The effects of LED light flicker on turkey hen production, health, and welfare to 11 weeks of age

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Overall Abstract

This study examined the impacts of light-emitting-diode flicker on Nicholas Select turkey hens (n=3267) to 11 weeks (wk) of age. There were three treatments: the 30 Hertz (Hz) treatment (30HZ) the 90 Hz treatment (90HZ) and the 195 Hz treatment (195HZ). Birds were randomly assigned to nine independently controlled rooms (364 birds/room at placement). Performance was evaluated through body weight (BW) at 0 days (d), 4, 8, and 11 wk of age. At 0d-4, 4-8, and 8-11 wk BW gain, feed intake (FI) and feed-to-gain ratio were examined. At 10 wk, a sample of 30 birds/room was individually weighed to examine uniformity. Mortality and culls were also collected twice daily. Video recordings were taken at 4, 8, and 10 wk for 18 hrs which were scan sampled at 20-minute intervals to determine the percentage of time (%t) birds spent performing different behaviours. At 10 wk, 20 birds/room were examined for mobility (gait score), footpad lesions, feather condition, and feather cleanliness. At 11 wk 4 birds/room were euthanized and the right eyeball was excised and measured for weight, medio-lateral diameter, dorso-ventral diameter (DV), and anterior-posterior size (AP). Heterophil-to-lymphocyte (H/L) ratios were determined using blood samples at 4, 8, and 11 wk (20 birds/room). Litter quality was examined at 11 wk. Incidences of aggressive damage on birds were monitored twice daily. For the first 7 d and at 4, 8, and 11 wk, a novel object test (NOT) was performed to examine fearfulness. Data were analyzed using Proc Mixed (SAS 9.4). Tukey's range test was used to separate means with significance declared when $P<0.05$. Birds in the 30HZ weighed less than those in the 195HZ at 8 wk with those in the 90HZ intermediate ($P=0.025$). The birds in the 30HZ had higher BW gain from 8-11 wk compared to those in the 90HZ with those in the 195HZ intermediate ($P=0.003$). From 0d-4 wk and 4-8 wk, birds in the 30HZ had reduced FI ($P\leq0.001$, 0.001, respectively). Mortality corrected feed-to-gain was improved from 8-11 and 0d-11 wk in the 30HZ compared to the 90HZ with the 195HZ intermediate ($P=0.048$, 0.040 respectively). Infectious mortality was reduced ($P=0.049$) in the 195HZ versus the 90HZ from 0d-11 wk with the 30HZ intermediate. Total mortality was reduced in the 195HZ from 0d-11 wk ($P=0.024$) and 0d-4 wk ($P=0.005$). From 4-8 wk, round heart mortality was higher in the 30HZ ($P=0.040$). Footpad scores of 0 were seen more frequently for birds in the 90HZ, resulting in better average footpad scores ($P=0.011$, 0.021, respectively). Average feather cleanliness was improved and scores of 1 were more frequent for birds in the 90HZ compared to those in the 195HZ with those in the 30HZ intermediate ($P=0.021$, 0.030, respectively). Flicker frequency did not affect litter quality or NOT. The DV (*P*=0.046) and AP (*P*=0.033) of birds in 30 Hz were increased compared to 195 and 90 Hz, respectively. Bird behaviour varied across treatments with no clear pattern. The %t birds spent gentle feather pecking was reduced at 4 and 8 wk in the 30HZ (*P*=0.040, 0.016 respectively). At 8 wk, %t wing flapping was reduced by birds in the 195HZ (*P*=0.004). The %t birds spent fighting (*P*=0.049) and aggressive pecking (*P*=0.022) was reduced in the 30HZ compared to the 90HZ at 8 wk with the 195HZ intermediate. Birds reduced the %t preening in the 30HZ compared to the 90HZ and 195HZ at 10 wk (*P*=0.034). Incidences of aggressive damage were reduced at all ages at various locations for birds in the 30HZ with the exception of damage directed towards the neck at 11 wk (*P*<0.001). The H/L ratio of birds in the 30HZ was reduced compared to those in the 195HZ at 11 wk with those in the 90HZ intermediate (*P*=0.044). Overall, visible light flicker had negative impacts on production early in a turkey's life; however, effects were not seen by the end of the production period. Aggression was reduced under the 30HZ; however, this may not be beneficial as other behaviours indicate this may have been due to a reduction in activity. Stress also seems to be reduced, based on H/L ratios, when lamps flicker at 30 Hz, though this is likely due to the reduction in aggression rather than a direct effect of flicker.

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List of Abbreviations

195 Hz treatment .... **(195HZ)**
30 Hz treatment .... **(30HZ)**
90 Hz treatment .... **(90HZ)**
Adrenocorticotropin hormone .... **(ACTH)**
Ammonia .... **(NH_3)**
Anterior-posterior size .... **(AP)**
Body weight .... **(BW)**
Carbon dioxide .... **(CO_2)**
Corticosterone .... **(CORT)**
Critical Flicker Fusion Frequency .... **(CFF)**
Day .... **(d)**
Dorso-ventral diameter .... **(DV)**
Feed intake .... **(FI)**
Feed-to-gain mortality corrected ratio .... **($F:G^m$)**
Feed-to-gain ratio........ **(F:G)**
Flicker Fusion Frequency .... **(FFF)**
Gentle feather pecking .... **(GFP)**
Hertz .... **(Hz)**
Heterophil-to-lymphocyte .... **(H/L)**
Hypothalamic Pituitary Adrenal .... **(HPA)**
Light-emitting-diode .... **(LED)**
Novel object test .... **(NOT)**
Percentage of time .... **(%t)**
Sudden death associated with perirenal hemorrhage .... **(SDPH)**
Sympathetic Adrenal Medullary .... **(SAM)**

1.0 Chapter 1: Literature Review: The effect of light flicker on the production, health, and welfare of turkey hens

1.1 Introduction

The investigation into artificial light and its effects on poultry has become increasingly focused on light-emitting-diodes **(LED)** in the wake of incandescent bulbs being discontinued internationally starting in 2012 (Waide, 2010). LED bulbs have become the most popular alternative to incandescent bulbs as they are more durable, energy-efficient, customizable, and have lower operating costs (Khan and Abas, 2011; Benson et al., 2013; Thomson and Corscadden, 2018). With the increased use of LED bulbs, any issues that arise with their use need to be investigated. While most research has focused on the effects of intensity and wavelengths that LED bulbs produce, the effects of flickering have been investigated minimally.

Light flicker is the rapid change in the intensity of lights. Flickering is common in fluorescent and LED bulbs but has been researched primarily in fluorescent bulbs with humans and European starlings. Flickering of fluorescent bulbs impacts the health of humans, inducing headaches and eyestrain (Brundrett, 1974). Flickering of LED bulbs, while studied less, is still thought to impact humans with photosensitivity by inducing conditions such as epilepsy (Fisher et al., 2005; SCHEER, 2018). There is minimal evidence of the impacts of flickering light on poultry production, and there are also no regulations on flicker or flicker frequency during production in Canada (NFACC, 2016). However, while LED bulbs do flicker, most research has been done on fluorescent bulbs, representing a knowledge gap with LEDs being used more frequently. This study strives to fill this gap by investigating how LED light flicker affects turkey hen health, welfare, and production using a wide variety of measures.

1.2 Vision in birds

Vision is one of the most important senses in birds, with the eyeball comprising approximately 50% of the cranial space (Bayon et al., 2007; Vallone and Kern, 2021). Birds have two types of photoreceptors: cones, which allow for vision in daylight and colour, and rods, which assist in low-light vision (Jones et al., 2007). The cones of domestic turkeys also contain oil droplets, which help make specific cones sensitive to different types of wavelengths by altering the spectral transmission (Hart et al., 1999; Jones et al., 2007). Domestic turkeys (*Meleagris gallopavo domesticus*) have further specialization of their visual system with five types of rhodopsin, visual pigments, within their retinae (Hart et al., 1999; Prescott et al., 2003).

Turkeys also have seven different subtypes of photoreceptors, allowing them to detect a greater range of wavelengths than humans (315-750 nm, 400-750 nm, respectively; Hy-Line, 2017). The anatomy of a bird's eye also allows them to have highly sensitive vision. Birds' fovea, which is a dip in the retina where the center of the field of vision is focused, has a high density of cones, which increases the sharpness of an image (Figure 1.1; Inzunza et al., 1991; Jones et al., 2007; Hendrix et al., 2021). Because of the ocular specializations which improve poultry vision, poultry species can be extremely susceptible to behavioural, welfare and health issues based on their heightened sensitivity to light.

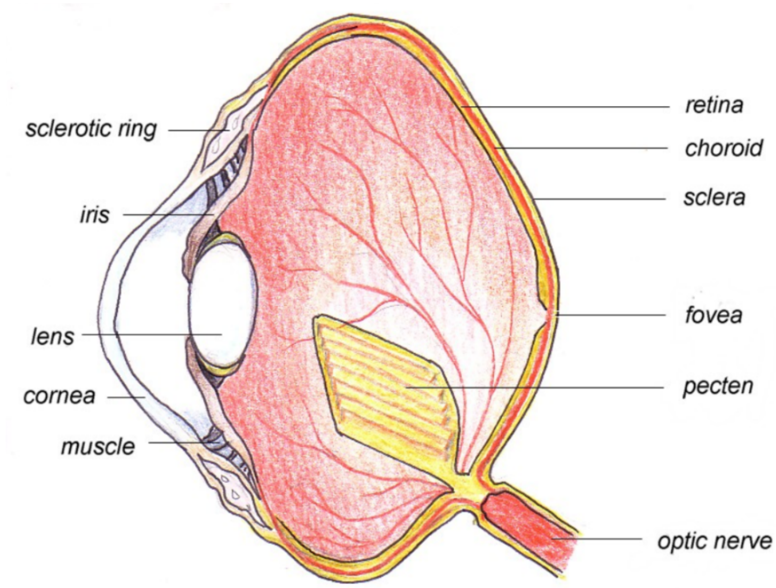

Figure 1.1 Avian eye anatomy adapted from Bleak (2008)

1.3 Poultry lighting

Artificial light is used within most commercial poultry operations to mimic natural daylengths (Allen et al., 1954; Prescott et al., 2003). More recently, different wavelengths have been investigated to mimic daylight as well. However, this is less commonly used within commercial poultry operations (Spindler et al., 2020).

1.4 Light flicker

Light flicker is typically caused by three conditions: fluctuations in supply voltage, improper bulb selection, and incompatible dimming systems (Chmielowiec, 2011; Wang et al., 2012; Drapela et al., 2018; Collin et al., 2019). The conditions mentioned above result in flickering, the rapid change in light intensity coming from the bulb (Collin et al., 2019). Fluctuations in supply voltage happen naturally, as alternating current occurs in a sinusoidal waveform (positive and negative). LED bulbs only allow current to pass through them in one direction, so electronic drivers are used to rectify and smooth out the waveform, creating a direct-current source, though it still fluctuates slightly (Wang et al., 2012; Drapela et al., 2018; Collin et al., 2019). Oscillations in light intensity can be caused by gradual or abrupt deterioration of the electronic components used to create the direct-current source. Fluctuations can also occur when an object with a high need for power draws more power from the system, which results in less power available for the functioning of the bulbs (Benson et al., 2013). Improper bulb selection can cause flickering as different electronic drivers within LED bulbs are suited for different applications. LED drivers are configured to work for different systems to minimize the effect that voltage fluctuations have on light output (Chen and Ron Hui, 2012; Wang et al., 2012). Finally, dimming systems cause flicker in LED light because some existing dimmer systems are incompatible with new LED bulbs and cause various issues including flickering and insufficient dimming (Javvadi, 2011). Flickering can occur while dimming an LED bulb as the electronic drivers cannot smooth out the modulation of the voltage, causing flicker to be perceptible to the human eye (U.S. DOE and Ashdown, 2008). Incompatible dimmer systems can also result in failure of an LED bulb to produce light if using a dimming switch on a non-dimmable LED bulb (Rand et al., 2007).

1.4.1 Flicker fusion frequency in poultry

Two measures are important when looking at the effects of light flicker in any species: flicker fusion frequency **(FFF)** and critical flicker fusion frequency **(CFF)**. The point at which a flickering light is seen as a continuous stream of light by an observer is the FFF for that observer (Lisney et al., 2011). The highest frequency, at any intensity, that flickering can be unconsciously detected by an observer is their CFF (Nuboer et al., 1992; Lisney et al., 2012). Flicker frequencies past the CFF are not perceived and are termed 'invisible flicker' as the light is still flickering, but the observer cannot consciously or unconsciously detect it. These measures are important for determining if a subject can detect light flicker and at what frequencies detection occurs. Lights have a standard flicker frequency of 120 Hertz **(Hz)** in the United States and 100 Hz in Europe, which were thought to be above the FFF of birds (105 Hz; Nuboer et al., 1992; Prescott et al., 2003). The CFF of birds depends on the species, light source, wavelength, illuminance, and individual species' physiology, resulting in a range from 39.2 Hz to 119 Hz (Nuboer et al., 1992; Jarvis et al., 2002; Lisney et al., 2012; Inger et al., 2014). As there are individual differences in the perception of flickering, the specific lighting regimes and anatomy of turkeys' eyes may impact their CFF.

The use of dim lighting in turkey management may impact the perception of flickering because flickering does not always relate linearly to the fluctuation in energy sources as the lights are dimmed (Prescott et al., 2003; Lisney et al., 2011). At lower intensities, the visual rods are the primary photoreceptors involved in vision and are more sensitive than cones, which may increase sensitivity to flickering at low light intensities (Lisney et al., 2011).

1.4.2 Light flicker in humans

While there is little to no experimentation on human sensitivity to LED light flickering, there is information available on the flickering of fluorescent bulbs. Brundrett (1974) found that human sensitivity to light flickering of fluorescent bulbs is highly variable has a tendency to reduce with age and decreasing pupil size. The CFF in humans is typically between 50 and 60 Hz, and humans prefer higher flicker frequencies, especially for extended periods (Wilkins et al., 1989; Nuboer et al., 1992). Using techniques such as electroretinograms, it has been found that the human eye will respond to flicker frequencies higher than the human FFF (Brundrett, 1974; Burns et al., 1992; Davis et al., 2015). While these higher flicker frequencies are not distinguishable by humans, they can have effects on the physical and psychological systems (Wilkins et al., 1984, 1989; Hazell and Wilkins, 1990; Veitch and McColl, 1995; Sandström et al., 1997). Physically, pain is typically observed in the eyes and brain, leading to fatigue of the eyes and brain, headaches, and potentially epileptic episodes (Wilkins et al., 1984, 1989; Harding and Jeavons, 1994; Veitch and McColl, 1995; Sandström et al., 1997). Psychological factors have been studied less; however, Hazell and Wilkins (1990) found that agoraphobic (extreme fear of entering open or crowded places) patients had more symptoms under fluorescent light, including 'difficulty speaking, eyes feeling irritated, legs feeling weak, and tight band around the head'.

1.4.3 Light flicker in turkeys

There is minimal research on light flicker and turkeys. In one study, Sherwin (1999) examined turkey preference for either fluorescent or incandescent light. The authors did not indicate the flicker frequency of the tested fluorescent light; however, they did note that the rates were typically past the point of human perception. It was determined that individual turkeys preferred the fluorescent lighting treatment regardless of the type of light they were reared under (incandescent or fluorescent; *P*=0.0001). Both light-reared groups preferred the fluorescent light (incandescent: *P*=0.0001 and fluorescent: *P*=0.0003). The authors concluded that if the turkeys could perceive the light flicker from the fluorescent bulbs, it was not aversive to them.

The impact of LED light flicker on the productivity and stress of turkey toms has also been examined (Raabe et al., 2024). The authors used three different flicker frequencies (165, 500, or 16,000 Hz) to see the impact on tom BW (1 day; **d,** 141d), FI, mortality/morbidity, injury, and feather corticosterone **(CORT)** concentration. The authors found no effect of light flicker on any of the measured parameters.

1.4.4 Light flicker in European starlings

One of the few avian species that have been studied more thoroughly under the influence of flickering light is European starlings. Maddocks et al. (2001) looked at how light flicker affected the stress of European starlings using CORT levels. This study used wild birds acclimatized to their environment under fluorescent lighting. A low flicker frequency (100 Hz) or high flicker frequency (35-40 kHz) were used during the photoperiod as the treatments. The authors found no treatment effect on the maximal level of CORT (*P*=0.561), but the basal level was higher, indicated more chronic stress (*P*=0.023) in the 100 Hz versus the 35-40 kHz treatment.

Greenwood et al. (2004) also tested European starlings to examine how fluorescent light flickering affects welfare. The birds were caught from the wild and allowed to acclimatize before the experiment was initiated to determine a baseline for CORT levels (Greenwood et al., 2004). Birds were exposed to low flicker (100 Hz) and high flicker (>30 kHz) to see how the stress of starlings was affected. They found no change in basal or maximal CORT (*P*>0.05). Greenwood et al. (2004) also examined the preference for high versus low flicker frequencies of fluorescent light. They determined that birds preferred the high-frequency (*P*<0.001) over the low-frequency flicker. The behaviours that Greenwood et al. (2004) observed were eating, drinking, moving, aggressive pecking, somersaulting, bathing, putting the head out of the cage, and preening. Observations were performed live for 30 minutes, and each behaviour was recorded as an event, so the frequency of behavioural events was determined during the observation time. It was concluded that treatment had no effect on any of the behaviours.

The use of wild birds by Maddocks et al. (2001) and Greenwood et al. (2004) may have contributed to the differences in stress responses regardless of the adjustment period rather than the treatment alone. Both experiments gave conflicting results, further emphasizing the need for research in poultry species under light flicker as there is clearly a knowledge gap in how birds are affected by flickering light.

1.4.5 Stress in poultry

Stress and fear in animals are difficult to quantify, which means that several techniques are often used to measure poultry stress. When birds are stressed, their body responds by stimulating the sympathetic adrenal medullary **(SAM)** pathway and/or Hypothalamic Pituitary Adrenal **(HPA)** axis (Borah et al., 2022). The SAM pathway is activated first when a bird experiences a stressor (Chu et al., 2022). The stressor stimulates sympathetic neurons, which induce the release of epinephrine and norepinephrine from chromaffin cells in the adrenal medulla (Chu et al., 2022). Once in the bloodstream, epinephrine and norepinephrine bind to specific receptors throughout the body, increasing vasoconstriction, smooth and cardiac muscle constriction, and heart rate (Chu et al., 2022). Other functions are reduced through the binding of epinephrine and norepinephrine, including intestinal mobility and bronchial dilation (Chu et al., 2022). In combination, this response results in the physiological changes necessary for the ‘fight or flight’ response (Borah et al., 2022).

The secondary stress response occurs through the activation of the HPA axis (Figure 1.2). The stressor results in the stimulation of the hypothalamus to release corticosterone-releasing hormone. Corticosterone-releasing hormone passes from the hypothalamus to the anterior pituitary gland, which stimulates it to release adrenocorticotropic hormone **(ACTH)**. ACTH passes through the bloodstream to the adrenal cortex, which is then stimulated to release CORT into the bloodstream. CORT can interact with target cells to induce gluconeogenesis and glycogen breakdown to release glucose for cells to use as energy (Løtvedt et al., 2017). Chronic activation of the HPA axis and, therefore, increased CORT levels causes a drop in the production of immune cells (Dohms and Metz, 1991; Shini et al., 2010).

1.5 Measures to determine impact of light flicker on birds

1.5.1 Productivity traits

Production metrics, such as growth, feed efficiency, and mortality of laying hens, turkeys, and broiler chickens are impacted by lighting parameters, including photoperiod, intensity, and wavelength (Rozenboim et al., 1999; Rogers et al., 2015; Mohammed et al., 2016; Vermette et al., 2016a; Oso et al., 2022). However, little information is available on the effects of light flicker on production traits.

1.5.1.1 Growth rate

Productivity in the form of body weight **(BW)** and BW gain have been minimally studied in avian species exposed to light flicker. Raabe et al. (2024) examined the effect of LED flicker (165, 500, or 16,000 Hz) on the BW of turkey toms and saw no effect at 0 d or 141 d. Evans et al. (2012) examined the short-term effects of high (>30 kHz) and low (100 Hz) flicker frequencies of fluorescent light on European starlings. Their birds were kept under high or low flicker and then switched to the opposite light flicker treatment, which they labelled as phases. The authors found that birds in their experiment lost weight during the first week **(wk)** of the trial regardless of the treatment type/phase.

1.5.1.2 Feed intake

There is minimal evidence of the impacts of flickering light on feed intake **(FI)** in poultry. Raabe et al. (2024) found no differences in the FI of turkey toms from 0-141 d when raised under LED flicker of 165, 500, or 16,000 Hz. Evans et al. (2012) found European starlings increased time feeding under high flicker (>30 kHz) compared to low flicker (100 Hz; $P=0.016$) in fluorescents. The authors concluded that the birds were compensating for increased energy consumption due to higher activity levels in >30 kHz than 100 Hz.

1.5.1.3 Feed efficiency

Feed efficiency is a common production measure taken in species to determine the effect a treatment has on the ability of an animal to convert feed into growth. Feed efficiency is typically measured using feed conversion ratio (feed-to-gain; **F:G**). A F:G is measured by collecting an animal's initial and final BW and comparing them to the FI. The represents how much feed is required to produce a unit of weight gain in the animal. F:G is commonly determined for poultry flocks as it is relatively easy to calculate. Average BW gain is calculated using a sample of animals. The BW gain is measured by weighing an animal at different times during a trial. One can determine BW gain wk to wk or from the start to finish of a production cycle. The FI is measured by taking the initial feed weight and comparing that to the weight of feed remaining in feeders and storage at the time BW is taken. The average BW gain and overall FI can be used to determine the average feed efficiency of a group of birds. To date, there are no published scientific data on the impacts of light flicker on the feed efficiency of poultry.

1.5.1.4 Uniformity

Uniformity is how close members of a flock of birds relate to each other with respect to BW. Uniformity is usually determined by individually weighing a representative sample of birds and then determining how many birds are close to the average weight of the flock (Beaulac et al., 2019). One can also measure the coefficient of variation of that group. Currently, there is to my knowledge no published research on how light flicker impacts flock uniformity.

1.5.1.5 Mortality

Mortality refers to the number of deaths in a population during a given time or place. However, within the poultry industry, mortality rates can also include those animals that are culled, often referred to as “mortality and morbidity”. Mortality can occur due to severe physical injury, stress, or compounded nutritional issues, which leads to culling (Aviagen Turkeys, 2015). Determining cause of death is important for producers to understand underlying causes, as there are many possibilities.

1.5.1.6 Causes of mortality

There are generally multiple causes of mortality/morbidity in commercial turkeys. These can be classified into groups, including metabolic, infectious, mechanical, behavioural, and unknown causes.

1.5.1.7 Metabolic causes of mortality

1.5.1.7.1 Round heart disease

Round heart disease, sometimes called dilated cardiomyopathy, was a primary cause of mortality within the turkey industry; however, its occurrence has decreased since the 1980s (Hargis, 2023). Round heart disease is a type of spontaneous cardiomyopathy with death typically occurring at the end of the second wk of age (Sautter et al., 1968), but can occur up to 14 wk old (Crespo and Shivaprasad, 2008). Upon post-mortem inspection, the heart is enlarged with both ventricles dilated, giving a rounded appearance (Crespo and Shivaprasad, 2008). The right ventricle is typically more dilated, with the left showing more hypertrophy (Stenzel et al., 2008).

Birds afflicted by round heart disease present with poor respiratory function, drooping wings, and ruffled feathers (Sautter et al., 1968; Czarnecki, 1984). Birds that do not die from round heart disease underperform as they grow (Sautter et al., 1968).

