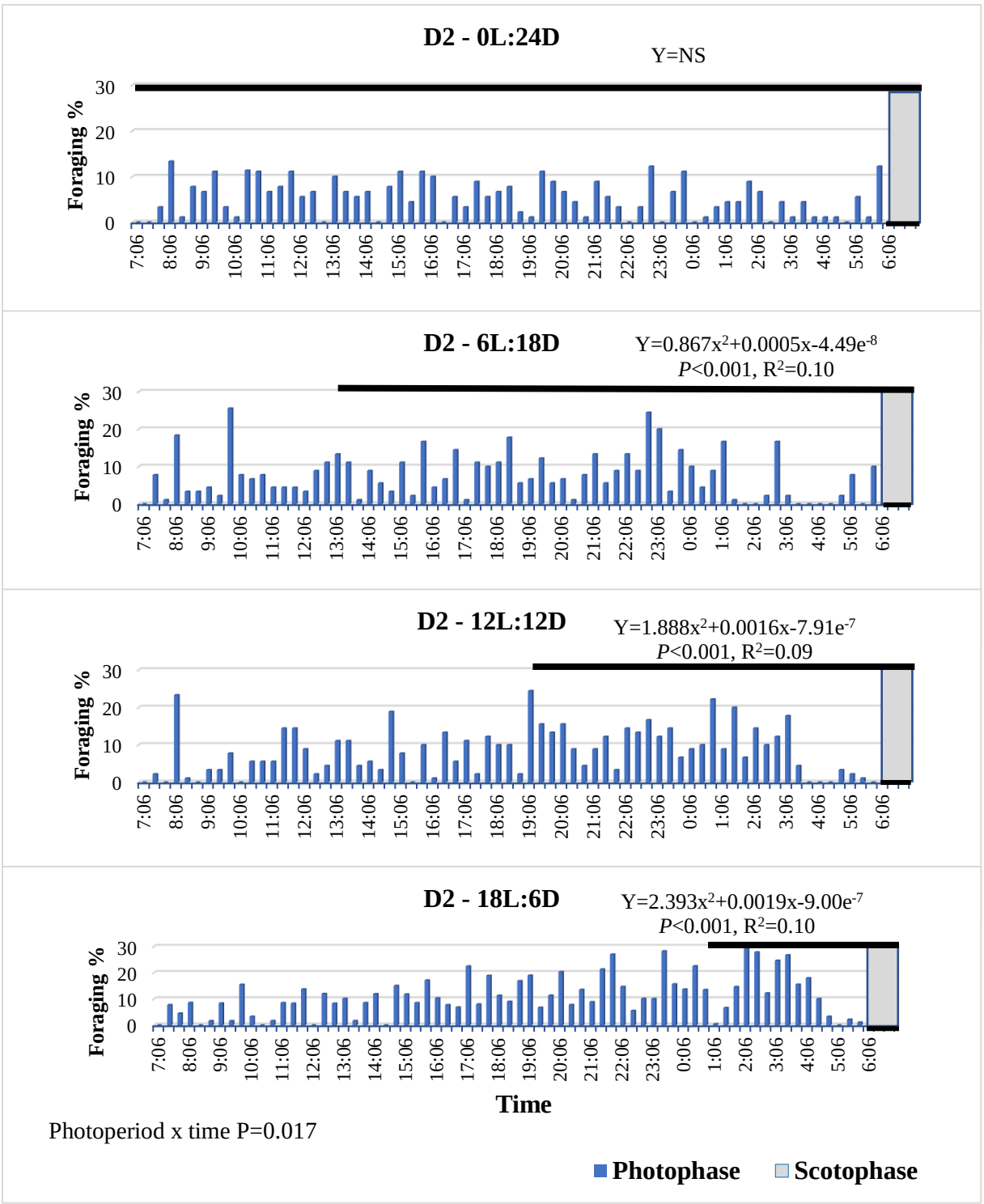

Figure 4.8. Foraging behaviour over the photophase at the age of 2 post-hatch from LSL pullets exposed to various in-ovo lighting photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