The cause of round heart disease is unknown. It has been associated with stressful events in birds (Sautter et al., 1968). There is some evidence of a genetic component and that high altitudes and cold weather may also have an effect (Frame et al., 1999). Because of the indication that stressful events may result in round heart disease, if light flickering is stressful to turkeys, it may increase the incidence of round heart.

1.5.1.7.2 Sudden death associated with perirenal hemorrhage

Sudden death associated with perirenal hemorrhage **(SDPH)** in turkeys is another metabolic disease that causes mortality. SDPH tends to affect males between 8 and 14 wk of age; however, females can suffer from the same condition (Mutalib and Hanson, 1990; Frank et al., 1991; Larochelle et al., 1992; Boulianne et al., 1993; Crespo and Shivaprasad, 2008). The main gross lesion found in birds that die from SDPH is mild to severe perirenal hemorrhaging (Mutalib and Hanson, 1990; Frank et al., 1991; Larochelle et al., 1992; Crespo and Shivaprasad, 2008). Several other lesions that can accompany hemorrhaging include an enlarged heart, pulmonary congestion, congestion of the spleen, and congestion of the kidneys (Mutalib and Hanson, 1990; Frank et al., 1991; Larochelle et al., 1992). The hemorrhaging is not thought to be the cause of death, and the actual cause is unknown. There are several theories as to the cause of SDPH, with acute cardiomyopathy of an unknown cause as the main theory (Larochelle et al., 1992). Mutalib and Hanson (1990) postulated that pulmonary congestion leading to respiratory acidosis causes ventricular fibrillation, which is the resultant cause of death. Birds dying from this disease usually have a full crop and gizzard and are well-muscled (Frank et al., 1991).

It is suggested that stress due to exercise, fighting, changing location, heat, and humidity exacerbate the heart failure (Mutalib and Hanson, 1990; Larochelle et al., 1992; Boulianne et al., 1993). If hysteria and/or aggression are increased by light flicker, there may be an increase in SDPH mortality of turkeys raised under flickering LED light.

While round heart and SDPH have similar etiology, the main difference seems to be the rate at which the disease causes death, with SDPH occurring more acutely (Larochelle et al., 1992).

1.5.1.8 Infectious causes of mortality

The potential influence of LED light flicker on stress, hysteria, and aggression could lead to an increase in the incidences of infectious diseases either in combination or individually. The primary infectious diseases that would be increased in a turkey flock under these conditions are osteomyelitis, pericarditis, peritonitis, polyserositis, yolk sac infection, and viral arthritis. Stress is known to reduce the immune system's effectiveness, increasing an animal’s susceptibility to infectious diseases.

At any given time, changes in a bird’s environment (light, temperature, air quality, environment cleanliness, and feed) can cause stress (Abo-Al-Ela et al., 2021). In general, all of these stressors impact the HPA axis (Figure 1.2). If a bird is exposed to a stressor for a short period of time, the increase in CORT subsequently leads to an increase in the mobilization of heterophils from the bone marrow (Shini et al., 2010; Abo-Al-Ela et al., 2021). This, in turn, causes more production of pro-inflammatory cytokines. These changes to the immune cells being produced assist birds in fighting off infections. If a bird is exposed to a stressor for a long period, there is a slightly different effect. Because of prolonged exposure to CORT and ACTH, the immune organs shrink in size, which causes those organs to produce fewer immune cells (Dohms and Metz, 1991; Shini et al., 2010). The immune system will, therefore, no longer be in a high-activity state; rather, it will enter an immunosuppressed and anti-inflammatory state (Dohms and Metz, 1991; Shini et al., 2010). These states result in lower antibody production caused by reduced B- and T-cell production. With fewer antibodies being produced, birds would be more susceptible to infectious diseases (Stier et al., 2009).

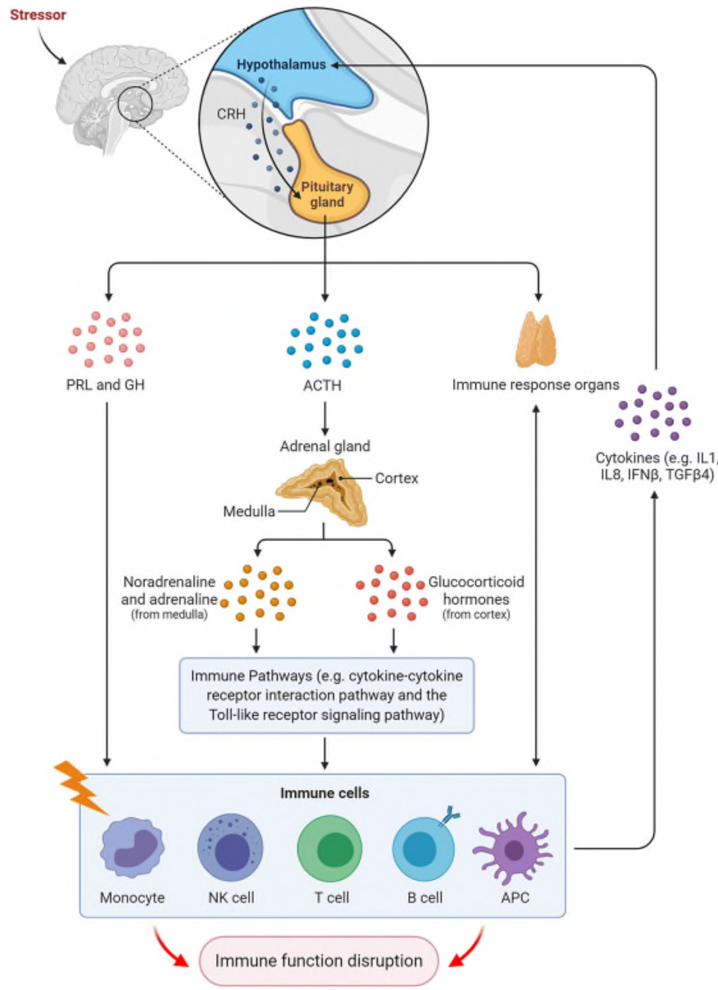

Figure 1.2. Response of the HPA axis to a stressor in order to influence immune function (Abo-Al-Ela et al., 2021)

1.5.1.8.1 Osteomyelitis

Osteomyelitis is the result of a bacterial infection that tends to impact turkey toms more than hens (Mutalib et al., 1996). Turkeys are generally affected between 9 and 20 wk of age (Huff et al., 2000). Osteomyelitis is recognized by the soft, yellow necrotic growth plate, and distortion and destruction on the end of the long bones (Nairn, 1973; Julian, 1985). Stress-induced immunosuppression may lead to increased incidences of osteomyelitis (Huff et al., 2000).

1.5.1.8.2 Pericarditis

Pericarditis is the inflammation of the pericardium, a protective membrane around the heart (“Pericarditis | Heart and Stroke Foundation,”). There are many causes of pericarditis, with viral infections the primary cause (Saif, 2008).

1.5.1.8.3 Peritonitis

Peritonitis is the inflammation of the peritoneum, the protective membrane surrounding the abdominal organs (Salzer, 2018). Bacterial diseases typically cause the inflammation; however, some viral infections cause peritonitis (Saif, 2008).

1.5.1.8.4 Polyserositis

Polyserositis is the inflammation of multiple serous membranes simultaneously (“Polyserositis Definition & Meaning | Merriam-Webster Medical,”). Polyserositis can be caused by many different types of infectious diseases and often accompanies other infectious causes of death (Zanella et al., 2000; Srinivasan et al., 2014; Jansen et al., 2015).

1.5.1.8.5 Yolk sac infection

Yolk sac infection impacts the poults in the first wk of life, caused by the infiltration of bacteria via the navel into the poult (Ashton, 1990). The infection can occur at many different stages, including in-ovum and in the hatcher. Poults that have yolk sac infection tend to huddle, appear ill, and have a distended stomach. The main signs upon post-mortem examination are the unabsorbed and inflamed yolk sac (Ashton, 1990).

1.5.1.8.6 Viral arthritis

Viral arthritis in turkeys can be caused by reoviruses, tenosynovitis, mycoplasma synoviae, and staphylococcus aureus infections in turkeys (Page et al., 1982; Jones, 2008). Viral arthritis caused by reoviruses is much less common in turkeys than in chickens (Jones and al Afaleq, 2007; Jones, 2008). Turkeys that are afflicted by viral arthritis are more commonly infected with tenosynovitis, mycoplasma synoviae and staphylococcus aureus. Viral arthritis can infect birds via skin lesions around the foot or hock joint (al Afaleq and Jones, 1990). Birds experiencing a higher level of leg lesions would, therefore, be more susceptible to infection with viral arthritis.

1.5.1.9 Skeletal causes of mortality

Skeletal diseases are common in meat-type turkeys as there are many influences on skeletal growth (Julian, 1998; Erasmus, 2018). In turkeys, rotated tibia and tibial dyschondroplasia are two common problems that result in culling (Julian, 1998).

1.5.1.9.1 Tibial dyschondroplasia

Tibial dyschondroplasia is the inability of cartilage in the tibial growth plate to ossify, leaving a cartilage plug (Figure 1.3; Pines and Reshef, 2015). There are several different theories as to the cause of tibial dyschondroplasia, including rapid growth, nutritional deficiencies, lack of critical growth factors, environmental conditions, and any factors that influence growth plate development (Pines and Reshef, 2015). Poultry suffering from tibial dyschondroplasia suffer from lameness, potentially leading to osteomyelitis (Hester, 1994; Rath et al., 1994). There is no research on the impacts of light flicker and any potential influence on tibial dyschondroplasia.

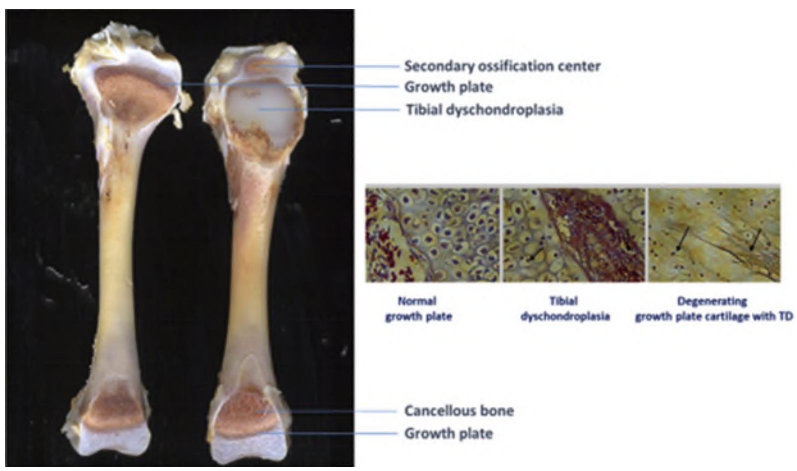

Figure 1.3. Gross lesions of tibial dyschondroplasia (Pines and Reshef, 2015)

1.5.1.9.2 Rotated tibia

Rotated tibia is the rotation of the tibia that causes the foot to be turned laterally (Riddell, 1980; Julian, 2005; Crespo and Shivaprasad, 2008). There is no damage to the joints or tendons that hold the tibia in place (Julian, 2005; Crespo and Shivaprasad, 2008). The true cause of rotated tibia is not known; however, slippery flooring and poor footing are thought to be some of the potential causes (Julian, 2005; Crespo and Shivaprasad, 2008). There is no known information on the potential impacts of light flicker on the occurrence of rotated tibias in poultry.

1.5.1.10 Behavioural causes of mortality

1.5.1.10.1 Aggressive damage

Aggressive damage through injurious pecking can be a significant cause of mortality in domestic turkey flocks (Lewis et al., 1998a, b; Moinard et al., 2001; Buchwalder and Huber-Eicher, 2005; Dalton et al., 2013). Injurious pecking, which is the repeated pecking at the feathers or head of a conspecific leading to the removal of skin or feathers, is typically the initial behaviour that then leads to cannibalism (Savory, 1995; Duggan et al., 2014). However, injurious pecking can be severe enough to lead to mortality or culling (Sherwin et al., 1999; Duggan et al., 2014). Cannibalism differs from injurious pecking as it involves the hemorrhaging of the skin and removal of blood and tissue, which may be consumed by the pecking bird (Hale et al., 1969; Dalton et al., 2013). In turkey toms, Raabe et al. (2024) saw no effect of LED flicker (165, 500, or 16,000 Hz) on the frequency of injuries or mortalities/morbidities caused by aggression. The authors did note that the injury pattern showed deep gouges to the head and necks of birds, suggesting that aggressive behaviours caused the injuries.

The type of lighting that birds are kept under may influence the incidence or location of injurious pecking (Denbow et al., 1990; Sherwin et al., 1999; Moinard et al., 2001). Denbow et al. (1990) found so few incidences of aggressive or injurious pecking in turkey hens that they did not analyze the data regarding the influence of fluorescent versus incandescent light. Moinard et al. (2001) found that birds raised under fluorescent light had fewer injuries caused by pecking on the wing and tail (P=0.03) compared to those raised under incandescent light. This result differed from those found by Sherwin et al. (1999), who found that there were no differences in the number of injuries due to wing and tail pecks in birds reared under fluorescent or incandescent light (P=0.22 and P=0.152, respectively). Sherwin et al. (1999) determined that turkeys raised under fluorescent light received fewer head-pecking injuries than those raised under incandescent light (P=0.02). Neither Sherwin et al. (1999) or Moinard et al. (2001) indicated that light flicker contributed to their results.

An increase in fearfulness or nervousness may contribute to injurious pecking, as birds may use it as a coping mechanism (Lindenwald et al., 2021). Environmental complexity may increase stress, which could increase the incidence of injurious pecking in domestic turkeys (Lindenwald et al., 2021). Lindenwald et al. (2021) found that birds provided environmental enrichment had significantly (P<0.05) increased head pecking at 14 d post-hatch in one trial repetition. The authors suggested that the increased complexity of the environment caused that increase in damage.

1.5.1.10.2 Emaciation or starve-out

Starve-out is defined as the failure of poults to perform feeding behaviours. Though it may be the direct cause of death, it may also be exacerbated by previous weakening of a poult (Enneking, 2010). The stress around placement may contribute to poults failing to consume feed. Poultry are known to have fight, flight, or freeze responses to alarming stimuli (Jones, 1996). It is possible that if birds are immobilized due to fear of an environmental stressor, such as light flicker, they will not perform feeding behaviours, leading to increased mortalities from starve-outs.

Lewis and Hurnik (1979) examined the use of flashing light as an attractant to feed for d of hatch poults. Bulbs were used on the feeders and flashed irregularly for 20-minute periods, followed by 40 minutes of no flashing (repeated over 24 hours). It was determined that flashing light decreased the time to initial feeding and increased the amount of time spent feeding during the first hour. There was an interaction ($P<0.01$) between treatment and period (flashing versus not flashing), which indicated that birds fed more during the flashing period than the non-flashing period. The same interaction occurred when looking at the number of poults within reach of the feeder. These results suggest that flashing light can be used as an attractant to feed. While there was increased feeding with the flashing light, there was no change to the mortality due to starve-outs.

1.5.1.11 Unknown causes of mortality

Unknown mortalities occur when the pathologist is unable to find lesions to support an explanation of the cause of death.

1.5.2 Behavioural expression

The behavioural expression of an animal is often indicative of its welfare. Different categories of behaviours can be used in conjunction with other measures to determine what affective state is being reflected by an animal.

1.5.2.1 Activity levels

Activity levels, both high and low, can be indicative of poor welfare. Abnormally high activity levels can be seen as hysteria or agitation, which can indicate that an animal is under stress and, therefore, experiencing negative welfare (Fraser, 2008). Alternatively, high activity can be associated with play behaviour, for example, frolicking, which indicates that the animal is experiencing a positive affective state (Baxter et al., 2019). Low activity levels may also indicate poor welfare if the animals are in a depressive or fearful state, where they are not able or willing to perform many of their natural behaviours (Bessei, 2006; Fraser, 2008). Other parameters should be used to determine the affective state of the animal before making conclusions regarding behaviours. Activity may be measured based on the observation of behaviours performed by birds (Table 1.1).

Table 1.1. Active and inactive behaviour measures and classifications. Adapted from Guhl (1958), Javid et al. (2016), Vermette et al. (2016b), and Beaulac and Schwean-Lardner (2018)

Behaviour	Definition
Active	
Walking	The bird takes two or more consecutive steps at a walking or running pace (Beaulac and Schwean-Lardner, 2018).
Standing	The bird is standing not performing another behaviour (Beaulac and Schwean-Lardner, 2018).
Frolicking	Spontaneous and rapid running and/or jumping and wing-flapping with no obvious intention, often with rapid direction changes. Running without wing-flapping is not classified as frolicking. A frolicking bout ends when the bird sits down or resumes another activity. Birds displaying frolicking directly leading to sparring are categorized as sparring to avoid misinterpretation of their movements (Guhl, 1958)
Pacing	A novel behaviour where birds open their wings and crouch then erratically and rapidly run back and forth (not necessarily) in a ‘pacing’ motion.
Jumping	Leaping with both feet off the ground. (Javid et al., 2016)
Inactive	
Resting	Bird is lying down, not performing any other behaviour. The bird may or may not be sleeping (Vermette et al., 2016b).

Hyperactivity, or hysteria, is characterized as a sudden onset of frightened cries accompanied by random running (Hughes, 1961; Hansen, 1976). The hysteria initially starts with an individual bird and then spreads through the flock. Hysteria can be caused by environmental factors or by the actions of one bird that create the ripple effect (Hughes, 1961; Hansen, 1976). There is evidence that high flicker frequency (26 kHz) of fluorescent light causes a significant (P<0.05) increase in the physical activity of broilers compared to low flicker frequency (100 Hz;

Boshouwers and Nicaise, 1992). This suggests that there may be an activity response that could lead to hysteria in birds kept under some flicker frequencies.

1.5.2.1 Comfort behaviours

Comfort behaviours are seen to be indicative of positive welfare when they are being performed. Conversely, if comfort behaviours are not being performed, it may indicate poor welfare (Zimmerman et al., 2011). The primary comfort behaviours noted in poultry are preening, dust bathing, wing flapping, perching and stretching (van Liere, 1991; Sherwin and Kelland, 1998; Mohammed et al., 2016; Li et al., 2020, 2021). Each of these behaviours is seen in the wild ancestor of the chicken when they are not under stress. This implies that these behaviours occur when the animal is in an environment that imitates stress-free natural settings.

1.5.2.2.1 Preening

Preening is a multipurpose act that birds perform to clean themselves or improve the bird's comfort (Kruijt, 1964; Black and Hughes, 1974; van Rhijn, 1977). Preening tends to occur in bouts and typically follows a sequence of movements, though the order and frequency of each movement can change (Table 1.2; Kruijt, 1964; Black and Hughes, 1974; van Rhijn, 1977; Sherwin and Kelland, 1998). The movements consist of pecking, combing, stroking, and nibbling, during which preen oil is applied to the feathers (Kruijt, 1964; Black and Hughes, 1974; Braun et al., 2018). Preening can be performed while the animal is sitting or standing (Sherwin and Kelland, 1998).

Preening has also been cited as a behaviour performed during stressful circumstances (Kozak et al., 2019). It has been noted that in these incidences, the preening action is likely used as a displacement behaviour to calm the bird (Delius, 1988; Spruijt et al., 1992; Fraser, 2008; Kozak et al., 2019). The difference between birds preening out of comfort and those during stressful situations is the presence or absence of elevated stress hormones (Kozak et al., 2019).

Table 1.2. Preening motions over intervals of time. Adapted from Kruijt (1964)

Interval	Motions	Definition
1	Pecking	Action toward the feathers or legs with the beak being removed from the body after touching legs or feathers
2	Nibbling	Action towards the base of feathers with a partially closed beak in small sideways circular movements. The beak is constantly in contact with feathers
3	Stroking	One or several feathers are pulled through a nearly closed bill
4	Combing	A half open bill, with light contact with feathers, makes rapid stroking movements

There is some information on the impacts of light flicker on the incidence of preening bouts, though no information is available for domestic turkeys. Greenwood et al. (2004) found that European starlings had no significant change (P>0.05) in the time spent preening under high (>30 kHz) or low (100 Hz) flicker frequencies. This differed from the results of Evans et al. (2012), who concluded that birds housed under low flicker frequency (100 Hz) decreased preening rates compared to those under high flicker frequency (>30 kHz).

1.5.2.2.2 Dust bathing

Dust bathing is considered a comfort behaviour when it is performed to maintain the integrity of plumage (van Rooijen, 2005). The actions performed during dust bathing assist in plumage and skin maintenance as well as bird comfort (Figure 1.4; Kruijt, 1964). The strong motivation to perform dust bathing regardless of previous exposure to bathing substrate indicates that this behaviour is important for the welfare and comfort of the birds (Wichman and Keeling, 2008). There is no known information on the impact that light flickering may have on dust bathing.

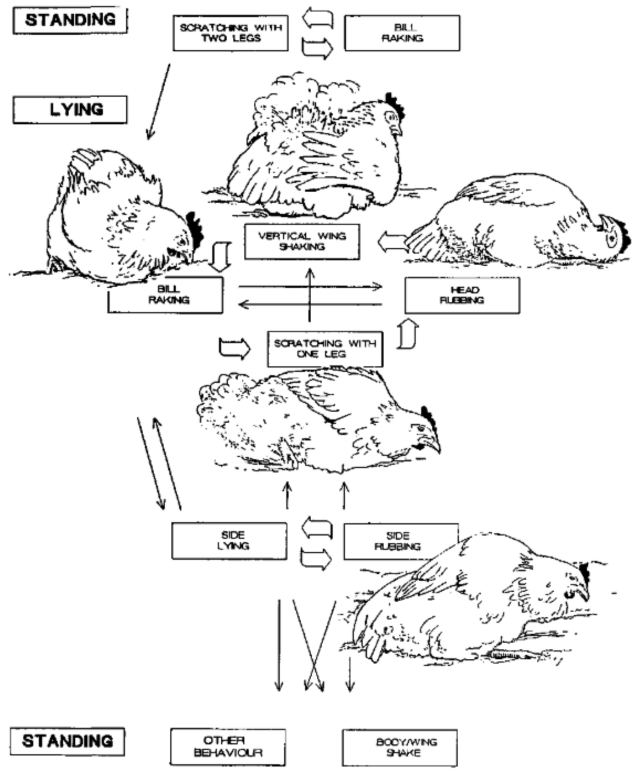

Figure 1.4. Sequence of dust bathing behaviours. Arrow width corresponds to high or low transition probability. Adapted from van Liere (1991)

1.5.2.2.3 Wing flapping

Wing flapping is the flapping of a bird's wings several times while standing still (Black and Hughes, 1974). Wing flapping is considered a comfort behaviour as it is performed in similar situations and patterns as other comfort behaviours (van Rhijn, 1977; Zimmerman et al., 2011). Wing flapping has also been found to be elicited when ACTH is injected, indicating that it may be a way of reducing stress via the HPA axis (Delius, 1988). Wing flapping often accompanies other behaviours, making it relatively difficult to distinguish (Kruijt, 1964). There is no known information on the influence that light flicker may have on wing flapping.

1.5.2.2.4 Perching

Birds have a high motivation to perch, and allowing them to do so reduces frustration and decreases stress (Campo et al., 2005; Bist et al., 2023). Perching is the act of sitting or standing high off the ground, typically on a structure (Mohammed et al., 2016). Wild birds use perching as a method of removing themselves from ground predators (Wright, 1914). Widowski and Duncan (1996) found that laying hens did not change the frequency of perching between low and high flicker frequencies (frequencies undefined). As perching is typically considered a comfort behaviour, the stress of novel environmental stimuli may impact the incidence of perching.

1.5.2.2.5 Stretching

Stretching is considered a comfort behaviour as it occurs during preening and dust bathing and may be indicative of good joint health (Kruijt, 1964; Schwean-Lardner et al., 2012a; Li et al., 2021). Stretching can occur in the wings, legs or a combination of both (Kruijt, 1964; Sinclair et al., 2015). Stretching is described as birds extending one or both legs or wings in a slow, prolonged manner, after which they return the limb to the original position (Nicol, 1987; Sinclair et al., 2015).

Light flicker may impact the presence and frequency of stretching (Evans et al., 2012). It was observed that stretching behaviours were increased in European starlings kept under low flicker frequency (100 Hz) compared to high flicker frequency (>30 kHz; Evans et al., 2012). As there is minimal information on the impact of light flickering on stretching, further study is required.

1.5.2.1 Nutritive behaviours

Nutritive behaviours are those associated with procuring and consuming food. In avian species, raising the head is not required for swallowing, which allows them to continuously eat (Hale et al., 1969). When drinking from cups, birds place their beak into the water to just under the nares then rapidly open and close their beaks to catch water (Hale et al., 1969). Once water is in the beak of the bird, they raise their head and again open and close their beaks to help swallow the water (Hale et al., 1969). When drinking from nipple drinkers, birds stretch up and allow water to run down their throats (Lott et al., 2001).

Accompanying the physical act of eating are the behaviours associated with when and why poultry eat. Feeding occurs most typically when the light turns on, based on diurnal rhythms, as light imitates morning (May and Lott, 1994; Schwean-Lardner et al., 2014). Poultry typically eat in bouts, which are interspersed with periods of other behaviours (Hughes and Grigor, 1996; Schwean-Lardner et al., 2012a).

The influence of light flickering on the nutritive behaviours of poultry has been minimally investigated (Greenwood et al., 2004; Evans et al., 2012). Evans et al. (2012)

examined the impacts of fluorescent light flickering, >30 kHz or 100 Hz, on European starling nutritive behaviours. The authors concluded that birds in the low-frequency-flicker treatment (100 Hz) spent less time feeding than those in the high-frequency-flicker treatment (>30 kHz). Greenwood et al. (2004) also examined the impact of high (>30 kHz) and low (100 Hz) frequency flickering of fluorescent light on European starlings. It was found that there was no influence of treatment on feeding or drinking ($P>0.05$). These results suggest that flicker likely has little impact on the nutritive behaviours of birds. However, as both studies examined European starlings, the influence of flicker on turkeys may differ.

1.5.2.1 Aggressive behaviours

Aggressive behaviours can be detrimental to poultry, where continued aggression can escalate to cannibalism (Classen et al., 1994; Hughes and Grigor, 1996; NFACC, 2016). Aggression is categorized into several different behaviours; these include severe feather pecking, aggressive or injurious pecking, and fighting.

1.5.2.4.1 Severe feather pecking

Severe feather pecking is the act of pecking that results in the removal of the feathers of one bird by a fellow (Savory, 1995; Sherwin and Kelland, 1998; NFACC, 2016; Dalton et al., 2018; Bartels et al., 2020). In female turkeys, severe feather pecking, specifically on the back of the neck, occurs during the creation of the social hierarchy (Hale et al., 1969). Other cases of severe feather pecking are thought to be a result of several factors, including redirected foraging and dust bathing (Savory, 1995; Hughes and Grigor, 1996; Martrenchar, 1999; Sherwin et al., 1999; Dixon et al., 2008; Dalton et al., 2013; Nicol, 2018).

Most commonly, severe feather pecking is thought to be a redirected attempt at foraging behaviours (Savory, 1995; Sherwin et al., 1999; Dixon et al., 2008). There is also a potential connection between increased severe feather pecking and an inability to dust bathe (Savory, 1995). Severe feather pecking may also be linked to stress, where turkeys use it as a coping mechanism (Hughes and Duncan, 1972). Some hypotheses about the cause of severe feather pecking have been tested and found to be lacking, at least in some areas. For example, Rudkin (2021) found that severe feather pecking was not associated with an inability to forage but an exploration behaviour.

1.5.2.4.2 Aggressive or injurious pecking

Aggressive or injurious pecking is a significant issue in turkeys as it occurs at high frequencies and can lead to cannibalism (Buchwalder and Huber-Eicher, 2005). Injurious pecking can occur in a number of different areas, including the wing, head, back, and tail (Lewis et al., 1998a, b; Sherwin et al., 1999; Dalton et al., 2018). The terms injurious and aggressive pecking are used in a variety of ways. Some research uses them interchangeably; however, some researchers indicate that aggressive pecking should be defined as only occurring between dominant and subordinate animals (Savory, 1995). Injurious pecking is characterized as pecking of fellow birds that can cause injury, feather removal or consumption, or vocalization (Savory, 1995).

It is not entirely known what causes aggressive pecking, though there seem to be several contributing factors which has led to several methods of reduction. Light intensity, duration, and type are some of the easiest factors to control when looking at reducing aggressive pecking (Lewis et al., 1998a, b; Sherwin et al., 1999).

1.5.2.4.3 Fighting

Fighting typically progresses in the method described by Estevez et al. (2002) and Sinclair et al. (2015). The action of fighting involves two birds that repeatedly press their bodies or necks together in a forceful jerk. The engaged birds then run in a tight circle with their bodies/necks still pressing together. After running, the birds peck at each other's face/neck and attempt to hold onto a portion of skin or feathers. The engaged birds stop the fighting behaviour when neither makes contact for 5 or more seconds. Often, fighting leads to injury, especially if the tugging stage occurs where the head can be damaged. Once damaged, injuries can lead to cannibalism within turkeys (Hale et al., 1969; Dalton et al., 2018). Raabe et al. (2024) did not see impacts of 165, 500 or 16,000 Hz LED flicker on the rate of injury in turkey toms, or the pattern of injuries as all were caused by deep penetrating beak blows, similar to those seen in fighting.

1.5.2.1 Exploratory behaviours

Exploratory behaviours are not well defined within literature; however, they are often characterized by movement, pecking, and scratching in the environment (Agnvall and Jensen, 2016; de Jong and Gunnink, 2019; Meuser et al., 2021). Exploratory behaviours are thought to work in opposition to fear behaviours, as exploration brings birds into contact with novel environments, situations, and objects (Agnvall and Jensen, 2016; Meuser et al., 2021). Researchers use this association to quantify fearfulness in birds by using types of novelty tests and then assessing willingness to explore the novel situation (Carter et al., 2013; Agnvall and Jensen, 2016; Meuser et al., 2021).

Evans et al. (2012) examined the impacts of fluorescent light flicker on exploratory behaviours of European starlings under high (>30 kHz) and low (100 Hz) flicker frequencies in different sequences. The authors found that exploratory behaviours were increased in the birds exposed to the 100 Hz prior to the >30 kHz treatment, regardless of which treatment they were in at the time of testing.

1.5.2.5.1 Gentle feather pecking

Gentle feather pecking **(GFP)** is the action of birds pecking at the feathers of another without drawing blood or removing feathers (Savory, 1995; Hughes and Grigor, 1996; Duggan et al., 2014). There are multiple theories as to the cause of GFP, including redirected ground pecking, foraging, or dust bathing (Blokhuis, 1986; Huber-Eicher and Weschsler, 1997, 1998). GFP can be performed to socially explore conspecifics (Riedstra and Groothuis, 2002). There are differing thoughts and results on whether GFP leads to severe feather pecking as birds age (Kjaer and Vesetergaard, 1999; Mcadie and Keeling, 2000).

1.5.3 Chronic stress

Heterophil-to-lymphocyte **(H/L)** ratio in blood plasma is typically used to determine chronic stress in poultry. Heterophils are white blood cells in avian species and are the equivalent of neutrophils in mammals (Genovese et al., 2013). Heterophils, which are produced in the bone marrow, are the major phagocytic cells in poultry (Genovese et al., 2013; Scanes, 2015, 2016). Lymphocytes assist in the destruction of pathogens via immunoglobulin production (Maxwell, 1993; Campbell, 1995; Davis et al., 2008).

Changes in the numbers of heterophils and lymphocytes occur as a response to stress in birds. Moderate to mild stress causes an increased number of heterophils (heterophilia) and a decreased number of lymphocytes (leukopenia), causing an increased ratio between the two (Gross and Siegel, 1983; Scanes, 2015, 2016). Increased CORT reduces heterophil apoptosis, resulting in an increased number of heterophils released from the bone marrow (Harmon, 1998; Ronchetti et al., 2018; Hatefi et al., 2021). Lymphocyte numbers are significantly (P<0.001) decreased by the release of ACTH during stressful situations (Davison and Flack, 1981; Scanes, 2015, 2016; Liu et al., 2017). The number of lymphocytes are also decreased when CORT is released (Gross and Siegel, 1983; Scanes, 2015, 2016). During extreme stress, such as extreme heat exposure or severe feed restriction, the number of heterophils can decrease through heteropenia (Gross and Siegel, 1983; Maxwell, 1993; Lentfer et al., 2015). In similar situations, lymphocytosis reactions can occur, which cause an influx of lymphocytes into the blood, which, in combination with heteropenia, reduces the H/L ratio (Gross and Siegel, 1983; Maxwell, 1993; Cotter, 2015; Lentfer et al., 2015). There is no research to this author's knowledge about the impact of light flicker on H/L ratios.

1.5.4 Fear

The affect of fear is complex and is comprised of behavioural, emotional, and physiological reactions to various stimuli (Jones, 1996). As a result, it can be difficult to measure. Fear testing is used in poultry to help determine the welfare of birds and their responses to environmental stressors. Birds that are in a state of fearfulness are thought to respond more intensely to fear-inducing situations or environments (Jones, 1996; Erasmus and Swanson, 2014). One of the methodologies used to measure fear in poultry is performing a novel object test **(NOT)** and is described below.

1.5.4.1 Novel object test

A NOT is used to determine the fearfulness of poultry through their typical avoidance, fight, flight, or freeze response (Jones, 1996). To perform a NOT, observers enter the home pen of the birds and place a novel object on the floor in the center of the pen. The birds are then observed for the time it takes for three separate individuals to make intentional contact with the object. The longer it takes a bird to make contact with the object, the more fearful they are thought to be (Erasmus and Swanson, 2014; Kulke et al., 2021). Erasmus and Swanson (2014) indicated that the type of object may be important for the bird's response. If light flicker changes how birds perceive an object or is an environmental stressor, it may alter the time it takes for birds to make contact; however, there is no current evidence of this.

1.5.5 Overall health and welfare

There are many aspects involved when considering the overall health of poultry. When looking for indicators of health, mobility, feather cleanliness, feather condition, and footpad scores can be used. Each of these scores can be influenced directly by the quality of litter that the birds are housed on. By scoring both the litter and the health parameters, an idea of what caused any potential health issues can be formed.

1.5.4.1 Litter quality

Litter quality is linked to many different aspects of bird health, including footpad dermatitis, feather cleanliness, feather condition, and mobility (Mayne et al., 2007; de Jong et al., 2014; Saraiva et al., 2016; Schreiter and Freick, 2023). By scoring the litter to determine moisture, the impact that the litter has on birds can be interpreted in conjunction with other bird-specific scores (i.e. gait, footpad, and feather scores).

In order to score litter quality, a visual and tactile method can be used to determine its approximate moisture content (Table 1.3; Welfare Quality Consortium®, 2009; Adler et al., 2021). While there is no known research on the impact of LED light flicker on litter quality, activities such as dustbathing, foraging, and scratching are known to turn litter, allowing for moisture to be released (Bessei, 2006; Lister, 2009; Calvet et al., 2011; Dunlop et al., 2015).

Table 1.3. Litter quality assessment. Adapted from Welfare Quality Consortium® (2009) and Adler et al. (2021)

Photo Reference					
Score	0	1	2	3	4
Criteria	Completely dry and flaky, easily moved	Dry but clumps form when compacted	Compacted ball forms and falls apart when pressure is removed	Readily sticks into a ball when compacted	Cap/crust must be broken and sticks in a wet ball. (Boot imprint may be left in litter under cap)

1.5.4.2 Mobility

Gait scoring is used to assess mobility, as birds who have severely altered gaits are likely to be in pain due to injury, abnormalities or weakness (Danbury et al., 2000; Schwean-Lardner et al., 2013; Vermette et al., 2016b). While the technique used to score turkeys, adapted by Vermette et al. (2016b), ranges from 0 to 5, scores from 3-5 are not typically seen at the time of testing, as birds with such an impediment would be culled (Hester, 1994; NFACC, 2016).

There is currently no research on the impacts of light flicker on the gait scores of turkeys. There are, however, some conflicting results on the impacts of activity levels on leg health and, therefore gait score of poultry (Newberry et al., 1985; Prayitno et al., 1997). Prayitno et al. (1997) found that broilers with lower activity had higher gait scores during 6 and 7 wk (P=0.02) than in the rest of the trial. This result is contrary to the findings of Newberry et al. (1985), who found that activity levels did not influence the incidence of leg abnormalities in broiler chickens. If light flicker causes changes in activity, there could also be changes to turkey mobility.

1.5.4.3 Footpad scores

Footpad scoring is used to assess the development of footpad lesions, which can be painful (Martland, 1984; Haslam et al., 2007). The presence of footpad lesions occurs when birds have extended contact with wet litter (Hester et al., 1987; Mayne et al., 2007; Škrbić et al., 2015). In terms of health, birds with more severe footpad lesions are subject to pain when walking, which impacts their ability to access feed and water with ease (Škrbić et al., 2012, 2015). There is no evidence of any potential impacts that light flicker may have on footpad lesions.

1.5.4.4 Feather condition

Feather condition in poultry is influenced by a number of different parameters and their interactions, such as behaviour and environment (Leeson and Walsh, 2004; Saraiva et al., 2016). Feather condition can be used as an indicator of health as feathers are integral to skin protection and thermoregulation, can indicate an infection, indicate nutritional deficiencies or behavioural abnormalities (Leong et al., 1959; Savory, 1995; Leeson and Walsh, 2004; Sarica et al., 2008; Olukosi et al., 2019).

Poor feather condition may be caused, in part, by feather pecking, a behaviour that can damage or remove the feathers of other birds depending on severity (Savory, 1995). The pulling of feathers can lead to cannibalism, reducing welfare and potentially causing the death of the affected bird (Savory, 1995; Hughes and Grigor, 1996).

Poultry under stress may also have poor feathering as CORT is thought to disrupt the feathering process (Savory, 1995; Campo et al., 2001; Romero et al., 2005; Strochlic and Romero, 2008). Romero et al. (2005) found that European starlings with CORT implants had significantly reduced feather growth from 5-15 d post implantation ($P<0.02$) compared to control birds. The follow-up study by Strochlic and Romero (2008) used psychological and physical stress to induce CORT production rather than exogenous CORT implants. It was found that psychological and physical stress alone did not reduce feather growth but did cause smaller areas of feather regrowth, abnormal feather growth, and asynchronous replacement. Campo et al. (2001) found that hens with poor feather condition had significantly ($P<0.05$) higher H/L ratios than those with perfect feathering.

1.5.4.5 Feather cleanliness

Feather cleanliness is important for overall health as it plays roles in thermoregulation and protection (Leeson and Walsh, 2004; Saraiva et al., 2016; Jacobs et al., 2017; Marchewka et al., 2019). Poor feather cleanliness may be caused by increased contact with wet litter, lack of preening, or lack of dustbathing (Greene et al., 1985; Vezzoli et al., 2015; Granquist et al., 2019; Marchewka et al., 2019).

Increased contact with poor-quality litter can lead to feathers that are damp or dirty. This reduces the ability of feathers to protect the bird from conditions such as contact dermatitis and may limit thermoregulation (Greene et al., 1985; Leeson and Walsh, 2004; Saraiva et al., 2016). Saraiva et al. (2016) found positive correlations between hock burns, footpad dermatitis, breast burns, and plumage cleanliness of broilers, which they concluded was due to increased contact with wet litter. Marchewka et al. (2019) found a positive correlation between increased lameness and dirty feathers ($P<0.0001$), which they concluded was caused by lame birds spending more time in contact with litter material.

Preening is a behaviour that is integral to feather maintenance. When preening behaviours are reduced, the cleanliness of feathers is as well. Chronic stress is known to cause a decrease in preening behaviours, which in turn would decrease the cleanliness of plumage (Wang et al., 2013). Wang et al. (2013) found that boilers exposed to chronic CORT had significantly decreased plumage cleanliness scores (*P*=0.004) which positively correlated with reduced preening behaviours.

Dust bathing is a comfort and maintenance behaviour performed by poultry to maintain feather condition and cleanliness (Kruijt, 1964). Wang et al. (2013) found that broilers given CORT reduced their incidences of dust bathing (*P*>0.05). As stress decreases the incidence of comfort and maintenance behaviours in general if light flickering is stressful, dust bathing and, therefore, feather cleanliness will likely be decreased.

1.5.4.6 Ocular morphology

The ocular morphology of poultry is known to be influenced by daylength, wavelength, and intensity of light (Ashton et al., 1973; Prescott et al., 2003; Blatchford et al., 2009; Schwean-Lardner et al., 2013; Olanrewaju et al., 2015; Vermette et al., 2016b; Remonato Franco et al., 2022). Schwean-Lardner et al. (2013) found that 23 hours daylight resulted in heavier eyes in broiler chickens compared to those reared under 14, 17, or 20 hours daylight (*P*=0.0001). This result is because the eyeball grows during the photoperiod in conjunction with melatonin release and, therefore, the circadian rhythm (Rada and Wiechmann, 2006). Vermette et al. (2016b) found that the eye weight of turkeys was influenced by daylength. Eye weight was significantly (*P*≤0.05) increased under 23 hours daylight at 18 wk of age, though no differences were seen in the 14, 17, or 20-hour treatment birds at 12 or 18 wk of age (Vermette et al., 2016b). Blatchford et al. (2009) determined that, when raised under incandescent light, the weight of 40 d old broiler eyes increased (*P*=0.002) under 5 lux compared to 50 and 200 lux. Olanrewaju et al. (2015) found that eye weight of broilers grown to 42 d of age were significantly heavier (*P*≤0.05) in those raised under incandescent and cool LED (cool LED 1) light compared to warm and cool (cool LED 2) LED light treatments (Figure 1.5). Olanrewaju et al. (2015) also noted that light intensity significantly impacted (*P*=0.0401) the eye weight of broilers grown to 42 d. Remonato Franco et al. (2022) investigated the impacts of blue, green, or white wavelengths on the eye morphology of broilers raised to 35 d. It was found that none of the wavelengths had a significant impact (*P*=0.13) on the weights of birds' eyes.

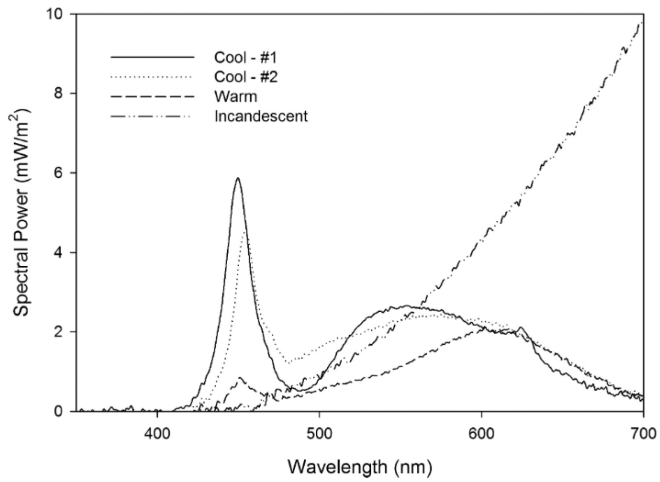

Figure 1.5. Spectrum of 2 cool LED, warm LED light, and incandescent light adapted from Olanrewaju et al. (2015)

The eye morphology based on measurements of the dorso-ventral **(DV)** diameter, medio-lateral diameter, and anterior-posterior size **(AP)** may change depending on light flicker (Lin et al., 2020), daylength (Vermette et al., 2016b), and wavelength (Remonato Franco et al., 2022). Lin et al. (2020) found that layer chicks subjected to flickering light (undefined frequency) had shorter axial length compared to unflickering light (undefined frequency; P<0.05). The definition of axial length used by Lin et al. (2020) is the same as AP, indicating that light flicker causes a shorter AP in chicks. Wavelength may alter the shape of an eyeball as different wavelengths focus at different points. This can cause the eye to change shape through emmetropization in order to minimize refractive error (Lin et al., 2020). Daylength, as described previously, alters the growth pattern of the eyeball. Vermette et al. (2016b) found significant (P≤0.05) increases in DV, medio-lateral diameter, and AP in turkeys kept under 23 hours of daylight at 12 wk of age, compared to those under 14, 17, or 20 hours of daylight. Similar effects were seen in turkeys aged 18 wk (P≤0.05), with birds raised under 23 hours light having greater eye measurements than those in the other treatments (Vermette et al., 2016b). These results indicate that daylength can significantly impact the dimensions of turkey eyes. There are, however, no significant (P<0.05) impacts of wavelength on the DV, medio-lateral diameter, or AP (Remonato Franco et al., 2022). As the only evidence of morphology changes caused by flicker is the AP, results on the rest of the morphology factors and eye weight are yet to be understood.

1.6 Objectives

The objective of this study is to determine the impact of flickering LED light on turkey hens in the following areas:

- General production traits including growth, feed intake, feed efficiency, level of mortality and morbidity, and their causes.
- Levels of the following behaviours: active (walking, frolicking, pacing, jumping, standing), comfort (preening, stretching, wing flapping, dust bathing, feather ruffle, head scratching, perching, head rubbing), nutritive (eating, drinking), aggressive (aggressive pecking, fighting, posturing), and exploratory (environmental pecking, feather pecking, litter scratching).
- Chronic stress response at early rearing, mid-way through growth, and at the end of production using heterophil-to-lymphocyte ratio.
- Fearfulness using novel object tests.
- Mobility using gait scores.
- Cleanliness and feather condition.
- Ocular health by assessing eyeball morphology for abnormalities.

1.7 Hypotheses

Overall, potentially visible (30 and 90 Hz) LED light flicker is hypothesized to negatively impact the behaviour, production, and welfare of turkey hens compared to flicker at 195 Hz.

Individually, it is hypothesized that:

- LED light flicker will negatively impact turkey hen feed intake due to the activation of the HPA axis in response to the aversive nature of visible light flicker (30 and 90 Hz). The increase in stress, as seen by general stress indicators (ex. fear response, behaviour), reduces feed intake and, therefore, growth.
- Visible light flicker (30 and 90 Hz) will increase the mortality of turkey hens through activation of the HPA axis, which reduces immune function, increasing the susceptibility of turkey hens to infectious mortality.

- 90 Hz light flicker will reduce the uniformity of turkey hens as some of the birds may be able to see the flicker while others may not, leading to differing responses to the stress of the flicker and, therefore, differing growth.
- Comfort behaviours of turkey hens will be reduced by visible light flicker (30 and 90 Hz) as the flicker will cause stress, resulting in activation of the HPA axis and SAM pathway, which in turn will alter the behaviour to those of fight or flight, reducing the expression of comfort behaviours.
- The 30 and 90 Hz treatments will increase the incidences of aggressive behaviours as stress will induce the flight or fight response, with 'fight' behaviours being expressed more frequently. The visual changes that may occur due to the flicker will also make it more difficult for individual birds to be identified, leading to instability within the social hierarchy and, therefore, increased aggression.
- The 30 and 90 Hz treatments will heighten the fear responses to novel object tests due to the increase in stress response which is indicated by avoidance behaviour, causing birds to be more wary of unknown objects in their environments.
- The heterophil-to-lymphocyte ratios will be increased as a stress response to the flicker treatments that should be visible (30 Hz and 90 Hz). Through activation of the HPA axis, the number of heterophils and lymphocytes will be altered as the leukocytes respond to the increased corticosterone.
- Increased CORT concentrations will reduce the number of feathers produced and increased feather pecking will cause the removal of feathers from conspecifics. These conditions will cause reduced feather condition of turkeys in the 30 and 90 Hz treatments.
- The feather cleanliness of turkeys in the visible light flicker (30 and 90 Hz) will be improved as reduced contact with the litter due to increased activity through hysterical reactions to the novelty of light flicker.
- The mobility of birds in the 30 and 90 Hz treatments will be improved through excessive activity through hysterical reactions to the novelty of light flicker.