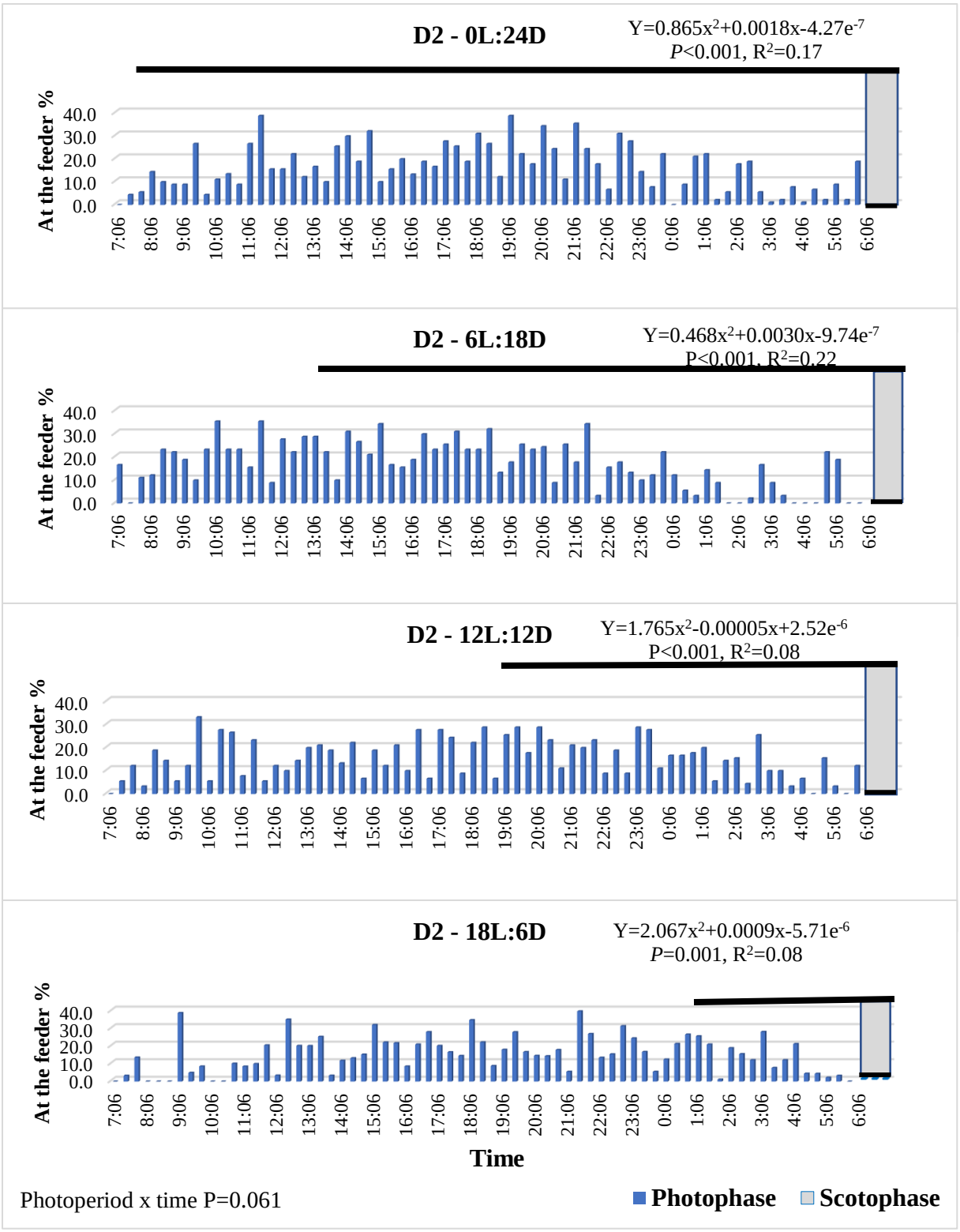

Figure 4.9. At the feeder behaviour over the photophase at the age of 2 post-hatch from LSL pullets exposed to various in-ovo lighting photoperiod during incubation. The horizontal bar on top of the graphs represents the scotophase period during incubation.

5.0 Chapter 5: Overall discussion and conclusion

5.1 Introduction

The increase in consumer awareness and changes in legislation have suggested that new pathways for poultry production management be considered, with particular attention focusing on the animal's wellbeing in their rearing environment. These changes are also impacting how chicks are managed at the hatchery. An example is the "HatchCare system" introduced by Hatchtech, a company based in the Netherlands with clients all over the world. The HatchCare system is based on providing light and feed at the hatching stage. This new technology is being implemented worldwide to improve the quality of chicks delivered to growers (Hatchtech, nd). However, the light has not yet been applied commercially in setters (Carla van der Pol, personal communication). The literature suggests that the use of light during incubation (setters) impacts embryo development (Shafey and Al-mohsen, 2002; Shafey, 2004; Cooper et al., 2011; Huth and Archer, 2015; Archer, 2015b). Those findings from various studies showed that in-ovo lighting improves the quality of chicks at hatch (Shafey, 2004; Archer, 2015a, 2015b; Huth and Archer, 2015), enhances the hatchlings' health and resilience to stress from new environments (Huth and Archer, 2015, Archer, 2015b) and improves growth post-hatch (Özkan et al., 2012b).