- The visible (30 and 90 Hz) light flickering will reduce the anterior-posterior size and alter the morphology of the eyeball. Changes in the morphology will occur through the process of emmetropization, where the axial length alters to reduce the amount of refractive error.

2.0 Chapter 2: Impact of flicker frequency on turkey hen production, health, and mortality

2.1 Abstract

This study aimed to determine the impact of flicker produced by white light-emitting-diode (LED) lamps on the productivity, general health, and mortality of Nicholas Select turkey hens reared to 11 weeks (wk) of age. Three different flicker treatments were used; the 30 Hertz (Hz) treatment (30HZ), 90 Hz treatment (90HZ) or 195 Hz treatment (195HZ). The experiment was arranged as a randomized complete block design with trial as the blocking factor (n=2) and analyzed as a one-way factorial analysis. Turkeys (364/room) were randomly placed into rooms (3 rooms/treatment/trial). Body weight (BW) was assessed at 0 day (d), 4, 8 and 11 wk; feed intake (FI) was measured in the periods between BW collections. Feed efficiency (feed-to-gain; F:G) and mortality corrected F:G (F:G^m) were calculated. Flock uniformity was measured at 10 wk (30 birds/room). The same birds were used at 10 wk to determine feather cleanliness (score 1=very clean, 4=very dirty), feather condition (score 1=no feather cover, 4=full intact plumage), footpad score (score 0=no lesion, 4=more than ½ necrotic footpad), and mobility (gait score 0=no impairment, 5=complete lameness). Litter quality (score 0=completely dry and flaky, 4=sticks into wet ball) and ocular weight and morphology (4 birds/room) were examined at 11 wk. Mortality and culls were collected twice daily, with cause of death determined by an independent laboratory. Data were analyzed using Proc Mixed (SAS 9.4) as a randomized complete block design with significance declared at *P*<0.05. At 8 wk, the BW of birds in the 30HZ was lower than those in the 195HZ (*P*=0.025). The BW gain of hens from 8-11 wk was increased when reared in the 30HZ compared the 90HZ (*P*=0.003). FI was reduced in the birds in the 30HZ during 0d-4 and 4-8 wk (*P*<0.001; 0.001, respectively). The F:G was not affected by flicker. At 8-11 and 0d-11 wk, F:G^m was improved for birds in the 30HZ (*P*=0.048; 0.040, respectively). Total mortalities were reduced in birds reared in the 195HZ compared to those in the 30HZ from 0d-11 wk (*P*=0.024). From 0d-4 wk, total mortalities were reduced in birds under 195 Hz (*P*=0.005). Infectious mortalities from 0d-11 wk were reduced in birds under the 195HZ compared to those in the 90HZ (*P*=0.049). Round heart mortalities were increased in birds under 30HZ (*P*=0.040) from 4-8 wk. Gait scores, feather condition, and litter quality were not influenced by flicker treatment. Average footpad scores were improved in the 90HZ birds (*P*=0.021). Average feather cleanliness was improved in the 90HZ birds compared to the 195HZ

birds (*P*=0.021). The dimensions of the right eyeball were altered with a larger dorso-ventral diameter observed in birds reared in the 30HZ versus in the 195HZ (*P*=0.046). The anterior-posterior size was also larger birds in the 30HZ compared to those in the 90HZ (*P*=0.033). Overall, there were minimal impacts on the productivity and health of birds raised under flickering light.

2.2 Introduction

Light flicker is a phenomenon that may be viewed in poultry housing systems but has not been investigated extensively in poultry species. Light flicker is visible to a viewer when it is within their flicker fusion frequency (**FFF**; Lisney et al., 2011). Light flicker can be unconsciously perceived when the frequency of the flicker is between the FFF and critical flicker fusion frequency **(CFF)**. The CFF is the point at which the viewer can no longer unconsciously perceive light flicker at any light intensity (Lisney et al., 2012). Few studies have attempted to determine the effects that light flicker has on poultry, including turkey toms, laying hens and broilers.

To this author's knowledge, one study has been conducted on light-emitting-diode **(LED)** flicker and its influence on turkey tom productivity (Raabe et al., 2024). The authors examined live body weight **(BW)**, feed intake **(FI)**, and mortality/morbidity of British United Turkeys 6 turkey toms under 165, 500, or 16,000 Hertz **(Hz)** from 1 day **(d)** to 141d. The results of this study found no differences in any of their measured performance parameters. One study has been conducted on light flicker and its influence on laying hen preference (Widowski and Duncan, 1996). Widowski and Duncan (1996) examined the impact of fluorescent light flicker at high or low frequencies (undefined) on Shaver 288 laying hens. It was determined that hens did not have a preference for either frequency (*P*>0.01). As different breed of poultry were used by Widowski and Duncan (1996) compared to the current study, their conclusions may not be applicable to turkey hens reared under LED flicker.

There is only one published study on the influence of light flicker on broiler chicken performance (Boshouwers and Nicaise, 1992). These authors studied the effects of fluorescent light flicker at high (26 kHz) or low (100 Hz) frequencies on the energy expenditure of Pilch broiler chickens. It was determined that the flicker did not affect the measured variable (*P*>0.05).

Evans et al. (2012) studied the effects of fluorescent light flicker at 100 or >30 kHz on European starlings. The only production parameter examined by these authors was BW, and light flicker had no impact (*P*=0.311).

To this author's knowledge, no study has examined turkey productivity in a flicker frequency below 165 Hz. There may be differences in relation to the perception of light flicker if it is within the CFF and FFF of birds. Research on non-turkey species may not give an accurate representation of turkey production in flicker frequencies below 165 Hz. We know that different species of poultry can respond differently to their environments. For example, turkeys can react differently to increased daylength than broiler chickens (Schwean-Lardner et al., 2012b; Vermette et al., 2016a). The BW of broiler chickens was found to have a quadratic relationship with increasing daylength, while turkey BW increased with increasing daylength at 21 and 42d (Schwean-Lardner et al., 2012b; Vermette et al., 2016a). The differences in how turkeys respond to their environments indicates that apart from Raabe et al. (2024), previous knowledge on light flicker in birds may not be applicable to turkey production.

The absence of information on the impacts of LED light flicker on the productivity, health, or mortality of turkey hens, indicates a gap in the scientific literature. The objective of this study was to determine the impacts of white LED light flicker on the general productivity, health, and mortality of turkey hens. It was hypothesized that:

- LED light flicker will negatively impact turkey hen feed intake due to the activation of the HPA axis in response to the aversive nature of visible light flicker (30 and 90 Hz). The increase in stress, as seen by general stress indicators (ex. fear response, behaviour), reduces feed intake and, therefore, growth.
- Visible light flicker (30 and 90 Hz) will increase the mortality of turkey hens through activation of the HPA axis, which reduces immune function, increasing the susceptibility of turkey hens to infectious mortality.
- The 90 Hz light flicker will reduce the uniformity of turkey hens as some of the birds may be able to see the flicker while others may not, leading to differing responses to the stress of the flicker and, therefore, differing growth.

- The feather cleanliness of turkeys in the visible light flicker (30 and 90 Hz) will be improved as reduced contact with the litter due to increased activity through hysterical reactions to the novelty of light flicker.
- The mobility of birds in the 30 and 90 Hz treatments will be improved through excessive activity via hysterical reactions to the novelty of light flicker.
- The visible (30 and 90 Hz) light flickering will reduce the anterior-posterior size and alter the morphology of the eyeball. Changes in the morphology will occur through the process of emmetropization, where the anterior-posterior size (axial length) alters to reduce the amount of refractive error.

2.3 Materials and methods

The following procedures of this experiment have been approved by the University of Saskatchewan Animal Care Committee under the protocol number 20210090 (AUP). Animals were cared for following the Guide to the Care and Use of Experimental Animals by the Canadian Council of Animal Care (2009).

2.3.1 Animal housing and husbandry

Two trials (January 11th to March 25th, 2022 and November 14th, 2022 to January 30th, 2023) researched the impact of the flicker of white LED lamps on the production, health, and mortality of Nicholas Select turkey hens from 0 to 11 weeks **(wk)** of age.

The experiment consisted of three treatments, the 30 Hertz (Hz) treatment (30HZ), 90 Hz treatment (90HZ), or 195 Hz treatment (90HZ), each replicated three times per trial. This resulted in six replicates of each treatment. The experiment was conducted at the University of Saskatchewan Poultry Centre Brooding and Rearing Facility. Daily care and management were provided by the staff at the University of Saskatchewan Poultry Centre Brooding and Rearing Facility, following the requirements of the Canadian Codes of Practice (NFACC, 2016). These requirements included monitoring bird health and environment and providing feed, water, and, if necessary, euthanasia. Birds were fed a commercial diet from a commercial feedmill (Table 2.1).

Table 2.1. Diet Schedule

Diet Type	Diet Form	Age Fed (wk)	Feed Consumption (kg/bird)
Starter #1	Fine Crumble	0-18 (d)	0.85
	Coarse Crumble	18 (d)-4	0.55
Starter #2	Coarse Crumble	4-5	1.01
	Short Pellet	5-6	0.79
Grower #1	Short Pellet	6-8	2.80
Grower #2	Pellet	8-10	3.80
Finisher	Pellet	10-11	*Ad libitum*

Nine identical light-tight rooms, each measuring 6.7 m x 10 m (67m^2, estimated final stocking density=45kg/m^2), were utilized to mimic industry environments. Each room contained 12 tube-type feeders with an initial pan size of 36 cm. After 27 d, the pan size was changed to 44 cm. Each room had 4 Lubing EasyLine™ pendulum nipple drinkers (Lubing, Cleveland, TN), totalling 12 drinker cups per room. Wood shavings were used at an initial depth of 7-10 cm for bedding. For the first 9 d, 3 brooder rings (5 m x 7 m) were used per room with supplemental feeders and drinkers. For the first 7 d, humidity was maintained between 50-70% via portable humidifiers, with whole room temperatures at 34ºC. After 7 d, humidity was not controlled but remained between 50-70%. Temperature was set at initial target of 31ºC with a gradual reduction to 16ºC by 11 wk (Aviagen Turkeys, 2015). Temperature was maintained via hot water pipes that ran along the walls of the rooms.

The photoperiod began at 23L:1D and was gradually reduced to 18L:6D by 9 d (Aviagen Turkeys, 2015), with darkness provided in one continuous period. The on and off of bulbs were controlled using timers (MC404 NOMA Outdoor Heavy Duty 24-Setting Timer, Canadian Tire Corporation, Toronto, ON) that were temporarily connected to the power source for each room. Light intensity (lux) was initially set at 40 lux and was gradually reduced to 10 lux by 8 d. Light intensity was manipulated via the duty cycle for each room using the DTD controller and AgriLamp Symmetry Control Dimmer (AgriLamp 11W ES26.27; Greengage Lighting Ltd., Edinburgh, UK). Light intensity was measured weekly (Lighting Passport Spectrometer, ASENSETEK) to ensure that the correct intensity was maintained throughout the trial period. If light intensity varied from the defined value, bulbs were cleaned to remove dust build up. If cleaning did not rectify deviations in light intensity, the duty cycle was manipulated to ensure the required intensity was met.

Within each trial, 3 rooms were randomly assigned to each treatment, with 364 birds/room (1092 birds/treatment) at placement. Treatment flicker frequencies were purposely created using a combination of AgriLamp bulbs, AgriLamp Symmetry Control Dimmer and DTD controller from Greengage Lighting Ltd. (AgriLamp 11W ES26.27; Greengage Lighting Ltd., Edinburgh, UK). The flicker equipment was temporarily connected to the existing power and lighting controls in each room, altering the power supply to the bulbs and creating the flickering. The 195 Hz rooms used the AgriLamp bulbs and Symmetry Control Dimmer; however, the flicker frequency was generated by the fluctuations in the power source for the Poultry Centre. This was used to mimic real-life conditions where flicker is beyond the CFF of birds. Flicker frequencies were checked weekly using a spectrometer (Lighting Passport

Spectrometer, ASENSETEK) for the 30 and 90 Hz treatments and an oscilloscope (TDS 210 Digital Real-Time Oscilloscope, Tektronix, Beaverton, OR, USA) and Lichtflimmer (LiFli) (Messgerat LiFli, Fauser Elektrotechnik, München, Germany) for the 195 Hz treatment. Two separate measurement techniques were used as neither were able to accurately check the full range of flicker frequencies used in this experiment. In order to ensure accurate and precise measurements were taken, the readings from both machines were compared to one another in the 90 Hz rooms.

Carbon dioxide **(CO_2)**, ammonia **(NH_3)**, and humidity were measured weekly to assess air quality. An environmental monitor was used to measure both humidity and CO_2 ($CO_2$40; Extech Instruments; Nashua, NH). A ToxiRAE II Personal Single-Gas Monitor (RAE Systems-Honeywell; San Jose, CA) or Dräger-Tubes with a handheld pump (Dräger, Inc.; Houston, TX) were used to measure NH_3.

2.3.2 Data Collections

2.3.2.1 Body weight

Group BW were taken (0d, 4, 8, and 11 wk) to allow calculations of average BW. At 4 wk, birds (20) were placed into crates and weighed. At 8 and 11 wk, groups of 25 or 15 birds, respectively, were weighed on a purpose-built scale (Figure 2.1). The group weights were then added together to get a whole room weight.

Figure 2.1. Purpose built turkey scale courtesy of Sameeha Jhetam (2019)

2.3.2.2 Feed intake

Feed weights were recorded at 4, 8, and 11 wk by weighing the remaining feed in all feeders per room. The weight of feed added to each room was recorded on a daily basis. Using the remaining feed weight and the added weight, the amount of FI for a whole room was calculated.

Feed intake= feeder weight(kg) + feed added(kg) – remaining feed weight(kg)…………………………………………………………………………………(Eq.1)

2.3.2.3 Feed efficiency

Feed efficiency was calculated for the following periods: 0d-4 wk, 4-8 wk, 8-11 wk, and 0d-11 wk. The FI per room, along with the average BW gain per room for each of the collection periods, were used to calculate feed conversion as a feed-to-gain ratio **(F:G)**. By accounting for mortality in each room during each period, mortality corrected F:G **($F:G^m$)** was also calculated.

F:G=feed intake(kg)/body weight gain(kg)…………………………………………………(Eq.2)

$F:G^m$=feed intake(kg)/(final body weight(kg)+weight of mortalities and culls-initial body weight)………………………………………………………………………………(Eq.3)

2.3.2.4 Flock uniformity

At 10 wk, 30 birds per room were randomly selected and individually weighed to assess flock uniformity. The individual weights were categorized as being within 5, 10, or 15% of the average BW per room. The coefficient of variation was also used to indicate deviation around the average flock BW.

2.3.2.5 Mortality/Morbidity

Birds that were sick were removed from treatment rooms and either placed in a hospital pen or were euthanized depending on the severity of sickness and likelihood of recovery. In order to make the decision for recovery versus euthanasia, the humane endpoints in the Poultry Centre’s Humane Intervention Point Checklist (Appendix A.3) were used.

Cervical dislocation was used as the method of euthanasia. Mortality and culls were sent to Prairie Diagnostic Services Inc. for necropsy to determine the cause of death. Causes were placed into one of six categories: metabolic (round heart, sudden death with peri-renal hemorrhage), infectious (yolk sac infection, osteomyelitis, peritonitis, polyserositis, arthritis/synovitis, pericarditis, general infection), mechanical (trauma, broken bone), aggressive damage, unknown, and other (emaciation/starveout, pendulous crop, decomposed, general). Values were expressed as a percentage of the number of turkeys placed (364) per room.

2.3.2.6 Mobility

Gait scoring was used to determine the mobility of 30 randomly selected birds per room (10 wk). Scoring was conducted by two observers using a scale (Garner et al., 2002; Vermette et al., 2016b; Beaulac and Schwean-Lardner, 2018). Scores ranged from 0-5 (score 0=bird walks normally, 5=bird unable to walk). Scores from 3-5 were not seen at the time of testing, as birds with such an impediment were culled (Appendix A.3). Scores were expressed as frequency of each score and then the average score for each treatment.

2.3.2.7 Footpad lesions

Footpad lesions were scored at 10 wk on the same 30 bird per room group. The right footpad of each bird was scored based on the scale by Hocking et al. (2008). The scale ranged from 0-4, where a score of 0=no lesions and 4=½ or more of the foot was covered by a lesion. Scores were expressed as frequency of each score and then the average score for each treatment.

2.3.2.8 Feather scores

At 10 wk, the same 30 birds per room were scored for feather condition using the system described by Davami et al. (1987), Sarica et al. (2008), and Beaulac and Schwean-Lardner (2018). The scorer observed the breast, wing, tail, and back and then assigned each area a score. The individual area scores were then totalled to give a total score for feather condition. The scoring system ranged from 1-4 with a score of 1=no feather cover and 4=full intact plumage. The total score for all locations ranged from 4-16. Values were presented as average scores.

Feather cleanliness was also scored at the same time, on the same birds, by the same observer. The scoring system described by Forkman and Keeling (2009) was used. The system ranged from 1-4, where a score of 1=very clean feathers and 4=very dirty feathers. The whole bird was scored, and values were expressed as the frequency of scores and average score for each treatment.

2.3.2.9 Ocular weight and shape

At 11 wk, 4 birds per room were weighed and then euthanized using an electric stunning knife at a power level of 8.5 (VS200, Midwest Processing Systems, Minneapolis, MN, USA). The right eyes were then removed, and excess tissue on the eyeball was removed. The cleaned eyes were weighed (ME4002E Precision and Analytical Balance, Mettler-Toledo, Greifensee, Switzerland) and measured, including medio-lateral diameter, dorso-ventral diameter **(DV)**, and anterior-posterior size **(AP)** using a digital caliper (Mastercraft Digital Caliper 6-in, Canadian Tire Corporation, Toronto, ON).

2.3.2.10 Litter quality

Litter quality was assessed using a visual scale adapted from the Welfare Quality Consortium® (2009) and Adler et al. (2021) at 11 wk. Four measurements were taken per room (middle rear, right back wall, under left front drinker, and middle front) to get an average score for the room. A handful of litter from the top layer was collected at each location then squeezed and examined for qualities of dryness and compactibility to determine litter moisture and, therefore, general quality.

2.3.3 Statistical analyses

Data were analyzed using a randomized complete block design with a one-way factorial analysis. The experimental unit was room (6 replicates/treatment total). Proc Univariate (SAS® 9.4, Cary, NC, USA) was used to test data for normality prior to analyses. If data were not normally distributed, they were log + 1 transformed. Proc Mixed (SAS® 9.4, Cary, NC, USA) was used to analyze differences among group means. All data were tested for block significance, with block removed as a random factor if it was not significant. If the ANOVA found significant differences between means, a Tukey's range test was used to separate the means. Differences were considered significant if $P<0.05$. Differences were considered a trend if $0.05<P\leq 0.10$.

2.4 Results

2.4.1 Body weight

The results of BW and BW gain are shown in Table 2.2. At 8 wk, the birds in the 30HZ weighed less (*P*=0.025) than those in the 195HZ, with those in the 90HZ at an intermediate weight. In the period from 8-11 wk, the birds raised in the 30HZ gained more weight than those in 90HZ with those in the 195HZ gaining an intermediary amount (*P*=0.003). There was also a weak trend for the birds in the 90HZ to gain less weight than those in either other treatment from 0d-11 wk (*P*=0.099).

Table 2.2. Effect of LED light flicker frequency on average body weight (kg) and body weight gain (kg) of turkey hens weighed at 0d, 4, 8, and 11 weeks of age

Age (wk)	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Average Body Weight					
0 d	0.06	0.06	0.06	0.001	0.592
4[*]	0.75	0.75	0.78	0.010	0.200
8[*]	3.77[b]	3.84[ab]	3.86[a]	0.019	0.025
11[*]	7.28	7.16	7.28	0.032	0.100
Average Body Weight Gain					
0d-4[*]	0.69	0.70	0.72	0.010	0.198
4-8	3.02	3.09	3.08	0.017	0.226
8-11	3.51[a]	3.32[b]	3.42[ab]	0.026	0.003
0d-11[*]	7.22	7.10	7.22	0.032	0.099

[*]Block differed significantly: included as a random factor (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Standard error of the mean

2.4.2 Feed intake

During the 0d-4 wk and 4-8 wk periods, turkeys in the 30HZ consumed less feed than those in the 90HZ and 195HZ (*P*≤0.001; Table 2.3). No other differences in FI were noted.

Table 2.3. Effect of LED light flicker frequency on turkey hen feed consumption (kg) from 0d-4, 4-8, 8-11, and 0d-11 weeks of age

Age (wk)	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
0d-4*	0.84[b]	0.89[a]	0.88[a]	0.009	<0.001
4-8*	4.64[b]	4.75[a]	4.75[a]	0.019	0.001
8-11*	6.99	6.73	6.90	0.080	0.185
0d-11*	12.46	12.37	12.53	0.069	0.619

*Block differed significantly: included as a random factor ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean.

2.4.3 Feed efficiency

Feed efficiency as F:G was not affected by light flicker. When corrected for mortality, during 8-11 wk and 0d-11 wk, birds in the 30HZ had improved feed efficiency compared to those in the 90HZ with those in 195HZ intermediate (P=0.048; 0.040, respectively; Table 2.4). No other differences were noted for F:G^m.

Table 2.4. Effect of LED light flicker frequency on turkey hen feed-to-gain ratio and mortality corrected feed-to-gain ratio from 0d-4, 4-8, 8-11, and 0d-11 weeks of age

Age (wk)	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Feed-to-Gain					
0d-4	1.227	1.279	1.224	0.0162	0.293
4-8	1.547	1.550	1.550	0.0049	0.972
8-11*	2.038	2.067	2.082	0.0235	0.197
0d-11*	1.748	1.759	1.761	0.0112	0.277
Feed-to-Gain Mortality Corrected					
0d-4	1.222	1.273	1.223	0.0159	0.320
4-8	1.518	1.529	1.529	0.0043	0.493
8-11*	1.964[b]	2.010[a]	1.989[ab]	0.0224	0.048
0d-11*	1.703[b]	1.725[a]	1.714[ab]	0.0113	0.040

*Block differed significantly: included as a random factor ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean

2.4.4 Flock uniformity

Flock uniformity was not impacted by light flicker treatment (% birds within 5, 10, or 15% of the average flock BW; Table 2.5). The coefficient of variation of BW also showed no influence of flicker.

Table 2.5. Effect of LED light flicker on turkey hen flock uniformity as the percent of birds within 5, 10, and 15% of mean body weight at 10 weeks of age

	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
% within x% of the mean					
5	49.33	53.89	52.00	2.215	0.671
10	79.33	86.67	86.00	2.205	0.346
15	92.67	96.67	96.00	1.325	0.439
Uniformity CV[2]					
CV	8.18	6.87	7.61	0.431	0.332

[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean
[2]Coefficient of variation

2.4.5 Mortalities/Morbidities

Over the flock life (0d-11 wk), mortality due to the infectious cause category was higher in birds raised in the 90HZ compared to those in the 195HZ with those in the 30HZ intermediate (P=0.049; Table 2.6). From 0d-11 wk, total mortality was reduced in the 195HZ birds compared to the 30HZ birds with the 90HZ birds intermediate (P=0.024; Table 2.6). Flicker treatment had no effect on specific causes of mortality within each category for that period (Table 2.6).

Table 2.6. Effect of LED light flicker on causes and categories of mortalities and morbidities as a percent (%) of birds placed in turkey hens from 0d-11 weeks of age

% of birds in category	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Aggressive Damage	3.30	2.88	3.30	0.294	0.785
Metabolic	0.55	0.37	0.33	0.075	0.623
Round Heart	0.44	0.32	0.27	0.047	0.508
Sudden Death with Peri-Renal Hemorrhage	0.11	0.05	0.05	0.040	0.843
Rotated tibia*	0.11	0	0.05	0.028	0.101
Infectious	0.27^{ab}	0.41^{a}	0.05^{b}	0.064	0.049
Yolk Sac Infection	0.05	0.09	0	0.028	0.421
Osteomyelitis	0.11	0.09	0	0.031	0.333
Peritonitis	0.11	0	0.05	0.028	0.276
Polyserositis	0	0.09	0	0.023	0.171
Arthritis/Synovitis	0	0.05	0	0.017	0.465
Pericarditis	0	0.05	0	0.017	0.465
General Infection	0	0.05	0	0.017	0.465
Mechanical	0.38	0.46	0.38	0.094	0.962
Trauma	0.16	0.23	0.33	0.075	0.723
Broken Bone	0.22	0.23	0.05	0.061	0.446
Unknown[2]	1.04	0.73	0.44	0.152	0.340
Other	0.82	0.50	0.66	0.123	0.570
Emaciation/Starveout	0.11	0.05	0	0.028	0.308
Pendulous Crop	0.66	0.27	0.55	0.102	0.276
Decomposed	0	0.09	0	0.023	0.171
General	0.05	0.09	0.11	0.033	0.819
Total	6.48^{a}	5.36^{ab}	5.22^{b}	0.307	0.024

*Block differed significantly: included as a random factor (P<0.05)

[a,b]Values with different letters within the same row differ significantly (P<0.05)

[1]Standard error of the mean

[2]Category consists of No Visible Lesions

Minor differences were noted in categories and causes of mortality at 0d-4 wk and 4-8 wk of age. Total mortality from 0d-4 wk was reduced in the 195HZ compared to the 30HZ and the 90HZ birds (0.55, 1.48, 1.37, respectively; P=0.005). From 4-8 wk round heart mortality was reduced in the 30HZ birds compared to those in the 90HZ and the 195HZ (0.27, 0.05, 0.05, respectively; P=0.040). No differences in categories or individual causes of mortality were noted from 8-11 wk.

2.4.6 Mobility (gait score) and footpad lesion scores

Treatment had no effect on gait scores (Table 2.7). Footpad lesion scores of 0 were more frequent for birds reared in the 90HZ compared to those in the other treatments (*P*=0.011). When averaged, footpad scores for birds reared in the 90HZ treatment were lower (improved) compared to those in the other treatments (*P*=0.021; Table 2.7).

Table 2.7. Effect of LED light flicker on average and frequency (% in each category) of mobility (gait scores; scale 0-5[1]) and footpad lesion scores (scale 0-4[2]) in 10-week-old turkey hens

Score	Treatments (Hz)			SEM[3]	*P*-value
	30	90	195		
Gait Scores					
0*	64.67	48.61	49.33	5.270	0.106
1*	30.00	42.22	40.67	4.070	0.176
2*	5.33	9.17	10.00	1.691	0.304
3*	0	0	0	-	-
4*	0	0	0	-	-
Average Score*	0.41	0.61	0.61	0.067	0.101
Footpad Lesion Scores					
0*	10.67[b]	24.44[a]	10.67[b]	3.855	0.011
1*	16.67	26.11	15.33	3.256	0.341
2	24.00	28.89	28.67	2.674	0.736
3*	27.33	14.44	25.33	3.347	0.147
4*	21.33	6.11	20.00	4.093	0.179
Average Score*	2.32[a]	1.52[b]	2.29[a]	0.262	0.021

*Block differed significantly: included as a random factor (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Score of 0=no impairment, 5=complete lameness (adapted from Garner et al., 2002 by Vermette et al., 2016b)
[2]Score of 0=no external signs of a lesion, 1=harder and denser footpad than score 0, 2=swelling and less than ¼ area necrotic, 3=swelling and ½ area necrotic, 4=more than ½ area necrotic (Hocking et al., 2008)
[3]Standard error of the mean

2.4.7 Feather scores

Flicker treatments had no effect on feather condition of 10 wk old turkey hens.

Table 2.8. Effect of LED light flicker on average feather condition scores (scale 1-4[1]) of back, wing, tail, and breast of turkey hens at 10 weeks of age

Average score	Treatments (Hz)			SEM[2]	*P*-value
	30	90	195		
Back	4.00	3.99	3.98	0.005	0.431
Wing	3.98	3.99	3.98	0.006	0.864
Tail	3.91	3.85	3.83	0.022	0.726
Breast*	3.82	3.83	3.80	0.021	0.324
Total*[3]	15.71	15.66	15.59	0.032	0.645

*Block differed significantly: included as a random factor (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Score of 1=no feather cover, 2=greater than 50% of plumage missing, 3=less than 50% of plumage missing, 4=full intact plumage (Davami et al., 1987 and Sarica et al., 2008)
[2]Standard error of the mean
[3]Sum of four parts: back, wings, tail, breast scored on a scale of 1-4

Feather cleanliness scores are displayed in Table 2.9. Scores of 1 were more frequent in the 90HZ birds than the 195HZ birds with the 30HZ birds intermediate (*P*=0.030). The average scores of the 90HZ birds were reduced (cleaner birds) compared to the 195HZ birds with the 30HZ birds intermediate (*P*=0.021; Table 2.9). The trends for scores of 2 and 3 (*P*=0.088, 0.071, respectively) showed that 90HZ birds were cleaner.

Table 2.9. Effect of LED light flicker on average and frequency of feather cleanliness scores (scale 1-4[1]) of turkey hens at 10 weeks of age

% of scores in category	Treatments (Hz)			SEM[2]	*P*-value
	30	90	195		
1*	42.00[ab]	62.78[a]	26.67[b]	7.722	0.030
2*	54.00	35.56	56.67	5.828	0.088
3	4.00	1.67	16.00	2.780	0.071
4	0	0	0.01	0.208	0.357
Average Score*	1.62[ab]	1.39[b]	1.91[a]	0.100	0.021

*Block differed significantly: included as a random factor (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Score of 1=very clean, 2=moderately clean, 3=moderately dirty, 4=very dirty (Forkman and Keeling (2009) as modified from Wilkins et al. (2003))
[2]Standard error of the mean

2.4.8 Ocular weight and morphology

The results for the influence of flicker treatment on the morphology and weight of the right eyeball are shown in Table 2.10. The DV was largest in the 30HZ birds and smallest in the 195HZ birds with the 90HZ birds intermediate (*P*=0.046). The AP was largest in the 30HZ birds and smallest in the 90HZ birds with the 195HZ birds intermediate (*P*=0.032). No differences were noted in weight or medio-lateral diameter.

Table 2.10. Effect of LED light flicker on eye weight (g), medio-lateral diameter (mm), dorso-ventral diameter (mm), and anterior posterior size (mm) of turkey hen eyeballs at 11 weeks of age

	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Eye weight	5.75	5.63	5.67	0.039	0.386
Medio-lateral diameter*	24.45	24.25	24.35	0.060	0.211
Dorso-ventral diameter*	24.56[a]	24.42[ab]	24.33[b]	0.069	0.046
Anterior posterior size	18.89[a]	18.60[b]	18.62[ab]	0.048	0.033

*Block differed significantly: included as a random factor (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Standard error of the mean

2.4.9 Litter quality

While there were no differences in the litter quality between the light flicker treatments, there was a trend for the litter quality to be poorer in the 195HZ rooms along the right-hand wall (*P*=0.076; Table 2.11).

Table 2.11. Effect of LED light flicker on average litter quality scores (scale 0-4[1]) of 4 locations in rooms of 11-week-old turkey hens

Location in room	Treatments (Hz)			SEM[2]	*P*-value
	30	90	195		
Back	0.60	0.17	0.20	0.120	0.285
Right Wall*	1.40	1.17	2.40	0.256	0.076
Middle	1.60	1.83	1.80	0.144	0.797
Under Drinker	4.00	3.83	3.80	0.085	0.639
Average	1.90	1.75	2.05	0.091	0.504

*Block was included as a random factor because it had significance (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Score of 0=completely dry and flaky, 1=dry but clumps form with compact, 2=ball forms but falls apart, 3=readily sticks into a ball 4=cap/crust must be broken and sticks into a wet ball (adapted from Welfare Quality Consortium® (2009) and Adler et al. (2021))
[2]Standard error of the mean
[3]Average of four locations: back, right wall, middle, under drinker scored on a scale of 0-4

2.4.10 Discussion

There is little information on the influence of light flicker on the productivity, health, and mortality of poultry species. What information is available may not be applicable to turkey hens under the measured flicker frequencies as species and sexes differ in their reactions to their environment (Schwean-Lardner et al., 2012b; Vermette et al., 2016a; Anandh, 2019; Dudusola et al., 2020). Therefore, the objective of this study was to determine the influence of white LED light flicker on the productivity, health, and mortality of turkey hens from 0d-11 wk.

A reduction in BW of poultry may suggest that a management practice or environmental factor is having a negative effect on the birds and compromising their welfare. With respect to growth, LED light flicker only influenced performance in the early life of turkey hens. Turkeys exposed to lower flicker frequencies appeared to adjust over time, and overall, the flicker treatments did not reduce BW. Overall, this result is similar to the result of Raabe et al. (2024), where no differences were seen in BW of turkey toms in LED flicker of 165, 500 or 16,000 Hz by 141 d. Raabe et al. (2024) did not examine BW early in life, so comments can only be made on the overall outcomes. Similarly, Evans et al. (2012) found no overall effect of light flicker on BW of European starlings. It should be noted that Evans et al. (2012) examined mature birds while this study examined growing birds, which could have led to differences. As Evans et al. (2012) used a different species of bird from the current study and did not use a flicker frequency less than 100 Hz, the results may not be applicable to turkey hens in the measured flicker frequencies.

The reduced BW in this study appears to result from the reduced FI during early life. This result does not align with the observations of Raabe et al. (2024), who saw no change in FI in early measures (1-14 or 15-35 d). The adjustment and subsequent increase in late-period growth by birds in the 30HZ was likely from the improved $F{:}G^m$ later in production. The differences in $F{:}G^m$ are unsurprising, as smaller birds tend to have better feed efficiency than larger birds (Fox and Bohren, 1954; Pym and Solvyns, 1979). The adjustment in production is further supported by the lack of differences in uniformity at 10 wk. Because the treatments did not differ with respect to BW at 11 wk, the early life impacts of light flicker should be focused upon in future research.

Changes in mortality may indicate benefits and disadvantages of management practices and environmental factors. The various differences in mortality in this experiment suggest that characteristics of light flicker (i.e. visible versus unconsciously perceived) impacted turkeys differently.

The differences in stress caused by visible flicker were hypothesized to increase the susceptibility of turkeys to infectious diseases. It is known that chronic stress has negative impacts on the immune system of animals (Shini et al., 2010; Abo-Al-Ela et al., 2021; Chu et al., 2022). In the immune system, chronic stress can reduce the number of immune cells, making birds more susceptible to infectious mortality (Dohm and Metz, 1991; Stier et al., 2009; Shini et al., 2010). To this author's knowledge, no study has examined the influence of visible light flicker on bird stress and/or mortality. Raabe et al. (2024) examined turkey tom mortality/morbidity and found no effect of light flicker. However, they did not comment on individual causes of mortality/morbidity. It has been documented that chronic exposure to visible light flicker has a negative impact on humans, leading to stressful events such as migraines and induction of epileptic episodes (Inger et al., 2014; SCHEER, 2018; Batra et al., 2019). With reductions in overall mortality for birds in the 195HZ, the visible/potentially visible light may have had more negative impacts on the health of the 30HZ and 90HZ birds. As differences were seen in infectious mortality in the 90HZ rather than the 30HZ, as hypothesized, there may have been differences in the visualization of flicker by those birds. This may have occurred because 90 Hz is near or at the laying hen FFF, meaning only some birds might be able to see the flicker (Nuboer et al., 1992; Lisney et al., 2011, 2012).

Chronic stress is also known to have negative impacts on the functioning of the cardiovascular (Aengwanich and Simaraks, 2004), gastrointestinal, and reproductive systems (Fischer and Romero, 2018). In the cardiovascular system, chronic stress can cause blood pressure to spike repeatedly, leading to increased risk of cardiac disease in humans (McEwen, 1998). The heart has also been shown to dilate and increase in size in poultry with chronic stress (Struwe et al., 1992; Aengwanich and Simaraks, 2004). These responses to chronic stress have been noted in broiler chickens under chronic stress (Aengwanich and Simaraks, 2004) and pullets with high levels of cannibalism because they were not beak-treated (Struwe et al., 1992). The differences in stressors, as well as species of poultry, means that those results may not be applicable to turkeys raised under flickering lamps. However, the change that was seen in round heart disease in this study (increased in the 30HZ from 4-8 wk) indicates that chronic stress may have caused changes to the cardiovascular system, making some birds more susceptible to mortality via round heart.

To this author's knowledge, the mobility, footpad lesions, litter quality, or feather condition/cleanliness of birds raised under light flicker have not previously been examined. The differences seen in the examined parameters of footpad lesions and feather cleanliness did not follow a pattern that indicated light flicker was the primary factor in those changes. Further research would have to be conducted to see if an uncontrolled factor was influencing those results.

Previous research has determined that there are factors of lighting that influence the morphology and size of bird eyeballs, including daylength, light intensity, and flicker (Thompson and Forbes, 1999; Vermette et al., 2016b; Leis et al., 2017; Lin et al., 2020). The findings of Lin et al. (2020) partially support those found in this study with reductions in the axial length (AP). As Lin et al. (2020) did not define flicker frequency and examined changes in chicks, it is hard to make direct comparisons to this study's results. Differences in eye morphology (myopia/hyperopia) through the shortening of the AP and increase in DV may alter the turkeys' vision, causing them to interact with their environments differently (Prescott et al., 2003; Leis et al., 2017). Changes in the eye morphology, without changes in their weight, may lead to increased intraocular pressure, causing cataracts or glaucoma (Whitley et al., 1984; Vermette et al., 2016b). Further research should be performed to determine the impacts that light flicker may have on bird vision.

In conclusion, visible light flicker had negative impacts on production early in life, evidenced by the BW and FI results. The birds appear to adapt and show compensatory gain seen in the BW gain, uniformity, and $F{:}G^m$. The reduction in overall mortality indicates that flicker past the point of unconscious perception is beneficial to production. Light flicker has minimal to no influence on the measured health parameters, and those differences observed are likely caused by factors that were not accounted for. While the morphology of the eye is influenced by light flicker, there is no evidence that bird vision is impacted. Overall, LED light flicker has minimal impacts on the production and health of turkey hens, and the mortality of turkey hens can be reduced under light flicker past the point of unconscious perception compared to visible light flicker.

3.0 Chapter 3: Impact of flicker frequency on turkey hen stress, fear responses, and behaviour

3.1 Abstract

This experiment studied the impact of white light-emitting-diode light flicker on the stress, fear, and behaviour of Nicholas Select turkey hens reared to 11 weeks (wk) of age. Three treatments were used, the 30 Hertz (Hz) treatment (30HZ), 90 Hz treatment (90HZ) or 195 Hz treatment (195HZ) and tested in a one-way factorial analysis designed as a randomized complete block using Proc Mixed (SAS 9.4). Birds were housed in nine rooms (3 rooms/treatment/trial) with 364 birds/room at placement. Behavioural expression at 4, 8, and 10 wk was measured by scan sampling 18-hour videos at 20-minute intervals during the photoperiod. Incidences of aggressive damage were monitored with live observation twice daily, with the location of damage recorded. Blood samples were taken from 20 birds/room at 4, 8, and 11 wk to determine heterophil-to-lymphocyte (H/L) ratios as a measure of chronic stress. Novel object test (NOT) was performed daily for the first 7 days (d) and at 4, 8, and 11 wk as a measure of fearfulness. At 4 wk, the percentage of time (%t) birds spent gentle feather pecking (GFP; P=0.040) and performing exploratory behaviours (P=0.049) was lower in the 30HZ compared to the 195HZ. At 8 wk, %t spent wing flapping was lowest for birds in the 195HZ (P=0.004). At 8 wk, the %t GFP (P=0.016), fighting (P=0.049), aggressive pecking (P=0.022), and performing aggressive behaviours were lower for birds in the 30HZ compared to those in the 90HZ. At 10 wk, birds in the 30HZ spent a lower %t preening (P=0.034) compared to those in the 90HZ and 195HZ. The birds in the 30HZ also spent a lower %t performing low incidence/other behaviours compared to those in the 195HZ (P=0.013). Incidences of aggressive damage were reduced (P=0.013) for birds reared in 30HZ versus those in 90HZ during 0d-4 wk. From 4-8 wk, aggressive damage directed at the tail (P<0.001) and overall damage (P=0.010) were reduced in the 30HZ birds versus the other treatments. Incidences of aggressive damage during 8-11 wk, when directed at the wing, were increased (P=0.001) in the 90HZ birds versus the other treatments. Aggression

directed at the neck from 8-11 wk was increased (*P*<0.001) in the 30HZ birds versus the other treatments. Aggression towards the snood was increased (*P*=0.050) in the 195HZ compared to the 30HZ birds during 8-11 wk. At 11 wk, H/L ratios of birds reared under 30HZ were decreased (*P*=0.044). There was no influence of flicker treatment on response to the NOT. Light flicker has minor impacts on most behaviours, with lower aggressive behaviours occurring in the 30HZ. Based on measures used in this work, fearfulness of turkey hens was not impacted by light flicker, and chronic stress was only reduced late in life.

3.2 Introduction

Light flicker is an aspect of light that has been explored to a limited degree, with very little known about its effect on poultry species. Flicker fusion frequency **(FFF)** is the point at which the viewer sees flicker as a continuous stream of light (Lisney et al., 2011). Critical flicker fusion frequency **(CFF)** is the point at which the light flicker is past a viewer's unconscious perception (at any intensity), meaning the brain no longer detects flicker (Lisney et al., 2012). Several studies have examined the FFF of birds and found that laying hens can consciously perceive flicker up to approximately 90-105 Hertz (**Hz**; Nuboer et al., 1992; Jarvis et al., 2002; Lisney et al., 2011, 2012). Lisney et al. (2012) further examined what laying hens can unconsciously perceive (invisible flicker) and determined that hens have a physiological reaction to flicker up to 118-119 Hz (CFF). What little information is available on light flicker and its impact on stress, fear, and behaviour has primarily focused on European starlings with minimal focus on poultry.

It is known that stressors cause poultry to release stress hormones through the Sympathetic Adrenal Medullary **(SAM)** pathway and Hypothalamic Pituitary Adrenal **(HPA)** axis. Direct examination of stress hormones, especially corticosterone **(CORT)**, is often used to determine if birds are stressed. However, stress hormones such as CORT, norepinephrine, and epinephrine cause changes to the behaviour of birds, bird fearfulness, and alter the number of circulating white blood cells (Dohms and Metz, 1991; Shini et al., 2010; Borah et al., 2022). Changes in fearfulness can be noted through fear tests such as NOT. Alterations to circulating white blood cells can be noted in heterophil-to-lymphocyte **(H/L)** ratios. Differences in these parameters can indicate the affective state of birds and their stress level.

To this author's knowledge, Raabe et al. (2024) is the only study to examine turkeys under light-emitting-diode **(LED)** flicker. They examined the feather CORT concentration of toms under 165, 500, or 16,000 Hz as a measure of long-term stress. It was determined that flicker had no effect on CORT measured through feather mass or feather length.

The stress response of European starlings under light flicker has been investigated, and the results are conflicting. Maddocks et al. (2001) examined the impact of fluorescent light flicker at 100 Hz versus 35-40 kHz on the stress response of these birds. They found that flicker did not impact maximal response of CORT (P=0.561); however, basal CORT was higher under the 100 Hz flicker (P=0.023) than the 35-40 kHz flicker. Evans et al. (2012) also examined the stress response of European starlings under fluorescent light flicker of 100 versus. >30 kHz. The authors concluded that birds under >30 kHz had higher basal CORT levels than those under 100 Hz. Greenwood et al. (2004) found similar results when examining the stress response of European starlings under the same lighting treatments as Evans et al. (2012), with no differences between the basal, maximal, or rate of rise of CORT (P>0.05).

There is little information on the behaviour of birds raised with exposure to light flicker. The rate of injured turkey toms due to aggressive behaviours was not impacted by LED light flicker at 165, 500, or 16,000 Hz (Raabe et al. (2024). Activity levels of broiler chickens were higher (P<0.05) under 26 kHz than 100 Hz fluorescent light flicker (Boshouwers and Nicaise, 1992). Greenwood et al. (2004) found that there was no difference in the percentage of time **(%t)** European starlings spent preening in either treatment tested (100 or >30 kHz). Evans et al. (2012) found that European starlings spent a lower %t preening under >30 kHz than 100 Hz (P<0.001). The authors noted that European starlings performed more aggressive and exploratory behaviours when exposed to 100 Hz flicker prior to >30 kHz, regardless of which treatment they were in at the time of observation (P=0.013). The authors concluded that this was because the 100 Hz had longer term negative impacts that were evident even when the light treatment changed.

To this author’s knowledge, there is no information available on the influence of light flicker on the stress, behaviour, or fear responses of turkey hens. It is hypothesized that:

- Comfort behaviours of turkey hens will be reduced by visible and potentially visible light flicker (30 and 90 Hz) as the flicker will increase the level of stress, diminishing the expression of comfort behaviours.

- The 30 and 90 Hz treatments will increase the incidences of aggressive behaviours as stress will induce the fight or flight response, with 'fight' behaviours being expressed more frequently. The visual changes that may occur due to the flicker will also make it more difficult for individual birds to be identified, leading to instability within the social hierarchy and, therefore, increased aggression.
- The 30 and 90 Hz treatments will heighten the fear responses to novel object tests due to the increased stress response, indicated by avoidance behaviours, causing birds to be more wary of unknown objects in their environments.
- The heterophil-to-lymphocyte ratios will be increased as a stress response to the flicker treatments that should be visible (30 and 90 Hz). Through activation of the HPA axis, the number of heterophils and lymphocytes will be altered as the leukocytes respond to the increased corticosterone.

3.3 Materials and methods

This experiment and its procedures have been approved by the University of Saskatchewan Animal Care Committee under the protocol number 20210090 (AUP). Animals used in this experiment were given care that followed the Guide to the Care and Use of Experimental Animals by the Canadian Council of Animal Care (2009).

3.3.1 Animal housing and husbandry

This experiment consisted of two trials (January 11th to March 25th, 2022 and November 14th, 2022 to January 30th, 2023). The two trials examined the impacts of white LED flicker on the welfare, stress, fear responses, and behaviour of turkey hens to 11 weeks **(wk)** of age.

The trials took place at the University of Saskatchewan Poultry Centre Brooding and Rearing Facility, abiding by the requirements of the Canadian Codes of Practice (NFACC, 2016). Staff at the Poultry Centre monitored birds twice daily for health, environment, feed, water, and if needed, provided euthanasia. The birds were fed a commercial diet from a commercial feed mill (Table 2.1). Three light flicker treatments were tested: 30 Hz treatment (30HZ), 90 Hz treatment (90HZ), or 195 Hz treatment (195HZ).