Those studies also showed contrasting results, which might be due to differences in experimental design and variables such as the source of light, light wavelength, light intensity, photoperiod and chicken genotype, resulting in difficulties standardizing the best in-ovo lighting management practices to use during poultry species incubation. Additionally, the genotype can impact the characteristics of the eggs, such as eggshell pigmentation (Shafey et al., 2005). The eggshell protects the embryo against threats such as environment adversities including light (Maurer et al., 2015) and can affect how light is transmitted and absorbed by the eggshell (Shafey et al., 2005; Maurer et al., 2015). Currently, there is no information to the author's knowledge regarding the behaviour output post-hatch of hatchlings impacted by in-ovo lighting wavelengths. Furthermore, most of the in-ovo lighting photoperiod studies looked into different photoperiods under white light, and the comparison of impacts on the embryo for graded in-ovo photoperiods under red light is limited. The evaluation of graded photoperiod is important to explain the variability in response to day length effects on embryo and chick post-hatch variables.

5.2 Objectives

The first study aimed to determine the behaviour output post-hatch of two strains of laying hen chicks impacted by in-ovo lighting wavelength during incubation, and evaluate the effects and interactions of the hatchlings under a near continuous (NC) and an intermittent (INT) photoperiod. The second study objective was to evaluate graded photoperiod lengths under dim to red (red) LED light and determine the impact on hatch traits, chick quality, chick health, chick development and behaviour post-hatch of those chicks reared under a singular photoperiod.

5.3 Overall discussion and conclusion

Although live chickens have a high sensitivity to light (Prescott and Whathes, 1999), the use of various light spectrums red, white, or blue during incubation of brown and white egg production layer eggs did not affect pullets early behaviour post-hatch. However, brooding the chicks under an INT photoperiod increased pullets activities regardless of in-ovo lighting wavelength (red, blue, or white).

The photoperiod length during incubation proved to have a more significant impact on post-hatch behaviour than light spectrum. Photoperiod length under red (LED) light impacted the timing of the start and end of the hatch, but it did not affect the time interval between the first and last chicks to hatch. Indeed, it can be concluded by the current research findings that light itself affected the incubation duration, however, the greatest impact was under a longer photoperiod (18L).

Although light duration affected the incubation time, the graded photoperiod length (red LED light) did not affect the percentage of the set and fertile White Leghorn eggs hatched. In disagreement with Archer`s (2015b) findings where the exposure of White Leghorn eggs to red (LED) light under a 12L photoperiod increased hatchability. However, incubation of White Leghorn eggs in a 12L photoperiod under white LED light (Huth and Archer, 2015b) or under a white fluorescent light for 21 days of incubation (Shafey, 2004) did not affect the percentage of chicks hatched. These contradictory findings might result from the differences in light sources and/or light intensity used during incubation. Although using the same variables such as LED`s light, red light wavelength and White Leghorn fertile eggs, the current study differed from Archer's (2015b) study, since that study applied a light intensity of 250 lux at eggs level, whereas the current

study applied 535-568 lux at the eggs level. Shafey et al. (2005) noted that high (1430-2080 lux) light intensity reduced the percentage of hatch from broiler eggs with light and medium eggshell pigmentation compared to low (90-138 lux) light intensities. However, light intensity did not impact hatchability of broiler eggs with darker pigmentation in their study.

The photoperiod length under red (LED) light impacted other White Leghorn chick traits such as body weight at hatch; it was noted that a photoperiod ≥ 6L under red light decreased chick weight at hatch. A similar result was reported by Archer (2015b) who observed reduced body weight at hatch endpoint in chicks exposed to red (LED) light under a 12L:12D photoperiod during the first 18 days of incubation compared to the chicks from a 12L photoperiod under white (LED) light or dark (0L) incubation. Additionally, exposure of White Leghorn eggs to a 12L photoperiod under white light for 21 days did not affect chick weight at hatch (Huth and Archer, 2015; Archer, 2015a). These findings could be an indication of red light affecting early hatching, and affecting chick weight which might be related to yolk sac residue absorption, the waiting time from hatch to the endpoint of the hatching process might have contributed to the increase in the difference in yolk sac residue observed at the hatch endpoint among early and late hatchers. The state of yolk sac residue right after hatch needs to be clarified in future research as the current study only evaluated yolk sac residue at the endpoint of the hatching process. Therefore measuring the residual weight of the yolk sac right after hatch might decrease variablity in weight when comparing to chicks incubated under dark, as chicks from lit incubation hatched sooner than dark incubated chicks which may increase consumption of yolk sac in comparison to chicks from dark incubation.