Open room housing was mimicked using nine light-tight rooms (6.7 m x 10 m=67m^2, estimated final stocking density 45 kg/m^2). Each room had 12 drinker cups on Lubing

EasyLine™ pendulum nipple drinkers (Lubing, Cleveland, TN) and wood shaving bedding at an initial depth of 7-10 cm. Each room had 12 tube type feeders (initial pan size 36; changed to 44 cm after 27 days; **d**). During the first 9 d, poults were kept in 3 ovular brooder rings per room (5 m x 7 m diameter), which contained supplemental feeders and drinkers. For the first d, the whole room temperature was kept at 34ºC with humidity at 50-70%. Humidity was maintained using portable humidifiers (55-70%). After the first d, temperature was gradually reduced to 16ºC by 11 wk, and humidity was uncontrolled, though it remained between 50-70% (Aviagen Turkeys, 2015).

The photo and scotoperiods started at 23L:1D and were reduced to 18L:6D by 9 d (Aviagen Turkeys, 2015) with darkness in one continuous period. The photo and scotoperiods were controlled using timers (MC404 NOMA Outdoor Heavy Duty 24-Setting Timer, Canadian Tire Corporation, Toronto, ON) that were temporarily integrated into each room's power supply. Light intensity was initially at 40 lux and was gradually reduced to 10 lux by 8 d. Light intensity was measured weekly (Lighting Passport Spectrometer, ASENSETEK) to maintain the correct value. If light intensities varied from the target, the bulbs in each room were cleaned. If cleaning was insufficient or the intensity exceeded the target, the duty cycle was manipulated to control intensity.

Of the nine rooms, three were randomly assigned to each treatment. Each held 364 birds (1092 birds/treatment) at placement. The 30 Hz and 90 Hz flicker frequencies were purposely created using Greengage Lighting Ltd. AgriLamp bulbs, an AgriLamp Symmetry Control Dimmer, and DTD controller (AgriLamp 11W ES26.27; Greengage Lighting Ltd., Edinburgh, UK). The 195 Hz rooms used AgriLamp Bulbs and Symmetry Control Dimmer; however, the flicker was a result of the general fluctuations from the Poultry Centre's power source. The equipment to produce and control the flicker was connected to each room's power and lighting systems for the trial. Once a wk, flicker frequencies were measured to ensure they were being maintained at the treatment set point using a spectrometer (Lighting Passport Spectrometer, ASENSETEK) and oscilloscope (TDS 210 Digital Real-Time Oscilloscope, Tektronix Beaverton, OR, USA) and Lichtflimmer (LiFli) (Messgerat LiFli, Fauser Elektrotechnik, München, Germany). The spectrometer was used to measure the flicker frequencies in the 30 and 90 Hz treatments, while the oscilloscope and LiFli were used in the 90 and 195 Hz treatments.

Two different tools were used for the different frequencies, as each could not accurately measure the full spectrum of treatments. The 90 Hz treatments were measured using both tools to ensure measurements were consistent.

Environmental conditions of relative humidity, ammonia **(NH_3)**, and carbon dioxide **(CO_2)** concentrations were measured once per wk to ensure they were within the requirements of the Canadian Codes of Practice (NFACC, 2016). Relative humidity and CO_2 were measured using an environmental meter ($CO_2$40; Extech Instruments; Nashua, NH). An NH_3 meter (ToxiRAE II Personal Single-Gas Monitor, RAE Systems-Honeywell; San Jose, CA) and Dräger-Tubes with a handheld pump (Dräger, Inc.; Houston, TX) were used to measure NH_3.

3.3.2 Data collections

3.3.2.1 Behavioural expression

Bird behaviour was recorded for 18 continuous hours at 4, 8, and 10 wk using infrared cameras mounted to the ceiling (Bosch WZ45 Integrated IR Dome; Bosch Security Systems, Inc., Fairport, New York, USA). The cameras were placed so that the field of view contained one drinker line, 4 feeders, and an edge of the room along the wall (Torrey et al., 2013a, b; Beaulac and Schwean-Lardner, 2018). Recordings were later observed (Genetec Omnicast Software, Genetec Inc., Montreal, Canada) and analyzed using a 20-minute scan sampling technique (photophase) to determine the specific mutually exclusive behaviours of the birds within the field of view. For a bird to be considered within the field of view, more than half of the bird needed to be visible. For nutritive behaviours, if only the head of the bird was visible and it was actively eating or drinking, it was considered to be within the field of view. Behaviours were defined, and birds were assigned to the correct behaviour based on their actions using an ethogram (Table 3.1). Inter and intraobserver reliabilities were tested, with requirements at 80% and 85%, respectively.

Table 3.1. Behavioural ethogram for turkey hens. Adapted from Guhl (1958), Kruijt (1964), Martrenchar (1999), Estevez et al. (2002), Javid et al. (2016), Mohammed et al. (2016), Vermette et al. (2016b), and Beaulac and Schwean-Lardner (2018), Baxter et al. (2019)

Nutritive Behaviours	
At the feeder	The bird is standing or sitting at the feeder with its head in the feeder (Vermette et al., 2016b; Beaulac and Schwean-Lardner, 2018).
At the drinker	The bird is standing or sitting at the drinker with its head in the drinker cup (Vermette et al., 2016b; Beaulac and Schwean-Lardner, 2018).

Active Behaviours	
Resting	Bird is lying down, not performing any other behaviour. The bird may or may not be sleeping (Vermette et al., 2016b).
Standing	The bird is standing not performing another behaviour (Beaulac and Schwean-Lardner, 2018).
Walking	The bird takes two or more consecutive steps at a walking or running pace (Beaulac and Schwean-Lardner, 2018).
Jumping	Leaping with both feet off the ground (Javid et al., 2016).
Pacing	A novel behaviour where birds open their wings and crouch then erratically and rapidly run back and forth (not necessarily) in a ‘pacing’ motion.
Frolicking	Spontaneous and rapid running and/or jumping and wing-flapping with no obvious intention, often with rapid direction changes. Running without wing-flapping is not classified as frolicking. A frolicking bout ends when the bird sits down or resumes another activity. Birds displaying frolicking directly, leading to sparring, are categorized as sparring to avoid misinterpretation of their movements (Guhl, 1958).
Aggressive Behaviours	
Fighting	Two or more birds, where one is posturing with its head back and breast thrust forward. The actions may also include one individual running and/or jumping at the other (Beaulac and Schwean-Lardner, 2018).
Aggressive Pecking	One bird forcefully pecks at a pen mate’s head, body, or snood while standing or resting. The pen mate will typically move away (Vermette et al., 2016b).
Posturing	Two birds stood staring at each other for >2s with necks upstretched and breasts pushed out (Estevez et al., 2002).
Comfort Behaviours	
Preening	The bird is manipulating its own feathers with its beak while standing or resting (Vermette et al., 2016b).
Stretching	The bird extends its wing(s) and/or leg(s) (Vermette et al., 2016b).
Wing Flapping	The bird flaps both wings (Beaulac and Schwean-Lardner, 2018).
Dust Bathing	Fluttering movement of the bird while in a lying position on the litter too pull loose substrate close to the body into the feathers (Kruijt, 1964).
Feather Ruffle	The bird shakes its whole body while standing or resting (Beaulac and Schwean-Lardner, 2018).
Head Scratching	Head and base of the beak are scratched, the leg passing under the wing (Kruijt, 1964).
Perching	Sitting or standing high off the ground, typically on a structure (Mohammed et al., 2016).
Head Rubbing	With one quick sweep, the side of the head is rubbed over the plumage of the back, shoulder, wing, or breast (Kruijt, 1964).
Exploratory Behaviours	
Environmental Pecking	Pecking at walls, drinker line (not the cups), or litter while standing or resting (Vermette et al., 2016b).
Feather Pecking	One bird pecks at pen mate’s feather while standing or resting. The pen mate does not typically move away (Vermette et al., 2016b).

Litter Scratching	Scratching at the ground from standing or walking (Baxter et al., 2019).
Disturbances	
Moderate Disturbance	A laying bird opens its eyes, lifts its head, or moves its body as a result of another bird walking in front of it, on top of it, touching it, or flapping near it (Martrenchar, 1999; Beaulac and Schwean-Lardner, 2018).
Severe Disturbance	A bird in a laying posture stands up as a result of another bird walking near it, on top of it, or flapping near it (Martrenchar, 1999; Beaulac and Schwean-Lardner, 2018).
Low Incidence/Other	Behaviours that occur extremely infrequently or were not occurring naturally.

3.3.2.2 Incidences of aggressive damage

Birds were monitored twice daily for signs of aggressive damage, including pecking damage, scratches, and general trauma to the body. Injured birds were caught, and pine tar was applied to the damaged area to reduce the chance of repeated aggression towards the area (Barnes and Greive, 2017). The location of the damage, date, and recurrence were noted (tail, wing, back, neck, head, snood, or skin tear). If the damage to the bird was severe, birds were either moved to a hospital pen or euthanized based on humane intervention points (see Appendix A.3).

3.3.2.3 Stress

Chronic stress of birds was examined using H/L ratios at 4, 8, and 11 wk. A random sample of 20 birds/room were selected, and approximately 2 ml of blood was drawn from the brachial vein into EDTA vacutainer tubes using 1 inch 22-gauge needles. Tubes were inverted to ensure no clots formed and were placed on an inversion table until smear stain slides were created. Each sample was used to create duplicate slides, resulting in 40 slides/room and 120 slides/treatment. Once slides were dry, they were stained using PROTOCOL TM Hema 3TM (Fischer Scientific; Ottawa, Canada). After staining, the slides air dried. Once dry, the best of each pair of slides was examined under a microscope (B-290TB; Optika©; Bergamo, Italy) using 100 x oil magnification. In order to calculate the H/L ratio, the number of heterophils and lymphocytes were counted until 100 cells total were found. The number of heterophils was then divided by the number of lymphocytes to calculate the H/L ratio.

3.3.2.4 Fear

The fearfulness of birds was tested using a novel object test **(NOT)** daily for the first 7 d and then at 4, 8, and 11 wk. Various objects (Figure 3.1) were used to ensure the object remained novel. During the first wk, two observers entered each room with 1 observer at the brooder ring in the rear left of the room and the other at the front left brooder ring. The same two observers were used in each room during a single day. The observers each wore a white lab coat to ensure that clothing would not serve as a distraction. The observers placed the object inside the brooder ring, at the same time, in a location where the majority of the birds could see it. Objects were placed at the same time to ensure that potential sounds when the object was placed would not influence reactions. The observer then timed until 3 individual birds made intentional contact with the object. Intentional contact was considered a peck or a bird using its foot (intentionally) to make contact with the object. The maximum time allowed per room was 10 minutes. After brooder rings were removed (post 7 d), one observer was stationed in the rear middle of the room and the other in the front middle and the same procedure was followed. If one observer had 3 birds make contact prior to the other, their object was quietly removed to not be a distraction.

Figure 3.1. Objects used to perform the NOT

3.3.3 Statistical analyses

Data were analyzed with one-way factorial analysis using a randomized complete block design. Trial (n=2) was the blocking factor, and the experimental unit was the room (3 replicates/treatment in each trial).

Proc Univariate (SAS® 9.4, Cary, NC, USA) was used to analyze data initially for normality. Log + 1 transformations were used if necessary for data to meet the normality assumptions. Data were tested for block significance, with block removed as a random factor when P>0.05. Proc Mixed (SAS® 9.4, Cary, NC, USA) was used to analyze differences between group means in a data set. If significant differences were found, a Tukey's range test was then used to separate the means. Differences were considered significant if P<0.05, and trends were noted if 0.05<P≤0.10.

3.4 Results

3.4.1 Behavioural expression

The results of the behaviour of 4 wk turkey hens are shown in Table 3.2. In the comfort and maintenance behaviour category, there was a trend for birds in the 90HZ to spend a greater %t preening than those in the 30HZ, with birds in the 195HZ intermediate (P=0.053). There was also a trend for birds in the 195HZ to spend a greater %t head scratching than those in the 90HZ (P=0.059).

In the exploratory behaviour category, birds in the 195HZ spent the greatest %t gentle feather pecking **(GFP)** versus those in the 30HZ with those in the 90HZ intermediate (P=0.040). The same pattern was seen in the total exploratory behaviour category (P=0.049).

At 4 wk, there were several weak trends for a variety of behaviours. Standing was performed for the greatest %t by birds in the 90HZ compared to those in 30HZ (P=0.094). There was also a trend for birds in the 30HZ to spend a greater %t at the feeder than those in the 90HZ (P=0.091). The same pattern was seen in the total nutritive behaviours (P=0.086).

Table 3.2. Percentage (%) of time spent performing behaviours by 4-week-old turkey hens within field of view (20-minute intervals) during the photoperiod

Behaviours	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Comfort and Maintenance					
Preening	6.76	7.90	7.11	0.322	0.053
Stretching	0.46	0.45	0.57	0.034	0.261
Wing flapping	0.07	0.04	0.07	0.016	0.726
Dust bathing	0.20	0.49	0.30	0.064	0.111
Head scratching	0.06	0.05	0.11	0.015	0.059
Feather ruffle	0.07	0.07	0.08	0.012	0.570
Perching	0.26	0.29	0.23	0.037	0.760
Head rubbing	0.05	0.03	0.03	0.013	0.983
Total	7.93	9.31	8.50	0.336	0.315
Mobility					
Walking	20.94	21.21	19.58	0.930	0.600
Frolicking	0.36	0.29	0.39	0.042	0.608
Pacing	0.10	0.03	0.03	0.014	0.173
Jumping	0.02	0.03	0.03	0.008	0.920
Standing	12.33	14.19	13.80	0.507	0.094
Resting	15.29	14.81	14.84	0.803	0.988
Total Active[2]	33.75	35.77	33.83	0.717	0.567
Exploratory					
Environmental pecking	5.57	5.81	6.78	0.290	0.220
Gentle feather pecking[*]	4.06[b]	4.28[ab]	5.82[a]	0.386	0.040
Litter scratching[*]	0.05	0.11	0.04	0.015	0.119
Total	9.68[b]	10.20[ab]	12.65[a]	0.503	0.049
Nutritive[*]					
Feeding	13.91	8.86	9.11	0.872	0.091
Drinking	2.10	2.39	2.27	0.251	0.703
Total	16.01	11.25	11.38	0.778	0.086
Disturbances					
Moderate disturbance[*]	1.17	1.24	1.29	0.143	0.449
Severe disturbance	0.22	0.25	0.23	0.022	0.686
Total[*]	1.40	1.49	1.52	0.153	0.427
Aggressive					
Fighting	0.30	0.64	0.78	0.177	0.406
Posturing	0.08	0.13	0.17	0.022	0.254
Aggressive pecking	1.25	1.08	0.92	0.093	0.445
Total	1.25	1.85	1.88	0.251	0.307
Low Incidence/Other[3]					
Total	0.93	0.85	0.87	0.070	0.935
Unknown[*]					
Total	14.66	15.32	15.41	1.164	0.962

[*]Block was included as a random factor because it had significance (*P*<0.05)
[a,b]Values with different letters within the same row differ significantly (*P*<0.05)
[1]Standard error of mean

[2]**Total Active:** Walking, frolicking, pacing, jumping, standing
[3]**Low Incidence/Other:** Eating novel object piece, tripping, accidental collision, tail twitching, frolicking, litter scratching, head scratching, perching, head rubbing, pacing, jumping

Table 3.3 depicts the behavioural results of 8 wk old turkey hens. In the comfort and maintenance category, birds in the 195HZ spent a lower %t performing wing flapping than in the other treatments (*P*=0.004).

In the mobility category, there was a weak trend for birds in the 30HZ to spend the greatest %t walking (*P*=0.087).

For exploratory behaviours, birds in the 30HZ spent the lowest %t GFP (*P*=0.016).

In terms of aggressive behaviours, birds in the 90HZ spent a greater %t performing fighting compared to those in the 30HZ with those in the 195HZ intermediate (*P*=0.049). The same pattern was seen in the %t spent performing aggressive pecking and total aggressive behaviours (*P*=0.022; 0.013, respectively).

Table 3.3. Percentage (%) of time spent performing behaviours by 8-week-old turkey hens within field of view (20-minute intervals) during the photoperiod

Behaviours	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Comfort and Maintenance					
Preening	8.14	8.00	8.53	0.370	0.107
Stretching*	0.57	0.48	0.54	0.051	0.981
Wing flapping*	0.18[a]	0.11[a]	0.07[b]	0.030	0.004
Dust bathing*	0.10	0.09	0.07	0.018	0.767
Head scratching	0.12	0.15	0.18	0.021	0.497
Feather ruffle	0.09	0.06	0.05	0.014	0.626
Perching	0.09	0.12	0.17	0.024	0.355
Head rubbing	0.12	0.05	0.08	0.014	0.249
*Total**	9.41	9.05	9.64	0.360	0.726
Mobility					
Walking	12.41	10.81	10.54	0.367	0.087
Frolicking*	0.13	0.10	0.09	0.020	0.604
Pacing	0.08	0.04	0.04	0.011	0.191
Jumping	0.02	0	0.01	0.008	0.470
Standing*	11.83	13.84	12.96	0.578	0.182
Resting	27.68	22.08	24.77	1.096	0.149
Total Active[2]	24.48	24.79	23.63	0.611	0.650
Exploratory					
Environmental pecking*	5.64	5.51	5.62	0.381	0.885
Gentle feather pecking*	4.26[b]	5.61[a]	6.38[a]	0.493	0.016
Litter scratching	0.02	0.02	0.02	0.007	0.894
*Total**	9.92	11.15	12.03	0.762	0.180
Nutritive					
Feeding	11.01	10.00	6.88	1.085	0.284
Drinking	1.92	1.91	2.07	0.280	0.897
Total	12.93	11.91	8.95	1.036	0.276
Disturbances					
Moderate disturbance*	2.40	2.30	2.26	0.212	0.809
Severe disturbance	0.32	0.22	0.24	0.023	0.758
*Total**	2.72	2.52	2.49	0.216	0.881
Aggressive					
Fighting	0.06[b]	0.17[a]	0.11[ab]	0.022	0.049
Posturing	0.06	0.13	0.07	0.023	0.216
Aggressive pecking	0.68[b]	1.20[a]	1.07[ab]	0.094	0.022
Total	0.80[b]	1.50[a]	1.24[ab]	0.112	0.013
Low Incidence/Other[3]					
Total	0.59	0.51	0.63	0.037	0.430
Unknown					
*Total**	15.83	16.97	17.22	1.695	0.876

*Block was included as a random factor because it had significance ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of mean

[2]**Total Active:** Walking, frolicking, pacing, jumping, standing
[3]**Low Incidence/Other:** Eating novel object piece, tripping, accidental collision, tail twitching, frolicking, litter scratching, head scratching, perching, head rubbing, pacing, jumping

Table 3.4 depicts the behavioural results of turkey hens at 10 wk. In the comfort and maintenance category, birds spent a greater %t preening in the 90HZ and 195HZ compared to the 30HZ (*P*=0.034).

In the mobility category, there was a weak trend for birds in the 195HZ to spend the greatest %t pacing (*P*=0.088). It was also seen that birds in the 90HZ spent the greatest %t standing (*P*=0.069). There was also a weak trend for birds in the 90HZ to spend the greatest %t performing total active behaviours (*P*=0.085).

In terms of exploratory behaviours, there was a trend for birds in the 195HZ to spend the greatest %t GFP (*P*=0.067).

It was also found that birds in the 195HZ spent a greater %t performing low incidence/other behaviours compared to those in the 30HZ with those in the 90HZ intermediate (*P*=0.013).

Table 3.4. Percentage (%) of time spent performing behaviours by 10-week-old turkey hens within field of view (20-minute intervals) during the photoperiod

Behaviours	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Comfort and Maintenance					
Preening*	8.29[b]	9.17[a]	9.31[a]	0.520	0.034
Stretching*	0.56	0.34	0.46	0.055	0.238
Wing flapping	0.09	0.09	0.12	0.023	0.830
Dust bathing	0.02	0.03	0.04	0.007	0.324
Head scratching	0.56	0.09	0.15	0.021	0.117
Feather ruffle	0.08	0.06	0.11	0.019	0.577
Perching	0.02	0.02	0	0.005	0.384
Head rubbing	0.05	0.04	0.09	0.013	0.350
*Total**	9.15	9.85	10.29	0.546	0.442
Mobility					
Walking	8.38	8.23	8.77	0.346	0.681
Frolicking*	0.03	0.06	0.02	0.011	0.340
Pacing	0.02	0.01	0.04	0.006	0.088
Jumping	0.01	0	0.02	0.006	0.142
Standing*	12.22	15.86	11.69	0.927	0.069
Resting*	25.86	23.71	23.19	0.573	0.268
Total Active[2]*	20.67	24.15	20.54	0.912	0.085
Exploratory					
Environmental pecking*	7.09	5.01	7.86	0.875	0.184
Gentle feather pecking*	4.63	5.61	6.42	0.582	0.067
Litter scratching	0	0.01	0.01	0.004	0.633
*Total**	11.72	10.63	14.29	1.400	0.373
Nutritive					
Feeding	9.41	6.53	7.17	0.735	0.497
Drinking	1.99	1.78	2.34	0.141	0.303
Total	11.40	8.31	9.51	0.721	0.384
Disturbances					
Moderate disturbance*	1.89	1.84	2.24	0.199	0.584
Severe disturbance*	0.28	0.19	0.22	0.036	0.311
Total	2.16	2.03	2.47	0.185	0.566
Aggressive					
Fighting	0.10	0.07	0.08	0.018	0.990
Posturing*	0.18	0.11	0.11	0.024	0.699
Aggressive pecking	0.50	0.66	1.04	0.120	0.196
Total	0.77	0.84	1.23	0.128	0.282
***Low Incidence/Other*[3]**					
Total	0.21[b]	0.27[ab]	0.36[a]	0.025	0.013
Unknown					
*Total**	18.24	20.43	18.47	1.231	0.571

*Block was included as a random factor because it had significance ($P<0.05$)

[a,b]Values with different letters within the same row differ significantly ($P<0.05$)

[1]Standard error of mean

[2]**Total Active:** Walking, frolicking, pacing, jumping, standing
[3]**Low Incidence/Other:** Eating novel object piece, tripping, accidental collision, tail twitching, frolicking, litter scratching, head scratching, perching, head rubbing, pacing, jumping

3.4.2 Incidences of aggressive damage

Flicker treatment had no effect on the incidences of aggressive damage during the 0d-11 wk period.

Table 3.5. Effects of LED light flicker on the incidences of aggression, as a percentage (%) of total incidents, and % of birds placed directed towards seven different locations on turkey hens from 0d-11 weeks

Location of Aggression	Treatment (Hz)			SEM[1]	*P*-value
	30	90	195		
Tail*	1.21	7.14	5.71	1.213	0.202
Wing	0.49	1.79	0.93	0.226	0.331
Back	0	0	0	-	-
Neck*	3.30	1.92	2.14	0.518	0.170
Head	0.38	0.41	0.82	0.121	0.444
Snood*	3.35	4.90	4.23	0.773	0.354
Skintear*	0.27	0.18	0.27	0.075	0.246
*Total**	9.01	16.35	14.12	2.404	0.119

*Block was included as a random factor because it had significance ($P<0.05$)
[1]Standard error of mean

Table 3.6 depicts the incidences of aggressive damage in 0d-4, 4-8, and 8-11 wk. From 0d-4 wk, more incidences of aggressive damage were noted on birds in the 90HZ compared to birds in the 30HZ ($P=0.013$) with those in the 195HZ intermediate. There were trends for the incidences of aggressive damage directed toward the tail and wing to be the lowest for birds in the 30HZ ($P=0.051$, 0.056, respectively).