Embryo mortality was not affected by in-ovo photoperiod, the current study results are supported by Archer (2015a, 2015b) the author reported that exposure of broiler and White Leghorn eggs to 12L photoperiod under white or red (LED) light for the first 18 days of incubation did not impact embryo mortality. Even exposure of the White Leghorn eggs to white (LED) light on a 12L photoperiod for 21 days did not affect embryo or chick mortality at hatch (Huth and Archer, 2015). Additionally, as mentioned previously navel healing was not affected by in-ovo lighting under red light. Even though chicks from the longer photoperiod hatched earlier, the navel of the early hatchlings showed the same healing level as chicks incubated under 0L photoperiod. Our findings concur with Archer (2015b) as the author did not observed effects of a 12L photoperiod under red light on White Leghorn chick's navel healing. However, exposure of White

Leghorn eggs to a 12L photoperiod under white (LED) light provided during the first 18 days (Archer, 2015b) or 21 days (Huth and Archer, 2015b) of incubation showed improvement in chicks navel healing at hatch. This suggests that the effects of light on navel healing are wavelength specific, as the current study used red wavelength and no effects were observed for any lighting treatment. Although in-ovo photoperiod under red light did not impact stress measure H: L and composite asymmetry at hatch nor at 21 days of age. A decrease in H:L ratio from 14 days old broilers exposed to white (LED) light under a 12L:12D photoperiod for 21 days of incubation have been reported (Huth and Archer, 2015).

Chicks' weight at hatch differed between in-ovo photoperiod in the current study, as 18L chicks were the lightest and 0L chicks were the heaviest with minimal changes over the 21 days growing period. In contrast broilers incubated for 21 days under a 12L photoperiod illuminated by white (LED) light did not differ in body weight at hatch nor 14 days post-hatch (Huth and Archer, 2015). Not even exposure to light in either the first 18 or 21 days of incubation under a 12L white (LED) light impacted broilers body weight at 45 days post-hatch (Archer, 2015a). The lower chick weight at hatch observed in the current study could be a result of experimental design, as chicks were weighed at the hatch endpoint, therefore chicks that hatched early had to wait longer without feed and water to be processed which could have resulted in body weight reduction due to the prolonged waiting time at the hatchers. Additionally, regardless of the differences in body weight in the current study the uniformity of the pullets up to day 21 post-hatch did not differ between treatments.

The length of the photoperiod during incubation affected chicks early behaviours. Incubation using ≥12 photoperiods increased chicks' locomotor behaviour post-hatch. A longer photoperiod such as 18L used during incubation increased activities such as standing, running, walking and foraging, on the other hand, a photoperiod ≤12L increased resting behaviour. Archer and Mench (2014b) did not observe an impact of 0L, 1L, 6L, or 12L photoperiods on chicken activities over the photophase post-hatch. Additionally, behaviour differences that initially were noted to occur in a diurnal rhythm were no longer found by 3 days of age, sugesting that an in-ovo lighting photoperiod entrainment might exist and that by the 3rd day the chicks are entrained to the new photoperiod.

It can be concluded that the variables of light wavelength and photoperiod might be used during incubation and is dependant on bird genotype. Red light under a daily photoperiod of

between 12 and 18 hours of light can be applied in laying hen white eggs incubation. This amount of light during incubation can decrease the incubation time without adverse effects on the hatched chick health and welfare. However, that reduction in incubation time might also impact chick weight at hatch when under red light. Photoperiod length also impacted chicken behaviour post-hatch, increased activities as day length increased while chicks hatched from a dark incubation spent a higher percentage of time resting. The hatchlings spent a higher percentage of the time during the photophase more active on the first day's post hatch when incubated under a longer photoperiod of up to 18L or providing an INT photoperiod post-hatch, which might stimulate finding feed and water due to the increased mobility on the first day's post-hatch. In short, the impact of light on some traits is wavelength, photoperiod, and genotype dependent. However light wavelength stimulus during incubation has minimum impact on early behaviour of pullets.

6.0 References

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