From 4-8 wk, there were fewer incidences of aggressive damage directed towards the tail for birds in the 30HZ ($P<0.001$). Total incidences of aggressive damage was lowest for birds in the 30HZ ($P=0.010$). There was a weak trend for there to be a greater number of skin tears on birds in the 30HZ ($P=0.086$).

From 8-11 wk, the incidence of aggressive damage directed towards the wing was the highest on birds in the 90HZ ($P=0.001$). More incidences of aggressive damage were directed at the neck of birds in the 30HZ ($P<0.001$). The incidence of aggressive damage directed towards the snood was higher (*P*=0.050) in the 195HZ birds than the 30HZ birds with the 90HZ birds intermediate.

Table 3.6. Effect of LED light flicker on incidences of aggressive damage and skin tears as a percent (%) of birds placed by location of damage in turkey hens at 0d-4, 4-8, and 8-11 weeks of age

% of birds tarred in each location	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
0d-4 wk					
Tail*	0	0.55	0.55	0.142	0.051
Wing*	0	0.14	0.05	0.031	0.056
Back	0	0	0	-	-
Neck	0	0	0	-	-
Head	0	0	0	-	-
Snood	0	0.09	0	0.023	0.171
Skintear	0	0	0	-	-
Total	0^{b}	0.78^{a}	0.60^{ab}	0.149	0.013
4-8 wk					
Tail*	0.99^{b}	5.63^{a}	4.18^{a}	0.942	<0.001
Wing	0.33	0.87	0.71	0.123	0.124
Back*	0	0	0	-	-
Neck*	1.76	1.42	1.32	0.337	0.131
Head	0.22	0.37	0.66	0.106	0.305
Snood*	2.42	2.93	2.20	0.472	0.780
Skin tear	0.11	0	0	0.023	0.086
*Total**	5.82^{b}	11.22^{a}	9.07^{a}	1.597	0.010
8-11 wk					
Tail*	0.22	1.01	0.99	0.239	0.288
Wing*	0.16^{b}	0.78^{a}	0.16^{b}	0.130	0.001
Back	0	0	0	-	-
Neck*	1.54^{a}	0.50^{b}	0.82^{b}	0.210	<0.001
Head	0.16	0.05	0.16	0.061	0.687
Snood*	0.93^{b}	1.88^{ab}	2.03^{a}	0.335	0.050
Skin tear*	0.16	0.18	0.27	0.069	0.420
*Total**	3.19	4.40	4.45	0.791	0.394

*Block was included as a random factor because it had significance (*P*<0.05)

[a,b]Values with different letters within the same row differ significantly (*P*<0.05)

[1]Standard error of the mean

3.4.3 Stress

Light flicker had an impact on chronic stress in this study. At 11 wk, birds in the 195HZ had higher H/L ratios than birds in the 30HZ with birds in the 90HZ birds intermediate (*P*=0.044; Table 3.7).

Table 3.7. Effect of LED light flicker on heterophil-to-lymphocyte ratios of turkey hens at 4, 8, and 11 weeks of age

Age (wk)	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
4	0.69	0.60	0.67	0.016	0.379
8[*]	0.69	0.67	0.69	0.020	0.945
11[*]	0.94[b]	1.03[ab]	1.07[a]	0.022	0.044

[*]Block was included as a random factor because it had significance ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean

3.4.4 Fear

There was no effect of light flicker on the response of turkeys to the NOT (Table 3.8).

Table 3.8. Effect of LED light flicker on average time (sec) for 3 turkey hens to contact a novel object at 1,2,3,4,5,6,7 days, 4,8, and 11 weeks of age

Age	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
1 d	46	89	85	13.643	0.495
2 d[*]	21	18	34	5.949	0.627
3 d[*]	76	49	108	21.719	0.514
4 d	95	28	60	19.837	0.348
5 d	23	20	22	3.567	0.534
6 d	39	58	40	14.137	0.982
7 d[*]	8	6	6	0.741	0.529
4 wk	486	333	422	41.720	0.331
8 wk	9	8	23	4.774	0.695
11 wk[*]	11	12	20	3.704	0.494

[*]Block was included as a random factor because it had significance ($P<0.05$)
[1]Standard error of the mean

3.4.5 Discussion

To this author's knowledge, no information is available on light flicker and its impact on turkey hen behaviour, welfare, and stress. For turkey toms, Raabe et al. (2024) examined the rate of injury and feather CORT concentrations. There has been some research on the influences that fluorescent light flicker has on the behaviour (Boshouwers and Nicaise, 1992; Evans et al., 2012) and stress (Maddocks et al., 2001; Greenwood et al., 2004; Evans et al., 2012) of European starlings. The current study was performed to help close the gap in the knowledge about light flicker and its influence on poultry.

It was hypothesized that visible light flicker would reduce the performance of comfort behaviours. This hypothesis was found to be partially accurate with greater %t spent preening by the birds in the 90HZ and 195HZ at 10 wk compared to birds in the 30HZ. There was also a trend for the same pattern to occur at 4 wk, with birds spending the greatest %t preening in the 90HZ and the least in the 30HZ. These responses are contradictory to those found by Greenwood et al. (2004), where light flicker did not impact preening. Evans et al. (2012) found that European starlings preened less under >30 kHz flicker than 100 Hz, which also contradicts the results from the current study. Because Greenwood et al. (2004) and Evans et al. (2012) used different species and flicker frequencies, their results may not be useful when drawing conclusions about turkey preening behaviour in this study. There is evidence that preening can also be seen when birds are under stress, termed displacement preening (Duncan and Wood-Gush, 1972; Hill, 1983; Pohle and Cheng, 2009). As behavioural observations were performed using an overhead camera, the minute differences between displacement and general preening may not have been noted. Wing flapping at 8 wk was the only other comfort behaviour influenced, though it occurred at very low incidences. As only two comfort behaviours were influenced by light flicker, the limitations of 20-minute scan samples, and the lack of a pattern in the results, it is difficult to draw conclusions based on these results. Other behaviours can give insight into birds' affective state and welfare if performed at abnormal frequencies. Within this study, GFP and aggressive behaviours were the other behaviours that were affected by flicker treatment and could give insight into the welfare of birds in this study.

Birds often use GFP as a form of social interaction and exploration (Savory, 1995; Dalton et al., 2018). The reduction in GFP by birds in the 30HZ at 4 and 8 wk can indicate a variety of behavioural changes, including a lack of interaction between birds or lack of movement. As turkeys are social animals, the lack of GFP may indicate that birds in the 30HZ were not moving through the room to engage in social interaction (Mench, 2009). While there was a trend for turkeys to be more active in the 90HZ at 10 wk, it was personally observed that birds in the 30 Hz were not as reactive/active throughout the trial. This observation may provide "unofficial evidence" for the idea that GFP was reduced because birds were not moving through the room to interact with conspecifics.

It was hypothesized that visible light flicker would increase aggressive behaviours and, therefore, incidences of aggressive damage. The reduction in the %t spent performing aggressive behaviours by turkeys in the 30HZ may have been a result of the reduced activity that was observed. However, this observation was not reflected in the results for active behaviours, potentially due to the behavioural analysis methodology used. While there is no quantitative evidence from this study, there is potential for the visual cues birds use to distinguish between one another to be altered by the flickering, causing birds to continuously attempt to establish a dominance hierarchy (Sherwin and Devereux, 1999; Moinard et al., 2001; Estevez et al., 2002; Dalton et al., 2013; Duggan et al., 2014). Evans et al. (2012) examined aggressive behaviours of European starlings under high (>30 kHz) and low (100 Hz) flicker frequencies in different sequences and found increases in aggressive behaviours if the birds were exposed to 100 Hz prior to >30 kHz (regardless of treatment at observation).

Aggression in turkeys can be substantially higher than in some other species, making it an important welfare indicator (Buchwalder and Huber-Eicher, 2005; Marchewka et al., 2013; Javid et al., 2016). Interestingly, in this study, the incidences of aggressive damage did not always follow the same pattern as aggressive behaviours. This was likely caused by differences in the observation methods used for these measures. At 8 wk, the greater %t performing aggressive behaviours by birds in the 90HZ compared to those in 30 Hz does align with the incidences of aggressive damage (lower in the 30HZ than the 90HZ). In contrast to this study's results, no effect of flicker was seen on injury rate of turkey toms under LED light flicker at 165, 500, or 16,000 Hz (Raabe et al., 2024). From 8-11 wk, the higher aggressive damage to the neck for birds in the 30HZ is worth noting, as that type of damage is indicative of fighting and did not follow the same pattern as other aggressive behaviours/damage. Interestingly, Raabe et al. (2024) noted that the injury patterns of turkey toms were similar, with deep injuries to the back of the head or neck. Even though there was a reduction in aggression by turkeys in the 30HZ, the underlying causes of this are indicative of reduced welfare of those birds (potentially reduced activity, changes in vision).

It was hypothesized that visible light flicker would cause an increase in the H/L ratio of birds in those treatments (30HZ and 90HZ). The results did not support this hypothesis, with a lower in the H/L ratio in birds under the 30HZ and an increase in birds under the 195HZ, with the 90HZ birds intermediate at 11 wk. This does not align with the results of Raabe et al. (2024), where no differences were noted in feather CORT. The difference in the type of stress examined (CORT versus H/L), though both are indicators of long-term stress, could account for this discrepancy. It is possible for a reduction in H/L ratios to be found in birds that have a lymphocytosis reaction in conjunction with heteropenia (Cotter, 2015). Lymphocytosis reactions occur when an animal is under extreme stress, which causes an influx of lymphocytes from the bone marrow (Harmon, 1998; Cotter, 2015). Heteropenia, which is the reduction of heterophils through exhaustion of the heterophil pool in the bone marrow, in conjunction with lymphocytosis reactions, can reduce the H/L ratio in stressed birds (Gross and Siegel, 1983; Maxwell, 1993; Lentfer et al., 2015). To interpret H/L ratios, it is useful to also examine other indicators of stress, such as behaviour or fearfulness. Using this technique, the reduced aggression by turkeys in the 30HZ may have influenced the reduced H/L ratios of those birds at 11 wk. It is not likely that the 30HZ flicker directly reduces chronic stress; rather, the observed lack of activity and lower aggressive behaviours/damage reduced the overall stress of those birds.

The results from the NOT indicated that flicker frequencies did not influence the fearfulness of birds. However, there was high variability within that data. The turkeys' response towards NOT also indicated that the test may not have functioned as intended. In some cases, the hens picked up the novel objects and ran with them, similar to what is known as the play behaviour "worm running". As turkeys are naturally inquisitive, NOT may not be the most accurate way of examining their fearfulness.

Overall, visible light flicker had minor effects on turkey hen behaviour, with the exception of aggression, through 11 wk. While the 30HZ seems to have benefits in reducing aggression, this is likely due to a lack of natural activity in those birds, which may negatively impact welfare. The 90HZ appeared to have the most negative influence on bird welfare through increased aggression particularly in the 4-8 wk period. Future research could focus on the early life of turkey hens, as there were observations of the birds in the 30HZ exhibiting hiding or avoidance behaviours from the visible flicker when in brooder rings. The lack of quantitative measures taken during the first week was a limiting factor of this experiment, so future experiments should take that into consideration.

4.0 Chapter 4: Overall Discussion

4.1 Introduction

Vision is an important sense for birds, with the eyeball comprising approximately 50% of the cranial space, allowing for increased visual specialization (Bayon et al., 2008; Vallone and Kern, 2021). These ocular specializations include oil droplets in the cones, increased visual pigments and seven subtypes of photoreceptors (Hart et al., 1999; Prescott et al., 2003; Jones et al., 2007; Hy-Line, 2017). Birds are, therefore, highly sensitive to light and the characteristics of light. One of these characteristics is light flicker, which is caused by fluctuations in the power supplied to the light. Two characteristics of the visualization of light flicker are the flicker fusion frequency **(FFF)** and critical flicker fusion frequency **(CFF)**. The FFF is the point at which the viewer sees light flicker as a continuous stream of light (Lisney et al. 2011). The CFF is the point at which the viewer no longer has unconscious perception of flickering light (invisible flicker; Lisney et al., 2012).

Light flicker has not been studied in birds as thoroughly as other characteristics. The few studies that have been conducted have primarily focused on the impact of fluorescent light flicker on European starlings, with even less focusing on domestic poultry species. To this author’s knowledge, only Raabe et al. (2024) has examined the effects of light-emitting-diode **(LED)** flicker on turkey toms. Therefore, this study aimed to determine the impacts of white LED light flicker on turkey hen production, health, and welfare when reared to 11 weeks **(wk)** of age.

The light bulbs in this experiment were set to flicker at one of three frequencies (30, 90, or 195 Hertz; **Hz**). The 30 Hz treatment **(30HZ)** was visible to humans as well as birds. Based on previous limited research, the 90 Hz treatment **(90HZ)** may have been visible to some turkeys as it is close to the FFF of some strains of laying hens (Lisney et al., 2011). The 195 Hz treatment **(195HZ)** should not have been visible or unconsciously perceived as it is well past the CFF of some strains of laying hens and humans (Brundrett, 1974; Nuboer et al., 1992; Jarvis et al., 2002; Lisney et al., 2012). Productivity in this study was evaluated by body weight **(BW)** at various ages, BW gain, feed intake **(FI)**, calculated feed efficiency (feed-to-gain ratio; **F:G** and mortality corrected F:G; **F:G^{m}**), flock BW uniformity, and mortality, along with the cause of mortality or morbidity. Turkey health was assessed using mobility, feather condition, feather cleanliness, litter and footpad scores, and ocular weight and morphology. Turkey welfare was examined using all the previous measures in combination with behavioural expressions, incidences of aggressive damage, fearfulness, and chronic stress.

4.2 Discussion

The BW of birds at the end of a production period is often used as a primary indicator of performance in meat turkey production. Previous research has indicated that LED light flicker did not influence the BW of turkey toms (Raabe et al., 2024). The same conclusion was drawn in this study, but only later in life. This study did see BW changes early in life, which Raabe et al. (2024) did not examine, under the 30HZ. The early reduced BW correlates to the reduced FI by birds reared under the 30HZ (0-8 wk). Interestingly, this was not related to the percentage of time **(%t)** at the feeder. In fact, there was a weak trend for turkeys in the 30 Hz to spend a greater %t at the feeder than those raised in the 90HZ. This contradicts the results of Raabe et al. (2024), who found no difference in FI of turkey toms. This variation could be due to the different flicker frequencies examined in the Raabe et al. (2024) study, which did not use frequencies below 165 Hz. The activity level of birds can impact the %t at the feeder. However, the activity data did not align with results for %t at the feeder, which suggests that activity had no effect on %t at the feeder or FI. Anecdotally, birds in the 30HZ may have been at the feeder without actively feeding with the observed lack of activity in that treatment.

Flock uniformity was not affected by flicker. Although not examined in turkeys previously, other research has indicated that other environmental challenges, such as stocking density, do not significantly affect turkey flock uniformity (Beaulac et al., 2019; Jhetam et al., 2022). In addition, there were minimal changes to behaviours such as %t at the feeder and general activity, both of which could impact uniformity (Collins and Sumpter, 2007). Activity in poultry can give insight into their affective state. Abnormally high levels of activity can indicate birds are ‘hysterical’ and potentially under stress, thus experiencing negative welfare (Fraser, 2008). However, increased activity levels within a more normal range can also indicate birds are experiencing positive welfare if other behaviours confirm this (i.e. play behaviours; Baxter et al., 2019). Very low activity levels can indicate birds are in a depressive state and experiencing negative welfare (Bessei, 2006; Fraser, 2008). The results from previous research about bird activity under light flicker are conflicting (Boshouwers and Nicaise, 1992; Widowski and

Duncan, 1996; Greenwood et al., 2004; Evans et al., 2012). Both Boshouwers and Nicaise (1992) and Evans et al. (2012) found an increase in active behaviours in broiler chicks and European starlings under high flicker frequencies (26 and >30 kHz, respectively) compared to low frequencies (100 Hz). Neither author concluded that the birds in their experiments were experiencing hysterical levels of activity. Widowski and Duncan (1996) and Greenwood et al. (2004) saw no differences in laying hen and European starling activity levels, respectively, due to flicker.

In the current study, it was hypothesized that visible and potentially visible flicker (30 and 90 Hz) would cause hysteria. The results did not support this hypothesis, as the active behaviours affected by flicker were not indicative of hysteria. Although not detected in behavioural analysis, staff commented on reduced activity in turkeys exposed to the 30HZ. Because this was a general observation, we cannot make a conclusion using this information. However, it is suggestive of those birds being in a depressive state and experiencing reduced welfare. It was also noted that in brooder rings, especially on the first day **(d)**, turkeys exhibited hiding/avoidance behaviours in the 30HZ. Birds appeared to use shadows within the brooder ring to escape the flicker, but again, this was not confirmed as measures were not taken at placement. In future, early behaviours could be examined, and a broader view (whole room) and more frequent scan sampling could be performed for more accurate results of behavioural expression.

Mortality is a significant indicator of bird welfare and drives economic return for producers. Raabe et al. (2024) found no differences in the overall mortality of turkey toms reared under LED flicker.

In the current study, there was a reduction in overall mortality in the 195HZ (0d-11 wk and 0d-4 wk). As previously stated, this is a major economic driver, making the reduced mortality a benefit of higher flicker frequencies. Notably, there were no obvious individual causes of mortality/morbidity that contributed to the overall differences between flicker treatments from 0d-11wk.

The reduced infectious mortality in birds raised in the 195HZ versus the 90HZ may be attributed to a combination of reduced immune response and increased injury from aggressive damage (90HZ), which increased the birds' susceptibility to infection (al Afaleq and Jones, 1990). The heterophil-to-lymphocyte **(H/L)** ratios did not support this conclusion. However, in extreme stress, heteropenia and lymphocytosis reactions can occur, which cause H/L ratios to be lower as birds use up their stores of heterophils and have an influx of lymphocytes (Gross and Siegel, 1983; Maxwell, 1993; Cotter, 2015; Lentfer et al., 2015). This may have occurred for turkeys in the 30HZ and the 90HZ treatments. Raabe et al. (2024) found no effect on feather corticosterone **(CORT)**, another indicator of chronic stress, which partially agrees with the results of this study.

The higher round heart mortalities in birds raised in the 30HZ may have been exacerbated by early life stress. Again, the H/L ratio data did not support this. As previously mentioned, poults were observed to hide on the d of placement in the 30HZ, which suggests they were stressed. However, the novel object test **(NOT)** also did not show differences. A NOT has been successfully used in many types of poultry (Jones, 1996; Erasmus and Swanson, 2014; Kulke et al., 2021). Turkeys, however, are naturally inquisitive, and their response to the NOT indicated it may not have functioned as intended. An example included incidences in this study where hens performed “worm running”, a play behaviour where they picked up the object and ran with it. It is difficult to make conclusions about early life stress in turkey hens using the examined parameters in this study.

Previous research that focused on avian species and light flicker has not examined health parameters apart from mortality. As there is no information regarding the physical health of birds raised in light flicker, the results of this study may inform areas for future research to concentrate on.

Litter quality can influence footpad lesions, feather cleanliness, and feather condition (Mayne et al., 2007; de Jong et al., 2014; Saraiva et al., 2016; Schreiter and Freick, 2023). There was no effect of flicker on the litter quality in the current study. This is in agreement with the lack of changes to behaviours that turn the litter and impact its quality, such as litter scratching, dust bathing, and general activity (Bessei, 2006; Lister, 2009; Calvet et al., 2011; Dunlop et al., 2015). Therefore, litter quality likely had little impact on the mobility, footpad lesions, and feather condition and cleanliness of turkeys in this experiment.

Feather condition of a bird can indicate behavioural abnormalities, infection, nutritional deficiencies, or stress (Leong et al., 1959; Greene et al., 1985; Savory, 1995; Leeson and Walsh, 2004; Sarica et al., 2008; Vezzoli et al., 2015; Marchewka et al., 2019). Feather condition scores, which indicate the severity of feather condition, were not affected by light flicker. Factors that may impact feather condition, including litter quality, behaviour, and infectious mortality, were also not impacted at the time feather scoring occurred (10 wk).

The feather cleanliness of birds can also indicate behavioural abnormalities and altered litter quality (Granquist et al., 2019; Olukosi et al., 2019). In this study, feathers were cleaner in birds reared in the 90HZ. As previously stated, litter quality likely did not influence feather cleanliness. Time spent in contact with litter (resting) was also not affected by flicker. The greater %t preening (10 wk) by birds in the 90HZ was likely the primary cause for improved cleanliness in those birds.

Mobility is often associated with leg development, especially as birds grow to heavy weights, and footpad lesions, which can be painful, as they can change the gait and, therefore, mobility of poultry (Lilburn, 1994; Prayitno et al., 1997; Reiter and Bessei, 2009; Škrbić et al., 2012, 2015). Gait scoring can be used in experimental settings to determine the mobility of birds. The gait of birds, and therefore mobility, was not influenced by light flicker. The footpad lesion scores, which indicate the severity of footpad lesions, showed less severe lesions on birds reared in the 90HZ. The footpad scores did not correspond with the gait scores, indicating that while lesions were present, they were not severe enough to alter gait. Other factors could have affected the gait of birds, including skeletal disease. However, flicker did not affect this, so in combination with no changes to gait score, we conclude that flicker did not affect mobility in this study.

Performance of comfort and maintenance behaviours can be indicative of the state of bird welfare, with preening, wing flapping, dust bathing, stretching, head scratching, feather ruffling, perching, and head rubbing falling within this category (van Rhijn, 1977; Delius, 1988; Zimmerman et al., 2011). Turkeys in this study spent a greater %t preening in the 90HZ and 195HZ (10 wk). Similarly, Evans et al. (2012) found that European starlings increased preening under 100 Hz versus >30 kHz. Conversely, Widowski and Duncan (1996) and Greenwood et al. (2004) found no differences in preening by laying hens or European starlings (respectively). The %t turkeys spend preening differs as they age (increases with maturation), which could have been why changes in preening were not seen early in life (Hughes and Grigor, 1996). While the change in preening in this study could indicate improved welfare for birds reared in the 90HZ, there were no changes in any other comfort behaviours, and the H/L data does not support a reduction in stress at this age (11 wk). Birds may have been displacement preening, which they do when stressed; again, however, the H/L data does not support this conclusion (Spruijt et al., 1992; Delius, 1998; Fraser, 2008; Kozak et al., 2019). As wing flapping occurred at very low incidences and was only impacted by flicker at 8 wk, it is not useful when interpreting the affective state of turkey hens in this study.

Exploratory behaviours can assist birds in understanding their environment as well as their conspecifics (Savory, 1995; Riedstra and Groothuis, 2002; Carter et al., 2013; Agnvall and Jensen, 2016; Dalton et al., 2018; Meuser et al., 2021). European starlings, when exposed to light flicker at high (>30 kHz) and low (100 Hz) in different sequences, increased exploration when exposed to 100 Hz prior to >30 kHz regardless of treatment at the time of measurement (Evans et al., 2012). Laying hens, however, did not differ in time spent ground or object pecking (Widowski and Duncan, 1996). Similar to Evans et al. (2012), turkeys in this study raised under 30 Hz (4 and 8 wk) reduced the %t gentle feather pecking **(GFP)**. Reduced activity could account for this the lower level of GFP. However, there were minimal effects on active behaviours in this study.

One of the theories for the cause of aggressive pecking is a progression of GFP (Kjaer and Vestergaard, 1999; Mcadie and Keeling, 2000). Using this theory, the reduced %t GFP by turkeys reared in the 30HZ (0d-4 wk) may explain the lack of aggressive behaviours in those birds at that age. However, the motivating factors behind aggressive pecking are numerous, so this may not be the case (Savory, 1995; Hughes and Grigor, 1996; Martrenchar, 1999; Dalton et al., 2013; Nicol, 2018). These factors include genetic differences (Flock et al., 2006), environmental and dietary challenges, group size, and behavioural frustration (Hughes and Grigor, 1996; Sherwin et al., 1999; Buchwalder and Huber-Eicher, 2005; Dalton et al., 2013).

Aggressive behaviours can influence the affective state of birds, with the recipients of aggression having a negative affective state (Classen et al., 1994; Hughes and Grigor, 1996). These behaviours can lead to injury or aggressive damage; however, this pattern was not always seen in this work. Aggressive damage was lower in birds raised in the 30HZ than in the other treatments (0d-4, 4-8, 8-11 wk) whereas, aggressive behaviours were only impacted by flicker at 8 wk. This disconnect may have occurred because of the constrictions on behavioural observation compared to live whole room monitoring for aggressive damage. The results of this study do not align with those of Raabe et al. (2024), who saw no effect of LED flicker at 165, 500, or 16,000 Hz on aggression related mortality or injury of turkey toms. This could be because Raabe et al. (2024) did not use visible flicker frequencies. Reduced aggressive damage may have improved the affective state of birds in the 30HZ, reducing stress; this is in agreement with the H/L data.

In this study, aggressive damage towards the neck was greater for turkeys in the 30HZ (8-11 wk). Similar damage was seen by Raabe et al. (2024). Aggressive damage toward the head and neck suggests that birds were fighting (Estevez et al., 2002; Dalton et al., 2013; Sinclair et al., 2015). This is often noted in wild turkeys at approximately the same age as they try to establish hierarchies (Healy, 1992; Buchwalder and Huber-Eicher, 2005; Bartels et al., 2020). It is generally understood that in groups >100, poultry are not able to create a stable dominance hierarchy because they cannot individually recognize that many conspecifics (Pagel and Dawkins, 1997; Martrenchar et al., 1999; Estevez et al., 2002; Keeling et al., 2003; Marchewka et al., 2013; Campderrich et al., 2017). In turkeys, aggression is still seen in commercial sized groups where hierarchies should not be stable. It has been suggested that this may occur as birds try to form a hierarchy without success (Buchwalder and Huber-Eicher, 2005; Bartels et al., 2020). Potentially, visual disruptions by 30 Hz flicker could have caused difficulty for birds to use visual cues to differentiate between one another (Sherwin and Devereux, 1999; Moinard et al., 2001; Dalton et al., 2013).

Ocular weight and morphology can be influenced by multiple aspects of lighting, including daylength (Ashton et al., 1973; Schwean-Lardner et al., 2013; Vermette et al., 2016b), wavelength (Olanrewaju et al., 2015; Remonato Franco et al., 2022), intensity (Prescott et al., 2003; Blatchford et al., 2009), and flicker (Lin et al., 2020). Differences in eye morphology can occur through emmetropization, where the eye grows differently to reduce the amount of refractive error (Prescott et al., 2003; Lin et al., 2020). Myopia, elongation of the eyeball, can lead to nearsightedness (Mayo Clinic, 2022), whereas hyperopia, shortening of the eyeball, can lead to farsightedness (Mayo Clinic, 2020). This study determined that the anterior-posterior **(AP)** size was larger in birds reared in the 30HZ, which contradicts the results of Lin et al. (2020), where the AP of layer chicks was smaller under flickering light. Because Lin et al. (2020)

did not define flicker frequency and tested layer chicks, the changes in eye morphology may be very different from 11 wk turkey hens in the tested flicker frequencies of this study. Likely, the ages at which Lin et al. (2020) and this study examined the eyes could have caused those differences. As birds age, their eyes grow, and their morphology can alter depending on the environment (Prescott et al., 2003). Therefore, a younger bird may have less time for the eye to change than an older bird (Prescott et al., 2003). In this study, the larger AP in birds reared in the 30HZ suggests that those turkeys may have been more nearsighted than those reared in the 90HZ. This could have affected their behaviour based on visual ability (Collins et al., 2011; Remonato Franco et al., 2022). As visual acuity of the turkeys in this study was not examined, any differences in sight could not be quantified. Further examination of bird vision under visibly flickering light could be performed to see what impact flicker might have. The larger dorso-ventral diameter **(DV)** in birds reared in the 30HZ is not indicative of changes to vision. However, it does suggest that the 30HZ may have more impact on eyeball growth than the other treatments.

4.3 Conclusion

In conclusion, white LED light flicker had minimal overall effects on the production, behaviour, health, and welfare of turkey hens to 11 wk, based on measures included in this work. However, some early life effects were noted. The 30HZ caused a reduction in growth early in hens' lives. Turkey hen health was minimally impacted in various ways by the tested frequencies. Turkey behaviour was also altered, with significant changes in the %t performing preening, aggressive, and GFP behaviours. In the visible and potentially visible (30HZ and 90HZ) flicker treatments, bird behaviour appeared to be more affected, leading to reduced welfare through the lack of activity (30HZ) and increased aggression/aggressive damage (90HZ). The changes in eyeball morphology also suggest that there may be changes in vision for birds in the 30HZ and 90HZ, causing alterations in how birds interact with their environment and therefore behaviour.

In future, studies should examine early life behaviour and stress response of turkey hens in visible flicker more thoroughly. Turkey vision could also be examined further. The results of this study could be used to inform future research for areas of interest with light flicker. Regulations regarding the frequency of light flicker in Canadian barns could also use this research as a starting point.

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6.0 Appendix A

Appendix A.1. Standard room temperature (Aviagen Turkeys, 2015)

Age (wk)	Target (ºC)	Minimum (ºC)	Maximum (ºC)
Day 1*	34		
1	31	27	31
2	29	25	29
3	28	23	27
4	24	22	26
5	22	20	24
6	21	19	23
7	20	18	22
8	19	17	21
9	18	16	20
10	17	15	19
11	16	14	18

Appendix A.2. Lighting regime (Aviagen Turkeys, 2015)

Age (d)	Light:Dark (hrs)	Lights On	Lights Off	Light Intensity (lux)
0-2	23L:1D	8:00 am	7:00 am	40
3	22L:2D	8:00 am	6:00 am	40
4	21L:3D	8:00 am	5:00 am	40
5-6	20L:4D	8:00 am	4:00 am	30
7	19L:5D	8:00 am	3:00 am	20
8	19L:5D	8:00 am	3:00 am	10
9-market	18L:6D	8:00 am	2:00 am	10

Appendix A.3. Humane Intervention Point Checklist for Poultry From Poultry Centre SOP Guide – Reviewed 2021

Score	Description
0	Normal. Bird is active, mobile, and curious. Preens feathers, normal plumage Move away quickly when approached. Body weight at expected weight, or more than 90% of flock average.
1	Hunched or asymmetric posture. Bird hesitates to move; may show mild lameness. Mild changes in food and water consumption. Does not forage for food readily Body weight equal to or greater than 70% of flock average, but less than 90%.
2	Hunching with unpreened plumage. Bird is slow to respond, or mildly depressed, but still responsive to environment. Bird is reluctant to stand, may show lameness or mild incoordination when it moves. May have moderate diarrhea. Superficial skin wounds. Body weight is greater than 50% but less than 70% of flock average.
3	Unable to stand or remain standing. Bird is depressed and not responsive to stimuli. Head is extended with open mouth breathing. Bird is very lame or incoordination and unable to reach for food or water; obvious leg defects or broken bones. Severe diarrhea. Large or severe wounds affective muscle layer. Infected wounds Body weight is less than 50% of flock average

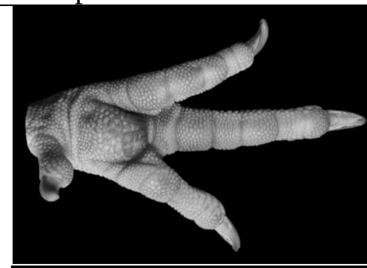
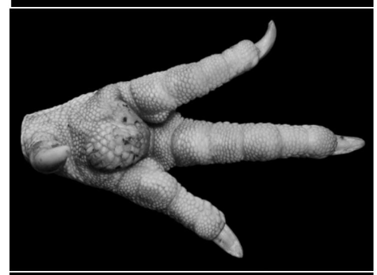
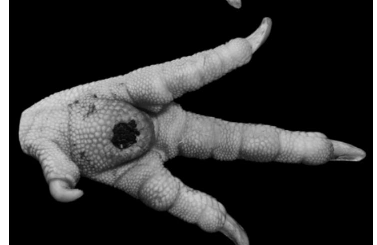
Expected body weights found in breed guidelines (Aviagen Turkeys, n.d.).
Birds with scores of 0-1 require no intervention. Continue to monitor.
Birds demonstrating any of the category 2 descriptions require close monitoring, investigation, and possibly intervention. Notify primary investigator and consult a veterinarian if needed.
Birds demonstrating any of the category 3 descriptions require immediate intervention. Consult a veterinarian or euthanize immediately

Appendix A.4. Gait scoring and descriptions adapted from Garner et al. (2002); Vermette et al. (2016b); Beaulac and Schwean-Lardner (2018)

Gait Score	Degree of Impairment	Description
0	None	Smooth, fluid locomotion. The foot furls when raised.
	Modified	Straight legs.
1	Detectable but with no identifiable abnormality	The bird is unsteady, or wobbles when it walks. However, the problem leg is unclear, or cannot be identified in the first 20s of observation. The bird readily runs from the observer in the pen. The foot may remain flat when raised, but the rest of the stride is fluid and appears unimpaired. Gait appears unstable, either shaky or stomping. The problem leg cannot be identified in the first 20 s of observation. Foot may appear flat when raised but the rest of stride is fluid.
	Modified	Gait appears unstable (shaky or stomping).
2	Abnormality is identifiable but has little impact on function	The leg producing the gait defect can be identified within 20 s of observation. If a problem leg is identified after 20 s of observed locomotor behaviour then the bird is classed as gait score 1. However, the defect seems to have only a minor impact on biological function. Thus, the bird will run from the observer spontaneously or if touched or nudged with the padded stick. If the bird does not run at full speed, it runs, walks or remains standing for at least 15 s after the observer in the pen has ceased to move towards or nudge it. Birds in this, and previous, scores are often observed to scratch their face with their feet-again indicating little impact on function. (The most common abnormality in this score is for the bird to make short, quick, unsteady steps with one leg, where the foot remains flat during the step.).
3	Abnormality is identifiable and impairs function	Although the bird will move away from the observer when approached or touched, or nudged, it will not run, and squats within 15 s or less of the observer in the pen ceasing to approach or nudge it. If the bird squats after 15 s have elapsed, it is classified as gait score 2.
4	Severe impairment of function but the animal is capable of walking	The bird remains squatting when approached or nudged. This criterion is assessed by approaching the bird, and if it remains squatting, gently nudging or touching the animal for 5 s. Animals may appear to rise but still resting upon their hocks. Only rising to stand on both feet within 5 s of handling is counted—a bird which takes longer than 5 s to rise, or which does not rise at all is scored as 4, while a bird that rises in 5 s or less is counted as a 3 (or lower if its gait is good). Nevertheless, the bird can walk when picked up by the observer and placed in a standing position, but

		squats immediately following one or two steps. (Squatting often involves a characteristic ungainly backwards fall.)Bird remains squatting for 5 s when approached. The bird may stand slightly, however the hocks still remain on the floor. If the bird takes less than 5 s to rise when approached it is counted as a score less than 4. If the bird takes more than 5 s to rise it is counted as a 4. If the bird can walk when picked up by observer and placed in standing position but squats immediately after 1 or 2 steps. Or if the bird requires wings to balance when standing or squatting.
	Modified	Bird requires wings for balance.
5	Complete lameness	The bird cannot walk, and instead may shuffle along on its hocks. It may attempt to stand when approached but is unable to do so, and when placed on feet unable to complete a step with one or both legs.

Appendix A.5. Footpad scoring system from Hocking et al. (2008)

Score	Description	Comparison
0	No external signs of footpad dermatitis. No swelling or necrosis is visible. Skin feels soft and unblemished	
1	Foot pad is harder and denser than a 0 score. Central part of the pad is raised, and the scales are separated. There is potential presence of black necrotic areas.	
2	Noticeable swelling on the foot pad. Reticulate scales are black with scale shaped necrotic areas. Scales around the outside edge of the necrotic area may be white. Area of necrosis is less than one quarter of the total area of the foot pad.	
3	Swelling evident and total foot pad is enlarged. Reticulate scales are pronounced, separated, and increased in number. Necrosis extends to half the foot pad.	
4	Same as score 3 but has more than half the foot pad covered by necrotic cells.	

Appendix A.6. Feather scoring system. From Davami et al. (1987); Sarica et al. (2008); Beaulac and Schwean-Lardner (2018)

Score	Description
1	No feather cover
2	More than half of the plumage is missing
3	Few or less than half of the plumage is missing
4	Full, intact plumage

Appendix A.7. Scoring system for feather cleanliness. From Forkman and Keeling (2009) as modified from Wilkins et al. (2003)

Score	Description
1	Very clean: more than 75% of the body feathers free from soiling
2	Moderately clean: 50-75% of the body feather are free from soiling
3	Moderately dirty: 25-50% of the body feathers are free from soiling
4	Very dirty: less than 25% of the body feathers are free from soiling

Appendix A.8. Litter quality assessment. Adapted from Welfare Quality Consortium® (2009) and Adler et al. (2021)

Photo Reference					
Score	0	1	2	3	4
Criteria	Completely dry and flaky, easily moved	Dry but clumps form when compacted	Compacted ball forms and falls apart when pressure is removed	Readily sticks into a ball when compacted	Cap/crust must be broken and sticks in a wet ball. (Boot imprint may be left in litter under cap)

Appendix A.9. Effect of LED light flicker on causes and categories of mortality and culls as a percent of birds placed in turkey hens at 0d-4 weeks of age

% of birds in category	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Aggressive Damage	0	0.09	0	0.034	0.465
Metabolic	0.16	0.23	0.16	0.041	0.721
Round Heart	0.16	0.23	0.16	0.041	0.721
Sudden Death with Peri-Renal Hemorrhage	0	0	0	-	-
Rotated tibia*	0.11	0	0.05	0.028	0.101
Infectious	0.22	0.37	0.05	0.057	0.070
Yolk Sac Infection	0.05	0.09	0	0.028	0.421
Osteomyelitis	0.11	0.09	0	0.031	0.333
Peritonitis	0.05	0	0.05	0.023	0.559
Polyserositis	0	0.09	0	0.023	0.171
Arthritis/Synovitis	0	0.05	0	0.017	0.465
Pericarditis	0	0.05	0	0.017	0.465
General Infection	0	0	0	-	-
Mechanical	0.05	0.05	0	0.023	0.639
Trauma	0.05	0.05	0	0.023	0.639
Broken Bone	0	0	0	-	-
Unknown[2]	0.71	0.46	0.16	0.135	0.262
Other	0.22	0.18	0.11	0.055	0.768
Emaciation/Starveout	0.11	0.05	0	0.028	0.308
Pendulous Crop	0.05	0	0	0.017	0.357
Decomposed	0	0.05	0	0.017	0.465
General	0.05	0.09	0.11	0.033	0.819
Total	1.48[a]	1.37[a]	0.55[b]	0.151	0.005

[*]Block differed significantly: included as a random factor ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean
[2]Category consists of No Visible Lesions

Appendix A.10. Effect of LED light flicker on causes and categories of mortality and culls as a percent of birds placed in turkey hens at 4-8 weeks of age

% of birds in category	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Aggressive Damage	1.87	1.69	1.54	0.219	0.696
Metabolic	0.33	0.09	0.05	0.050	0.051
Round Heart	0.27[a]	0.05[b]	0.05[b]	0.043	0.040
Sudden Death with Peri-Renal Hemorrhage	0.05	0.05	0	0.023	0.638
Rotated tibia*	0	0	0	-	.
Infectious	0.05	0.05	0	0.023	0.639
Yolk Sac Infection	0	0	0	-	-
Osteomyelitis	0	0	0	-	-
Peritonitis	0.05	0	0	0.017	0.357
Polyserositis	0	0	0	-	-
Arthritis/Synovitis	0	0	0	-	-
Pericarditis	0	0	0	-	-
General Infection	0	0.05	0	0.017	0.465
Mechanical	0.11	0	0.05	0.028	0.276
Trauma	0.05	0	0.05	0.023	0.559
Broken Bone	0.05	0	0	0.017	0.357
Unknown[2]	0.22	0.14	0.05	0.043	0.308
Other	0.33	0.18	0.22	0.061	0.601
Emaciation/Starveout	0	0	0	-	-
Pendulous Crop	0.33	0.14	0.22	0.063	0.457
Decomposed	0	0.05	0	0.017	0.465
General	0	0	0	-	-
Total	2.91	2.15	1.92	0.234	0.194

[*]Block differed significantly: included as a random factor ($P<0.05$)
[a,b]Values with different letters within the same row differ significantly ($P<0.05$)
[1]Standard error of the mean
[2]Category consists of No Visible Lesions

Appendix A.11. Effect of LED light flicker on causes and categories of mortality and culls as a percent of birds placed in turkey hens at 8-11 weeks of age

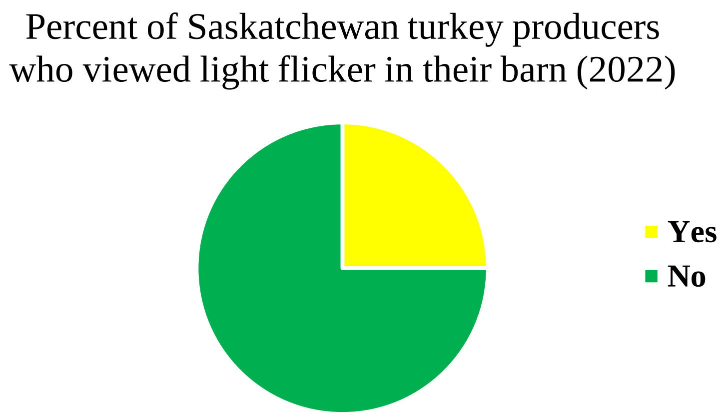
% of birds in category	Treatments (Hz)			SEM[1]	*P*-value
	30	90	195		
Aggressive Damage	1.43	1.10	1.76	0.220	0.372
Metabolic	0.05	0.05	0.11	0.031	0.690
Round Heart	0	0.05	0.05	0.023	0.639
Sudden Death with Peri-Renal Hemorrhage	0.05	0	0.05	0.023	0.559
Rotated tibia*	0	0	0	-	-
Infectious	0	0	0	-	-
Yolk Sac Infection	0	0	0	-	-
Osteomyelitis	0	0	0	-	-
Peritonitis	0	0	0	-	-
Polyserositis	0	0	0	-	-
Arthritis/Synovitis	0	0	0	-	-
Pericarditis	0	0	0	-	-
General Infection	0	0	0	-	-
Mechanical	0.22	0.41	0.33	0.084	0.696
Trauma	0.05	0.18	0.27	0.055	0.320
Broken Bone	0.16	0.23	0.05	0.061	0.538
Unknown[2]	0.11	0.14	0.22	0.061	0.839
Other	0.27	0.14	0.33	0.049	0.279
Emaciation/Starveout	0	0	0	-	-
Pendulous Crop	0.27	0.14	0.33	0.049	0.279
Decomposed	0	0	0	-	-
General	0	0	0	-	-
Total	2.09	1.83	2.75	0.214	0.208

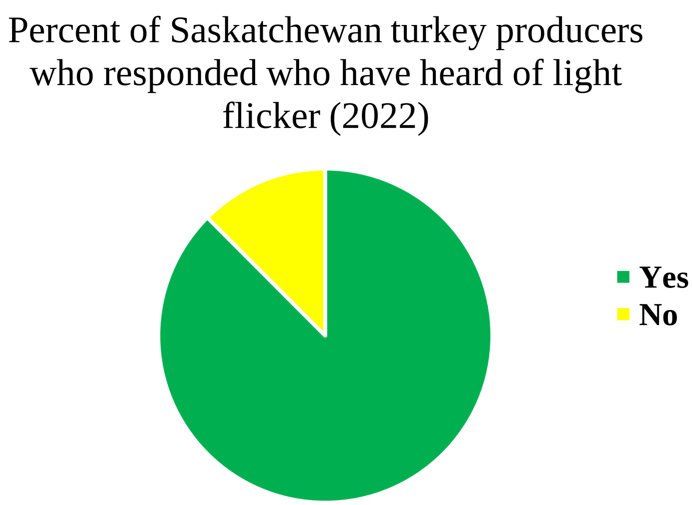
[*]Block differed significantly: included as a random factor ($P<0.05$)
[1]Standard error of the mean
[2]Category consists of No Visible Lesions

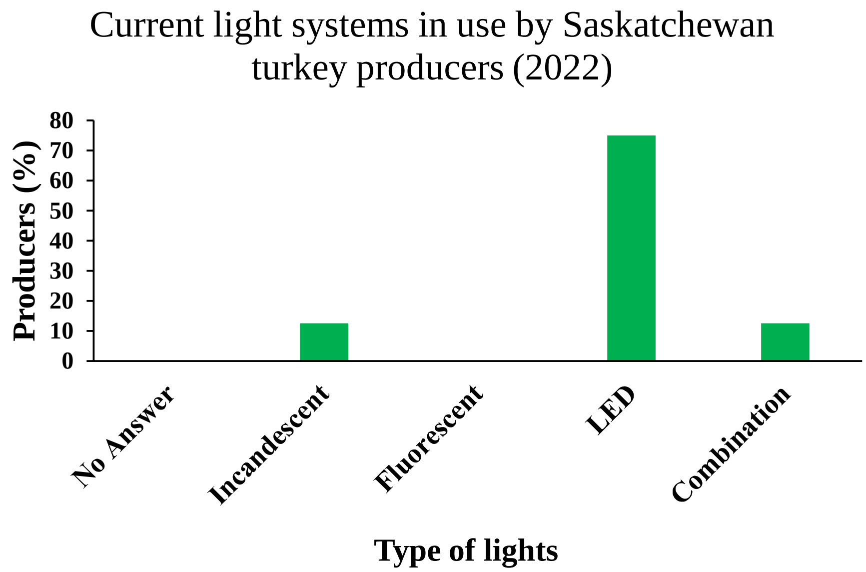
Appendix A.12. Weekly minimum and maximum temperatures (°C) for each trial (Environment and Climate Change Canada, 2022)

Date	Min (°C)	Max (°C)
Trial 1		
January 11th – 17th 2022	-15.8	0.6
January 18th - 24th 2022	-35.0	1.9
January 25th - 31st 2022	-33.2	1.6
February 1st – 7th 2022	-36.5	3.6
February 8th -14th 2022	-19.4	4.0
February 15th -21st 2022	-29.0	3.1
February 22nd -28th 2022	-31.3	-3.3
March 1st -7th 2022	-24.1	1.8
March 8th -14th 2022	-34.2	3.5
March 15th -21st 2022	-7.9	6.2
March 22nd -25th 2022	-10.1	9.4
Trial 2		
November 14th -20th 2022	-17.1	0.5
November 21st -27th 2022	-14.2	3.3
November 28th -December 4th 2022	-32.2	-3.4
December 5th -11th 2022	-36.9	-4.2
December 12th -18th 2022	-26.2	-7.6
December 19th -15th 2022	-37.7	-14.4
December 26th -January 1st 2022	-24.6	-7.8
January 2nd – 8th 2023	-22.1	-5.1
January 9th -15th 2023	-20.4	-7.7
January 16th -22nd 2023	-16.4	1.3
January 23th -29th 2023	-28.9	3.7
January 30th 2023	-29.6	-19.0

7.0 Appendix B






Current light systems in use by Saskatchewan turkey producers (2022)
Producers (%)
80
70
60
50
40
30
20
10
0
No Answer
Incandescent
Fluorescent
LED
Combination
Type of lights